REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE

Université des Frères Mentouri Constantine1
Faculté des Sciences de la Nature et de la Vie
Département de Biologie Animale

N° d'ordre :02/BA/2021
N° de série :34/DS/2021

Thèse de Doctorat
Présentée pour obtenir le diplôme de Doctorat en sciences
Option : **Biologie et physiologie moléculaire**

Présentée et soutenue par :

SAKHRI Fatma Zahra

Intitulé :

Effect of *Phoenix dactylifera* and *Cydonia oblonga* fruits on some diseases induced by stress

Membres du jury :

Président :	**TEBIBEL Soraya**	**Prof**	**Université frères Mentouri –Constantine1**
Directeur de thèse :	**ZERIZER Sakina**	**Prof**	**Université frères Mentouri –Constantine1**
Examinateurs :	**ABDENNOUR Cherif**	**Prof**	**Université Badji Mokhtar-Annaba**
	ZELAGUI Amar	**Prof**	**Université Larbi Ben M'Hidi-Oum el Bouaghi**
	DEHAMNA Saliha	**Prof**	**Université Ferhat Abbas-Sétif**
	CHETTOUM Aziz	**MCA**	**Université frères Mentouri –Constantine1**

Année universitaire 2020/2021

To all dead people during the COVID-19 pandemic

To all my little mice and rats that were sacrificed for this research.

To my lovely parents, sisters, brother for their love, support, and encouragement

throughout my life and academic career.

Acknowledgements

I would like to thank my supervisor Pr. S. Zerizer for her trusting me with the project, for her comments, advices, during this period.

With great pleasure and profound sense of gratitude, I express my most cordial and humble thanks to Pr. M. Sunagawa who provided access to his lab without which portions of this study would have been impossible to conduct, and for giving me the opportunity to work with Dr. N. Adachi who always has time to listen to my ideas and answer my unlimited questions. All the support is grateful acknowledged. I also want to thank all the staffs and PhD students at Prof. Sunagawa lab and all the staffs of Showa university for creating a nice and friendly atmosphere.

My special thanks and my profound gratitude are addressed to Pro. T. Hisamutsu president of Showa university, for his kindness, support and encouragement during my stay in Japan.

Thank you to Pr. Z. Kabouche for her comments and advices.

Thank you to Dr. C. Bensouici for his help.

Thanks to Pr. S. Tebibel, Pr. A. Cherif, Pr. A. Zelagui, Pr. S. Dehamna and Dr. A. Chettoum, for accepting to evaluate my PhD thesis.

A deep thank to my best friends; Hakim, Kheyra, Yaakoub, Mansour, Master, Benyamine, Wakachan, Yusukichan, Shichan, Mami and Meiko for their support and unforgettable memories.

CHAPTER II MATERIAL AND METHODS

II MATERIAL AND METHODS

ACTH: Adrenocorticotropic Hormone

AGIs: α-Glucosidase Inhibitors

ALT: Aspartate Aminotransferase

ALP: Alkaline Phosphatase

ANOVA: Analysis of Variance

BHA: Butylated Hydroxy Anisole

BHT: Butylated Hydroxytoluene

BSA: Bovine Serum Albumin

CA1: Cornu Ammonis 1

CA3: Cornu Ammonis 3

CORT: Corticosterone

CRH: Corticotropin-Releasing Hormone

CUPRAC: CUPric Reducing Antioxidant Capacity

DAPI: 4′,6-diamidino-2-phenylindole

DCX: Doublecortin

DPPH: 1,1-Diphenyl-2-Picryl-Hydrazyl free radical

DG: Dentate Gyrus

DMSO: Dimethyl Sulfoxid

DTNB: 5,5′-dithiobis-2 nitrobenzoic acid

EDTA: Ethylene Diamine Tetra Acetic Acid

FST: Forced Swimming Test

GABA: Gamma-aminobutyric Acid

GSH: Reduced Glutathione

GSSG: Gluthathione Disulphide

GR: Glucocorticoid Receptors

HPA: Hypothalamus-Pituitary-Adrenal

i.p: Intraperitoneal

IC_{50}: The half maximal inhibitory concentration

IL-1: Interleukin 1

IL-2: Interleukin 2

IL-6: Interleukin 6

LD50: Medial Lethal Dose

MCV: Mean Corpuscular Volume

MCH: Mean Corpuscular Haemoglobin

MCHC: Mean Corpuscular Haemoglobin Concentration

MDD: Major Depressive Disorder

MR: Mineralocorticoid Receptors

NOS: Nitric Oxide Synthases

NPG: p-Nitrophenyl-a-D-glucopyranoside

NS: Not Significant

NSPCs: Neural Progenitor/Stem Cells

OECD: Organisation for Economic Cooperation and Development

OFT: Open Field Test

PBS: Phosphate Buffered saline

PFA: Paraformaldehyde

PTSD: Posttraumatic Stress Disorder

PVN: Paraventricular Nucleus

RBC: Red Blood Cells

ROS: Reactive Oxygen Species

SAM: Sympathetic-Adrena1 Medullary

SEM: Standard Error of the Mean

SGZ: Sub Granular Zone

SPSS: Statistical Package for Social Science

SPT: Sucrose Preference Test

SVZ: Subventricular Zone

TCHOL: Total cholesterol

TG: Triglycerides

TNFα: Tumor Necrosis Factor

WBC: Wight Blood Cells

WHO: World Health Organization

List of Tables

Introduction

Introduction

Chronic stress has long been associated with the development of mental disorders such as anxiety disorders and depression. World Health Organization (2017) reports that more than 300 million people are suffering from depression, equivalent to 4.4% of the world's population; with a high risk in the population of (15-40) years old. In Algeria, neuropsychiatric disorders contribute to 13.1% of the global burden of disease (World Health Organization, 2011), with a suicide rate of 15/100.000 habitats.

Depressed patients are usually accompanied by dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Chan et al., 2017), mood disturbance, weight changes, sleep abnormalities (Nollet et al., 2012), anhedonia, and anxiety (Micheli et al., 2018). Moreover, clinical studies showed a reduction of hippocampal volume, the atrophy of the hippocampus and prefrontal cortex (Banasr et al., 2011; Micheli et al., 2018; Toda et al., 2019), and/or reduction in glial density in the amygdala in depressed patients (Bowley et al., 2002).

It has now been established that adult neural stem/progenitor cells (NSPCs) located in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus proliferate and give rise to new neurons. The potential roles of adult neurogenesis have been widely reported in cognitive processes such as learning and memory (Parihar et al., 2011; Hueston et al., 2017; Toda et al., 2019), olfaction (Micheli et al., 2018), the regulation of the HPA axis, and antidepressant actions (Alves et al., 2018). This process occurs in the brain of mammals, including humans, throughout the life-span (Yun et al., 2010; Parihar et al., 2011).

Importantly, Morphological change in the hippocampus and the lower level of adult hippocampal neurogenesis are possibly associated with stress-induced depression and cognitive dysfunction (Parihar et al., 2011; Hueston et al., 2017; Toda et al., 2019).

Antidepressant treatment can ameliorate both the stress induced behavioral changes and the suppression of neurogenesis in the DG of the hippocampus (Surget et al.,2008). It is, therefore, considered that the hippocampal neurogenesis is an indicator of the depressive state and could be a key target of the medical treatment for major depressive disorder patients (MDD).

Because of the technical limitations of human studies, limited availability, and the variable conditions of human samples, several rodent models of depression have been developed to facilitate our understanding of the neurobiological link between depression and hippocampal neurogenesis. Numerous studies (Gregus et al., 2005; Murray et al., 2008; Rosa et al., 2014; Ali et al., 2015; Lee et al., 2015) have indicated that an animal model of depression induced by repeated glucocorticoids administration was a useful and reliable model to investigate behavioural, endocrine, and neurobiological changes underlying stress-related disorders and exploring the pharmacological. Another commonly used rodent models to investigate the impact of stressful events in the adulthood on body and brain is chronic immobilisation stress, as it leads to characteristic phenotypes, such as anhedonia and anxiety-like behaviours, and neuronal alterations observed in depressive patients (Chen et al., 2008; Zhu et al.,2008; Chirg et al.,2009). Importantly, the reduced volume in the hippocampus can be reproduced in this rat model of depression. These changes are alleviated by the administration of human antidepressants (Surget et al., 2008). It is also noteworthy that blocking or suppressing

hippocampal neurogenesis resulted in the loss of the antidepressants' effect in animal models of depression (Santarelli et al., 2003; Surget et al., 2008; Banasr et al., 2011), suggesting that neurogenesis is required for antidepressants to evoke behavioural changes.

Besides advances in antidepressant drugs, other approaches are also important in the treatment of depression. For instance, electroconvulsive therapy has been one of the most effective treatment methods so far. Other neurostimulation techniques, including deep brain stimulation, transcranial magnetic stimulation, and vagus nerve stimulation, have been recently proposed in the treatment of MDD. Despite their different therapeutic targets, these therapies have limited applicability due to several reasons, such as delayed response, side effects, and lack of efficacy in a large percentage of patients (Santarelli et al., 2003; Ampuero et al., 2015). These circumstances are forcing researchers to look for new solutions concerning new therapeutic possibilities, besides a better understanding of the etiology of depression.

Because nothing is better than keeping ourselves away from diseases, the importance of herbal compounds, dietary fruit, and vegetable supplementation is currently enjoying a renaissance worldwide. Pharmacological studies revealed that popular compounds abundantly present in natural plants, such as curcumin (Xu et al., 2007), resveratrol (Liu et al., 2016), proanthocyanidin (Xu et al., 2010), blueberry polyphenols (Casadesus et al., 2004), Naringenin (Tayyab et al., 2019), and quercetin (Mehta et al., 2017), have been shown to improve the symptoms of depression and deficits in learning and memory by affecting the hippocampal region with different molecular mechanisms.

Regular consumption of natural bioactive compounds from dietary plants including fruits may be associated with protection against oxidative damage and lowered the risk of chronic diseases (Commenges et al., 2000; Al-Farsi et al.,2005; Flores et al., 2020). Polyphenolics and flavonoids constitute an important class of secondary metabolites that act as free radical scavengers and inhibitors of low-density lipoprotein, cholesterol oxidation, and DNA breakage, they can also form complex with minerals and hence, reduce mineral bioavailability (Al-Farsi et al.,2005; Aiyegoro and koh, 2010; Baliga et al 2011; Doungue et al.,2018). Furthermore, many plants are well-known to have immunomodulating activities. In addition to treating infectious diseases, the immunomodulatory responses of medicinal plants are also used for the treatment of arthritis, allergy, asthma, degenerative diseases, analgesic, anti-convulsive (Gao X et al.,2004).

Due to the richness and diversity of the metabolites in *Phoenix dactylifera L* and *Cydonia oblonga* Mill fruits and the lack of studies regarding the preventive effect of daily fruits consumption against daily stressful events leading to depression, the purpose of this thesis was to investigate the effect of these fruits' extracts on chronic stress-induced neurobehavioural changes in a rat model of depression.

To achieve this, the following objectives were determined:

- Preparation of the hydro-ethanolic extracts of *Phoenix dactylifera* and *Cydonia oblonga* fruits
- Evaluation of the antioxidant activity of prepared extracts *in vitro*

- Evaluation of the antidiabetic activity of *Cydonia oblonga* fruit extract *in vitro*
- Testing the safety of the extracts using acute toxicity tests in animals.
- Evaluation of the immunomodulatory effect of *Cydonia oblonga* fruit extract using carbon clearance assay.
- Evaluation of the antioxidant effect of *Cydonia oblonga* fruit extract using the GSH reduced dosage *in vivo*
- Investigate whether the chosen chronic stress paradigms (corticosterone injection/immobilisation stress) induce depression-like behaviours in rats.
- Evaluation of the anti-depressive activity of *Phoenix dactylifera* extract in a rat model of depression induced by chronic corticosterone injection using a forced swimming test.
- Investigation of the effect of chronic immobilisation stress and *Cydonia oblonga* extract treatment on physiological changes.
- Evaluation of the antidepressive effect of *Cydonia oblonga* fruit extracts in a rat model of depression induced by chronic immobilisation stress using behavioural test.
- Evaluation of the chronic stress effect and treatment with *Cydonia oblonga* fruit extract on hippocampal neurogenesis

Chapter I

Literature Review

I-1 Stress

I-1-1 Definition of stress

Stress, in the biological context, is the body reaction to any physical, mental, or emotional factors that a sensed threat to homeostasis. Stresses can be external (from the environment, psychological, or social situations) or internal (illness, or from a medical procedure). In 1976, Han Selye illustrated a neat definition of stress:

"Stress is part of our daily human experience, but it is associated with a great variety of essentially dissimilar problems, such as surgical trauma, burns, emotional arousal, mental or physical effort, fatigue, pain, fear, the need for concentration, the humiliation or frustration, the loss of blood, intoxication with drugs or environmental pollutants, or even with the kind of unexpected success that requires an individual to reformulate his lifestyle. Stress is present in the businessman under constant pressure; in the athlete straining to win a race; in the air-traffic controller who bears continuous responsibility for hundreds of lives; in the husband helplessly watching his wife's slow, painful death from cancer; in a racehorse, its jockey and the spectator who bets on them."

According to the duration of stress, stresses may be divided into two classes: acute stress (for example: speech tasks, running late for work, medical examination) and chronic stress (such as high-pressure jobs, financial difficulties, challenging relationships) which could be subdivided into disconnected and persistent stress.

I-1-2 The stress response

Despite the large variety of stressors, two main pathways are required to maintain homeostasis during a response to stress; the immediate response by the autonomic nervous system through the sympathetic-adrenal-medullary (SAM) axis followed by the delayed neuroendocrine changes through the HPA axis.

The activation of SAM leads to the release of catecholamines, noradrenaline, and adrenaline from the adrenal medulla into the bloodstream. This, in turn, bind to the α- and ß-adrenergic receptors in the heart muscle and blood vessel walls and lead to increase

heart rate and blood pressure and generating a “fight-or-flight” response (Herman et al., 2016).

Neuronal signals associated with a stressor activate the parvocellular neuroendocrine cells within the paraventricular nucleus (PVN) of the hypothalamus to release corticotropin-releasing hormone (CRH) and vasopressin which in turn stimulates the anterior pituitary to trigger the synthesis of adrenocorticotropic hormone (ACTH). Secreted ACTH stimulates via binding to its specific receptor on the adrenal cortex (*zona fasciculata*) the synthesis and the release of the stress hormones, (cortisol in humans and corticosterone in rodents), into the bloodstream with peak plasma levels occurring approximately ten to fifteen minutes after the initiation of stress (Jacobson, 2005; Smith and Vale, 2006; Locatelli et al., 2010; Gjerstad et al., 2018).

The release of glucocorticoids is ultimately inhibited through a negative feedback mechanism of glucocorticoids onto their receptors in the anterior pituitary and the paraventricular nucleus of the hypothalamus: the mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). These two receptor types differ in the affinity of glucocorticoids, thereby their functional role (Smith and Vale, 2006; Gjerstad et al.,2018). Low cortisol levels activated the MR which are thought to be primarily involved in maintaining basal HPA activity, when these levels rise and MR become saturated, GR moderate glucocorticoid activity and are responsible for the termination of the stress response resulted in decrease glucocorticoids concentrations to pre-stress levels via negative feedback inhibition (Locatelli et al., 2010; Gjerstad et al., 2018).

It is through this network known as the HPA axis that glucocorticoids, released into the systemic circulation, allow the body to recover and adapt to a stressor via different pathways such as stimulating energy mobilization, gluconeogenesis, lipolysis, dampening of immune system reaction, and the direct negative feedback of HPA axis to ovoid stress responses from overshooting (Smith and Vale, 2006). The adaptive processes that underlie the stress response have been collectively termed as allostasis.

While the acute stress response is an important and necessary mechanism to adapt to environmental changes and survival, hyperactivation of the HPA system, during poor

adaptation to stress and prolonged or excessive stress, can generate an allostatic load, which have detrimental effects on its integrators (nervous neurotransmitters (Locatelli et al.,2008), endocrine hormones (Olff et al.,2006), and immune system secondary lymphoid organs accompanied by the increased risk for the development of psychiatric disorders, such as anxiety, depression and posttraumatic stress disorder (PTSD). Despite the compelling evidence outlined above, it is still unknown whether HPA axis abnormalities are a primary cause of depression or, instead, secondary to depressed mood.

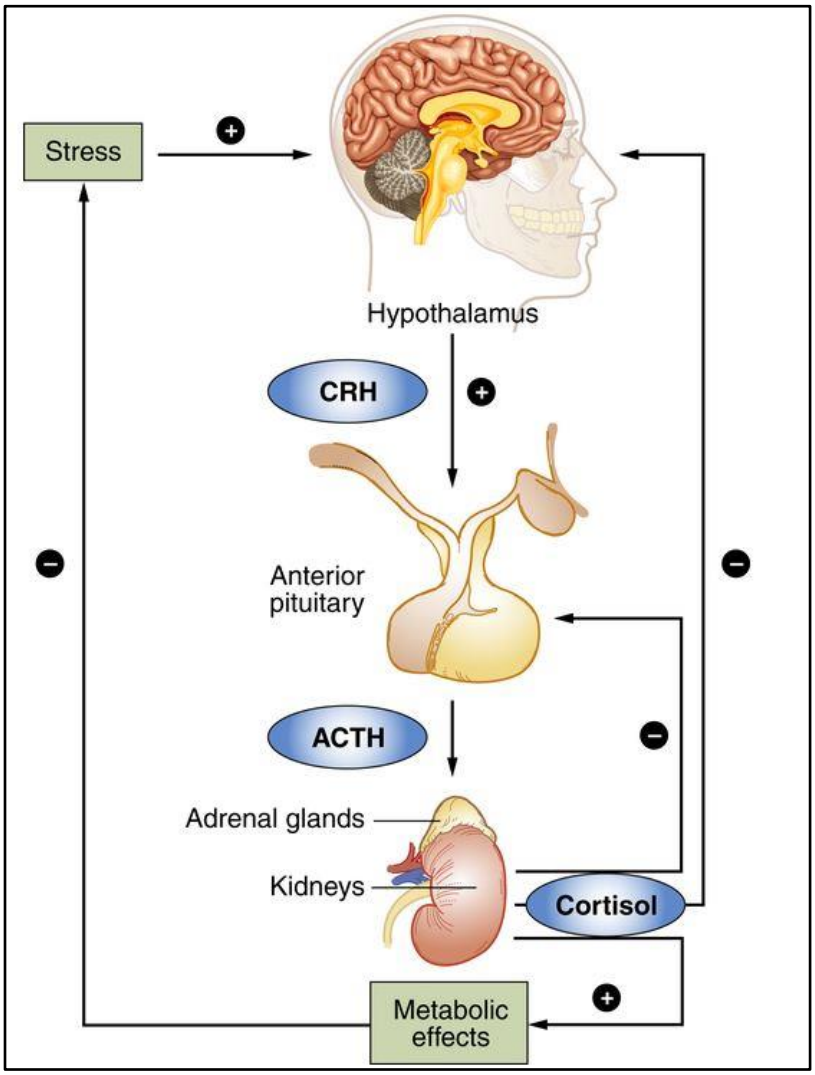

Figure 01: Hypothalamic-Pituitary-Adrenal (HPA) Axis. The response to stress begins in the brain. The hypothalamus is the control center in the brain for many hormones including CRH, Adrenocorticotropic hormone (ACTH) (https://basicmedicalkey.com/stress-and-disease)

I.2. Adult hippocampal neurogenesis

The hippocampal is a part of the brain, its structure is comprised of functionally heterogeneous subfields: the *Cornu Ammonis* (CA1-CA3), DG, and subicular complex. Adult neurogenesis refers to the birth of functional new neurons from neural stem cells in the adult brain that occurs throughout the life course in the adult mammalian brain, including that of humans. It occurs in two specific brain region areas: the SVZ of the lateral ventricles, and the SGZ in the DG of the hippocampus (Santarelli et al., 2003; Zhao et al., 2008; Christie and Turnley, 2013).

Adult neurogenesis involves a multi-step process starting with the proliferation of progenitor cells, followed by morphological and physiological maturation, and finally functional integration, these new cells express several markers (Figure 2) (Zhao et al., 2008; Krugers et al.,2010; Christie and Turnley, 2013). The total time to achieve a mature morphological and electrophysiological phenotype is around two months in rodents (Toda et al.,2019)

The new neurons generated in the SVZ during adulthood migrate along the rostral migratory stream to the olfactory bulb, where they differentiate into interneurons and contribute to the olfactory function (GABAergic inhibitory interneuron). However, in the case of pathological conditions such as stroke or brain trauma, the SVZ new neurons are redirected toward the damaged area where they contribute to repair (Christie and Turnley, 2013; Micheli et al., 2018).

Numerous studies suggest that new-born granular neurons in adult hippocampal are involved in cognitive homeostasis, the acquisition of spatial learning and memory, pattern separation, and antidepressant actions (Garthe et al., 2009; Krugers et al.,2010, Parihar et al., 2011; Jeon et al.,2016; Alves et al., 2018; Micheli et al., 2018; Toda et al., 2019) and the dysregulation of adult hippocampal neurogenesis may be associated with cognitive decline in neurological disorders and psychological symptoms in psychiatric and mental disorders (Parihar et al., 2011; Alves et al., 2018; Toda et al.,2019).

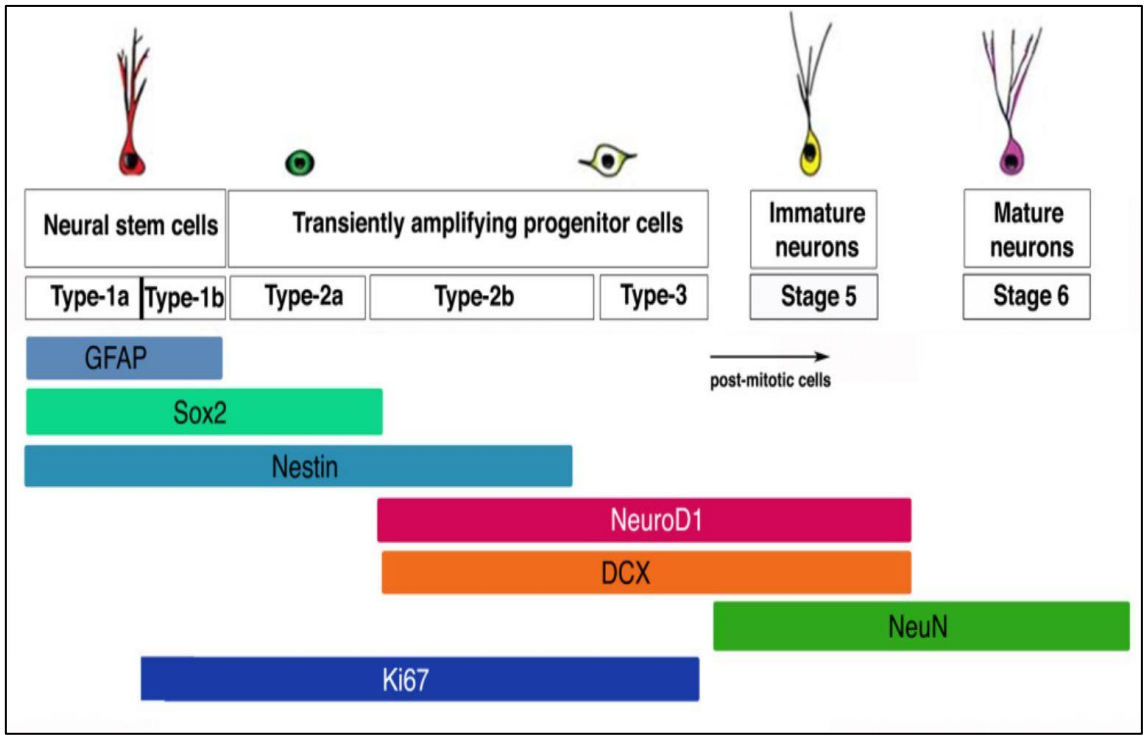

Figure 02. Immature neuronal markers expressed during neurogenesis (Farioli-Vecchioli et al., 2014)

I-3 Chronic stress and the hippocampal neurogenesis

It is now obvious that chronic stress can lead to atrophy of the brain mass and decrease its weight and affects different aspects of the nervous system. Chronic restrain stress significantly decreased the length and branch of CA3 pyramidal neurons, as well as contributes to the reduction in hippocampus volume. However, the exact cellular pathways mediating the inhibitory effect of chronic stress on neurogenesis are largely unknown (Zhao et al., 2008; Zhu et al., 2008; Christie and Turnley, 2013; Toda et al.,2019)

Regulation of neurogenesis could occur at several different stages, including cell proliferation, differentiation, and survival, it thought that stress can be diverse, ranging from apoptosis of progenitor cells to slow down the cell cycle by the upregulation of neuronal marker; an increase of the cell cycle inhibitor p27Kip1, induced cell cycle arrest. (Heine et al., 2004; Andreu et al., 2015)

Repeated stress also induced the expression of several proinflammatory cytokines by glia cells in the brain such as interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-α inducible nitric oxide synthases (NOS) (Golovatscka et al.,2012; Johnson et al., 2019), that are often negatively influenced the adult neurogenesis.

I-4 Natural products

Natural products, containing medicinal plants, fruits, vegetables, grains, etc., are an important source for the discovery of novel chemical substances that can possibly be developed into clinical products with potential therapeutic effects.

The study of Commenges et al. (2000) showed that an average intake of 14.4 mg/day antioxidant flavonoids (mainly from fruits and vegetables) leads to a significant reduction in the risk of dementia in a population of 3777 men and women over 65 ages in France. Several other studies (Chatzigeorgiou et al., 2017; Doungue et al.,2018, Daskalova et al.,2019; Flores et al., 2020) have been conducted to prove the existence of a relationship between fruit consumption and the prevention of neurological diseases.

I-4-1 *Phoenix dactylifera*

The botanical name of the date palm, *Phoenix dactylifera L*., is presumably derived from a Phoenician name "*phoenix*", which means date palm, and "dactylifera" derived from a Greek word "*daktulos*" meaning a finger, illustrating the fruit's form (Al-Alawi et al., 2017).

Another source refers the botanical name of date to the legendary Egyptian bird, "Phoenix", which lived to be 500 years old, and cast itself into a fire from which it rose with renewed growth. This resemblance to the date palm, which can also re-grow after fire damage, makes the bird and the date palm share this name, while "*dactylifera*" originates from the Hebrew word "dachel" which describes the fruit's shape (Zaid and De Wet, 1999).

4-1-1 Botanical classification

Kingdom	Plantea
Sub kingdom	Tracheobionta
Class	Monocotyledonae
Order	Arecales
Family	Areacaceae (Palmaceae).
Genus	*Phoenix*
Species	*Phoenix dactylifera*

4-1-2 Description

Phoenix dactylifera L. (Palmaceae) is one of the oldest trees grown under arid conditions primarily in the Middle East, North Africa, and the United States. Egypt, Iran, Saudi Arabia, Algeria, Iraq, are the top five date producing countries (FAOSTAT, 2018). The production of date fruit in Algeria was 934 377 metric tons in 2014 this production is characterized by the predominance of the famous date cultivar "Deglet Noor" which represents about 52% of total date palm production (MADR, 2015). Besides the economic property of date fruit, it is an important food item on a daily basis of the diet in Algeria. Its sweet fruit with a single seed, can be eaten before the final stage of ripening, which is called Rotab (50% soft brown colour and 50% hard yellow or red colors), or consumed after complete ripening and offered as Tamr (100% soft brown colour).

4-1-3 Medicinal uses

The importance of the date in human nutrition comes from its rich composition of bioactive components such as anthocyanins, phenolics, carotenoids, procyanidins, and flavonoids which offer protection against oxidative stress. It contains also fibers, vitamins such as ascorbic acid, niacin, pyridoxine, and traces elements such as potassium, phosphorus, magnesium, calcium, selenium, and iron (Mohamed 2000; Al-Shahib and Marshall, 2002; Al-Farsi et al., 2005; Mansouri et al., 2005).

Several studies have reported a wide range of medicinal properties to date fruit such as antioxidant activity (Biglari et al., 2008; Al-Mamary, 2014), antimutagenic (Vayalil,

2002), antibacterial (Al-Shwyeh, 2019), antiviral (Jassim and Naji, 2010), antifungal (Boulenouar et al., 2011), antihyperlipidemic (Ahmed et al., 2016), anti-inflammatory (El Hilaly et al., 2018), anticancer (Ishurd et al.,2004), gastroprotective(Al-Qarawi et al.,2003), hepatoprotective (Saafi et al., 2011), nephroprotective (Wang et al.,2019), immunostimulant and gonadotropic activities (Baliga et al., 2011). Miller et al. (2003), reported that the consumption of dates might be of benefit in glycaemic and lipid control of diabetic patients.

Figure 03. *Phoenix dactylifera* fruit -Ghars variety- (Personnel Photo)

I-4-2 *Cydonia oblonga*

Cydonia oblonga Mill. belong to the group of the oldest cultural plants, it is native to Iran, Turkey, and possibly Greece and the Crimean Peninsula and is cultivated in India, South Africa, Middle East, and Europe (Rop et al.,2011; Ashraf et al.,2014). Quince fruits called a variety of other names: Arabic: sefarjal; Chinese: wen po; English: quince; French: cognassier or coing; German: quitte or quittenbaum; Portuguese: marmelo; Russian; ajva; Spanish: membrillero; Swedish: kvitten, among others (de Almeida Lopes et al., 2018).

After Turkey, China, Uzbekistan, Iran, Morocco, Azerbaijan, Argentine, and Serbia, Algeria is the ninth largest producer of quince in the world with a production of more than 11,630 tons (FAOSTAT, 2018). However, as compared with other species, this fruit tree species is less popular throughout the world (Rop et al., 2011).

4-2-1 Botanical classification

Kingdom	Plantae
Sub kingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Rosales
Family	Rosaceae
Genus	Cydonia Mill
Species	*Cydonia oblonga* Mill

4-2-2 Description

Cydonia oblonga Miller; commonly known as quince, Sfarjel, Bahi dhana; is a pome with numerous seeds. The fruits are big (10-12 cm in diameter with an average of 150-200g), with variable dimensions and asymmetric shapes, and exhibit a characteristic fragrance. Quince is less suitable for direct consumption (Silva et al., 2004) because of its hardness, bitterness, and astringency; for that, it is mostly consumed as fruit liquor, glutinous starch syrup, jams, jellies, or candies.

4-2-3 Medicinal uses

The fruit, leaf, and seeds of the quince have long been used in folk medicine for the treatment and prevention of several diseases such as bronchitis, diuretic, cystitis, cardiovascular diseases, diarrhea, hepatitis, vomiting (Zhou et al., 2014; Mirmohammadlu et al., 2015; Umar et al., 2015; Ashraf et al.,2016). Several *in vitro* (Hamauzu et al., 2005; Magalhães et al., 2009; Rop et al., 2011) and *in vivo* (Hamauzu et al.,2006; Aslam and Sial, 2014; Zhou et al., 2014; Umar et al., 2015) studies

demonstrating the health benefits of the fruit against various diseases. The medicinal value of the plant is related to the presence of phytochemical components such as phenolic acids and flavonoids compounds (Caffeoylquinic acids, kaempferol and quercetin glycosides), vitamins (A, C, E, riboflavin, folic acid, and K), and minerals like calcium, potassium, and phosphorus (Silva et al.,2005; Rop et al., 2011; Zhou et al., 2014). Even C. oblonga fruit is a rich source of secondary metabolites, it has not been explored well as fresh fruit.

Figure 04. *Cydonia oblonga* Mill (Personnel Photo)

Chapter II
Material and Methods

II Material and Methods

II-1 Plant material

II-1-1 Collection

- ***Phoenix dactylifera* fruit**

The Ghars variety from *Phoenix dactylifera* fruit was used in this experiment. The sample was collected from a commercial farm in Biskra-Algeria.

- ***Cydonia oblonga* fruit**

The quince fruits were purchased from the local market of Constantine- Algeria in the month of December 2017.

Another sample of this fruit was purchased in the month of December 2018 at several markets in Algeria (Constantine, Batna, and Algiers).

The fruits having uniform size, maturity, without injury from insects and fungal infections, were selected to prepare the different extracts.

II-1-2 Preparation of the extract

For each sample, the fruits were manually separated from their seeds, cut into thin slices and freeze-dried. 500g of dried slices was macerated in the volume of ethanol/water (1:6; 70/30) for 24 hr. The macerate was then filtrated and centrifuged (10 min/3,000 rpm). The process was repeated three times, then the total extract was concentrated under reduced pressure at 40°C using a rotary evaporator and stored in the freezer until use.

II-2 Qualitative phytochemical analysis

All extracts were subjected to preliminary phytochemical screening following standard methods, as shown in Table 1, to determine the presence or absence of different

chemical composition and to estimate biological activities that might be due to the presence of secondary metabolites in the extracts (Aiyegoro et al., 2010; Ismail et al.,2016).

Table1. Test methods for screening the extracts for major classes of phytochemicals

Phytochemical class	**Add reagent**	**Expected results**
Phenols	$FeCl_3$(1%) +K_3(Fe $(CN)_6$) (1%)	Green-blue colouration
Flavonoids	KOH (50%)	Yellow precipitate
Saponins	Agitation	Formation of foam (1cm)
Tannins	$FeCl_3$ (1%)	Blue green colouration
Alkaloids	Dragendorff reagent	Red or Orange precipitate

II-3 Evaluation of biological activities

II-3-1 Determination of the antioxidant activity

- ***DPPH free radical scavenging assay***

The free radical-scavenging activity of the extract against stable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was assessed according to the method of Blois (1958) with slight modifications. Increasing concentrations of extracts were prepared in methanol (12.5-800 µg/ml). 0.3mM solution of DPPH in methanol was prepared and 160µL of this solution was added to 40 µL of sample extracts. The resultant mixtures were incubated in dark at room temperature for 20 min and the absorbance was measured at 517 nm. BHA and BHT were used as antioxidant standards to compare the activity.

As antioxidants can react with the violet coloured stable free radical DPPH, and convert it into a yellow coloured α,α-diphenil-β-picrylhydrazine, this assay is based on quantifying the change of the reaction mixture colour as a readout of the scavenging capacity of antioxidants towards DPPH.

The DPPH-scavenging ability was given as the half maximal inhibition concentration (IC_{50}) and calculated according to the following equation:

$$\%Inhibition = [(Abs_{(c)} - Abs_{(s)})/Abs_{(c)}] \times 100$$

$Abs_{(c)}$is the absorbance without extract; $Abs_{(s)}$ is the absorbance of the extract or standard.

- ***ABTS radical scavenging assay***

The ABTS scavenging assay was determined using the method of Re et al. (1999). The ABTS radical cations ($ABTS^{+}$) were produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulphate, stored in the dark at ambient temperature for 12–16 h before use. The resulting solution was diluted to get an absorbance of 0.703 ± 0.025 at 734nm with distilled water. 160µl of the ABTS radical solution was added to 40 µL of the sample at different concentrations (12.5-800 µg/ml). After 10 min and the absorbance at 734 nm was recorded immediately. BHA and BHT were used as antioxidant standards for comparison of the activity.

When the medium contains an antioxidant; the ABTS will be reduced and consequently the mixture will be discoloured. Therefore, the degree of discolouration can be expressed as the inhibition percentage of ABTS. The results were given as IC_{50}.

$$\%Inhibition = [(Abs_{(c)} - Abs_{(s)})/Abs_{(c)}]$$

$Abs_{(c)}$is the absorbance without extract; $Abs_{(s)}$ is the absorbance of the extract or standard.

- ***Cupric reducing antioxidant capacity (CUPRAC)***

The cupric reducing antioxidant capacity was measured according to Apak et al. (2004) with slight modifications. For each well, in a 96-well plate, a mixture of 50 µL (10 mM Cu (II)), 50 µL L 7.5 mM neocuproine, and 60 µL NH 4Ac buffer (1 M, pH 7.0) solutions were added. 40uL of each extract at different concentrations (12.5-800 µg/ml) was added to the initial mixture. One hour after, the absorbance at 450nm was recorded using a 96-well microplate reader.

The presence of antioxidant compounds reduced the cupric ions to cuprous ions, resultant in the formation of a stable complex between neocuproine and cuprous ions. A higher absorbance indicates a higher reducing capacity of antioxidants.

Results were given as ($A_{0.50}$), BHA, and BHT were used as antioxidant standards for comparison of the activity.

- ***Metal chelating activity***

The chelating activity of the extracts on Fe^{2+} was estimated by the method of Decker and Welch (1990), with slight modifications. Briefly, the extract solution (80 µL dissolved in ethanol at different concentrations) was added to 40 µL 0.2 mM $FeCl_2$. The reaction was initiated by the addition of 80 µL 0.5 mM ferene. The mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was measured at 593 nm. Ethylenediamine tetraacetic acid (EDTA) was used as antioxidant standard for comparison of the activity. The presence of a chelating agent disrupts the formation of the complex which leads to a decrease in the red colour.

The metal chelating activity was calculated using the following equation:

$$\text{Metal chelating activity} = [(A_c - A_s)/A_c] \times 100$$

where (A_c)is the absorbance of control devoid of sample and (A_s)is the absorbance of the sample in the presence of the chelator.

II-4 Determination of the antidiabetic activity of *Cydonia oblonga* extract

The α-glucosidase inhibitor activity of quince extract was assayed *in vitro* in accordance with Lordan et al. (2013). A reaction mixture containing 50µL of extract solution at different concentrations (12.5-25-50-100-200-400-800 µg/ml), 50 µL of p-nitrophenyl-a-D-glucopyranoside (NPG) solution 5mM (in phosphate buffer 100mM, pH 6.9) was incubated at 37°C for 10min, and then 100µL of alpha-glucosidase (0.1 U/mL) was added to the mixture. The absorbance was recorded at 405 nm for 30min using an Enzyme-linked immunosorbent assay (ELISA) microplate reader. Acarbose was used as a positive control.

The presence of an inhibiting agent disrupts the formation of the complex which leads to discolouration of the sample solution. The inhibition percentage of the sample was calculated as follow: $\%Activity= [(A_c- A_s)/A_c] X 100$

II-5 Evaluation of the toxicity of the extracts

II-5-1 Animals

Female *Wistar albinos'* rats (7 weeks old) and female *Mus Musculus* albinos mice (7 weeks old) from the animal house were used to examine the toxicity of the extracts. Animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 25±1C with 12:12 light: dark cycles). Food was provided in the form of dry pellets (SARL Production Locale, Bouzareah, Algeria) and water *ad libitum*.

II-5-2 Experimental Procedure

The oral acute toxicity test was assessed using the acute toxicity class method in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines (OECD, 2002).

- ***Phoenix dactylifera* extract**

Animals were fasted overnight, marked, and weighed before the oral administration of the vehicle/extract (Table 2). Each mouse was observed for mortality and clinical signs of toxicity for 14 days. The body weight was recorded daily.

Table2. Treatment of mice in acute toxicity test of *Phoenix dactylifera* extract

Group	Treatment	Number of animals	Doses
GI	Distilled water	5	2ml/250g
GII	*Phoenix dactylifera* extract	5	2,000mg/kg b.w

- ***Cydonia oblonga fruit* extract**

Prior to the administration of the extract, animals were marked, fasted overnight, and weighed (Table 3).

Table 3. Treatment of rats in acute toxicity test of *Cydonia oblonga* extract

Group	Treatment	Number of animals	Doses
GI	Distilled water	5	2ml/250g
GII	*Cydonia oblonga* extract	5	2,000mg/kg b.w
GIII	*Cydonia oblonga* extract	5	6,000mg/kg b.w

After the administration of doses on day 1, animals were observed individually in polycarbonate cages after 30min, two, and four hours and then two, four, and six hours after providing the feed. During the following days (2-14 days) observations were made once a day and animals were weighed each 4days. The observation focused on behavioural pattern (tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma) death, changes in the skin, and fur, eyes, and respiratory (OECD, 2002).

On day 15, blood samples were collected in EDTA tubes for haematological and biochemical analyses including red and white blood cells counts, platelets count,

haemoglobin, aspartate aminotransferase (ALT), alkaline phosphatase (ALP), blood urea, creatinine, glucose, triglycerides (TG), total cholesterol (tCH), and HDLc. All animals were sacrificed by decapitation under light anesthesia; liver, lungs, kidney, heart, stomach, spleen, and adrenal glands were removed, weighed, and followed by a macroscopic necropsy examination.

II-6 Evaluation of the immunomodulatory activity of the *C. oblonga* extract

II-6-1 Animals

Thirty adult female mice (32–40g, 8 weeks old) were obtained from the central pharmacy institute Constantine-Algeria. The animals were housed in polyacrylic cages (five animals each) with free access to food and water and maintain to acclimatize with standard conditions of our animal facility 10 days before the experiment. Food was provided in the form of dry pellets (SARL Production Locale, Bouzareah, Algeria).

II-6-2 Experimental procedure:

6-2-1 Measurement of phagocytic index

The phagocytic activity of quince extract was evaluated *in vivo* by the carbon clearance test (Biozzi et al., 1953). For this, animals have received an intraperitoneal injection (i.p) of the vehicle (control group) or the extract at different doses (table 4) on the first day. After 48h, carbon ink suspension (3ml black carbon ink, 4ml saline, and 4ml 3% gelatin solutions) was injected intravenously to the animal in a volume of 0.1ml/10g through the tail vein.

Blood samples were drawn from the orbital vein at 5 and 15min and lysed with 4ml of 0.1% sodium carbonate solution (Na_2CO_3) to lyse the erythrocytes.

The absorbance of the samples was measured at 675 nm using a spectrophotometer.

The animals were sacrificed after the last sampling and the liver was removed immediately.

Table 4. Treatment of mice in carbon clearance rate test of *Cydonia oblonga* extract

Group	Treatment	Number of animals	Doses	Carbon ink
GI	NaCl 0.9%	5	500µl/mouse	0.1ml/10g b.w
GII	*C. oblonga* extract	5	12.5mg/kg b.w	0.1ml/10g b.w
GIII	*C. oblonga extract*	5	25mg/kg b.w	0.1ml/10g b.w
GIV	*C. oblonga* extract	5	50mg/kg b.w	0.1ml/10g b.w
GV	*C. oblonga* extract	5	100mg/kg b.w	0.1ml/10g b.w
GVI	*C. oblonga* extract	5	200mg/kg b.w	0.1ml/10g b.w

The phagocytic activity is expressed by the phagocytic index K that measures all the reticuloendothelial system function in the contact with the circulating blood. The clearance rate is expressed as the half-life period of the carbon in the blood ($t_{1/2}$, second). Calculations were performed by the following equations:

$$K = (\ln (OD_1) - \ln (OD_2)) / (t_2 - t_1)$$

$$t_{(1/2)} = 0{:}693/K$$

Where, OD_1 and OD_2 are the optical densities at times t_1 and t_2, respectively.

6-2-2 Measurement of reduced glutathione (GSH)

- **Preparation of liver homogenate**

The weight of 0,5g of each mouse's liver was homogenized in 2ml of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4) and centrifuged (9000g/15 min) at 4˚C. The supernatant was used to measure the GSH.

- **Measurement of protein content**

The protein content of liver homogenate was determined by the method of Bradford (1976) by mixing 0.1 ml of the homogenate with 5 ml of Bradford reagent. After 5 min the optical density was measured at 595 nm and the protein concentrations were determined by comparison to that of bovine serum albumin standard (BSA) realized in the same conditions (Appendix).

- **Measurement of reduced glutathione**

The GSH level was determined according to the method of Weckbecker and Cory, with slit modifications. 800µl of each homogenate sample was deproteinized with 200µl of 5-sulfosalicylis acid (0.25%) and centrifuged at 1000 rpm for 10 min. 1ml of tris-HCL buffer (0.4 M, pH 9.61 containing 20 mM EDTA) and 25µl of DTNB (0.01M; 5,5′-dithiobis-2 nitrobenzoic acid) were mixed with 500µl of the supernatant and left at room temperature for 5min. The developed yellow colour was measured at 412 nm, and the glutathione concentration was calculated by the following formula:

$$\text{GSH (µmol GSH/mg protein)} = \frac{\text{OD* 1*1.525}}{\text{13100*0.8*0.5*mg proteins}}$$

OD: Optical Density.

1: total volume of the protein decomposition solutions (0.8 ml the homogenate + 0.2 sulfo-salicylique acid).

1.525: volume of the total solutions used for the SH dosage at the supernatant level (0.5 ml supernatant + 1 ml Tris-EDTA + 0.025 ml DTNB).

13100: group –SH absorbance coefficient at 412 nm.

0.8: homogenate volume.

0.5: supernatant volume

II-7 Evaluation of the anti- depressant activity of *Phoenix dactylifera* extract

II-7-1 Animals

Male *Wistar albino* rats aged 7 weeks, were acclimatized for 1 week before starting the experiment. Animals were kept under standard conditions of temperature (25 ± 1°C) and humidity (40–50%) and 12:12h light/dark cycle. Food (CLEA Japan, CE-2, Tokyo, Japan) and water were available *ad libitum* to animals.

II-7-2 Drugs and treatment administration

Corticosterone (Sigma– Aldrich) was administered daily subcutaneously to the rats at a dose of 40 mg/kg for the first 10days, then the dose was decreased to 30 mg/kg. The solution of corticosterone was prepared by suspending corticosterone in corn oil containing 1% Dimethyl Sulfoxide (DMSO).

II-7-3 Experimental design.

Rats were randomly divided into four groups of five animals as mentioned in table 5. In groups 3 and 4: rats were injected with corticosterone and treated with date extract at the dose 600 and1000mg/kg respectively and daily for 21 days. The body weight was measured daily.

Table 5: Treatment of rats in chronic corticosterone injection-induced depression experiment

Experimental group	Corticosterone Injection	Treatment	Number of rats
GI	-	Distilled water	5
GII	+	Distilled water	5
GIII	+	*P.dactylifera* extract 600mg/kg b.w	5
GIV	+	*P.dactylifera* extract 1000mg/kg b.w	5

II-7-4 Forced swimming test (FST)

After the treatment period, animals were subjected to the forced swimming test to evaluate the anti-depressive activity of the extract.

The rats were forced to swim in translucent plastic cylinders (50 cm height × 20 cm diameter) filled with 33cm warm water at 24°C for 10 min. The immobility time was recorded automatically using SMART software for the last 5 min. Immobility was defined when the rats make just the necessary movements to keep their head above the water.

II-8 Evaluation of the antistress activity of *Cydonia oblonga* extract

II-8-1 Animals

Male *Wistar albino* rats aged 7 weeks were housed at the animal facility 10 days before the beginning of the experiment. Animals were maintained on a 12 h light-dark cycle (light on at 7 a.m.), under controlled conditions of temperature (25 ± 1°C) and humidity (40–50%). Food (CLEA Japan, CE-2, Tokyo, Japan) and water were available *ad libitum*.

II-8-2 Treatment administration

The extract of *C. oblonga* at a dose of 300 mg/kg b.w freshly prepared was orally administered to the rats at 8:30 a.m. for 21 days.

II-8-3 Experimental design

The animals were divided into four experimental groups (Table 6). Rats of stressed groups were completely immobilised for 6 h per day (9:00 -15:00) in a rodent immobilisation box, for 21 consecutive days. Unstressed rats remained in their home cages without access to either food or water during the period of immobilisation stress. The body weight and food/water consumption were measured daily.

Table 6. Treatment of rats in the immobilisation stress experiment

Experimental group	Immobilisation stress	Treatment	Number of rats
GI	-	Vehicle	5
GII	+	Vehicle	5
GIII	+	*C. oblonga* extract 300mg/kg b.w	5
GIV	-	*C. oblonga* extract 300mg/kg b.w	5

II-8-4 Behavioural tests:

After 21days, animals were subjected to the behavioural tests in the order OFT, SPT, and FST.

- **Open field test (OFT)**

The OFT was performed to measure spontaneous activity in rodents. The apparatus used for the OFT consists of a 90 × 90 cm square field with 40 cm-high walls. Each rat was placed individually in the center of the field and allowed to freely explore the arena for 5 min. Trials were video-recorded by a suspended, overhead camera and the rat movements were recorded using SMART software (version 3.0.05, Panlab, Spain). The total distance, time spent in the center (45 × 45 cm, center zone) were analysed.

- **Sucrose preference test (SPT)**

Rats were individually housed and allowed to drink water from two bottles maintain in each cage. Food and water were removed 12 h before the test. During the test, animals were presented with two bottles of drinking solutions, one was sweetened with 2% sucrose and the other one was normal drinking water. Intake was measured by weighing the bottles containing water or 2% sucrose solution after 24 h. Sucrose preference was expressed as: (the weight of consumed sucrose solution) / [the weight of consumed (sucrose solution + drinking water)] × 100.

- **Forced swimming test (FST)**

The FST, for this experiment, is a 2-day procedure in which rats swim in translucent plastic cylinders (50 cm height × 20 cm diameter) filled with 33cm warm water at 24°C. On the first day, the rats were forced to swim for 15 min and then dried with towels and placed in a warmed enclosure before the return to their home cages. After 24 hours, rats were examined for 5 min swimming during the actual test session. Data acquisition and analysis were performed automatically using SMART software.

II-8-5 Plasma concentration of corticosterone

Blood samples collected on the final day were centrifuged immediately (3,000 g for 10 min at 4°C) and the plasma was stored at −80°C until used.

Corticosterone (CORT) levels were measured by Enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Science, NY, USA) according to the manufacturer's instructions.

II-8-6 Immunohistochemistry

Each rat was deeply anesthetized and transcardially perfused with phosphate buffer solution (PBS, pH=7.4) followed by paraformaldehyde (PFA) fixative (pH= 7.4).

After perfusion, brains were removed (surgical tools used to dissect the brain are shown in the annex) and post-fixed in PFA solution for 24h, equilibrated in sucrose solution (30%), and then stored at −80°C. The hippocampal sections of 30 μm were obtained using a cryostat (CM1860; Leica Biosystems, Germany). Sections were rinsed in PBS, blocked with 10% goat serum and 0.3% Triton X-100, and then incubated with primary antibodies including anti-doublecortin (DCX) antibody or anti-Ki67 antibody and anti-Neu N

After incubation with the primary antibodies, sections were rinsed in PBS and incubated with secondary antibodies for 1 h at room temperature. Secondary antibodies were prepared in the same blocking solution at a dilution of 1:200.

Tissues were rinsed, mounted on glass microscope slides, and coverslipped with mounting medium containing DAPI (4′,6-diamidino-2-phenylindole, Santa Cruz Biotechnology, CA, USA).

Table 07. Details of antibodies used for immunohistochemistry

Protein of interest	Primary antibody	Company/product code	Secondary antibody
Ki67	Rabbit Polyclonal anti Ki67	Abcam/ ab15580	Goat Anti-rabbit Alexa-Fluor 488
DCX	Rabbit Polyclonal anti-DCX	Abcam/ab18723	Goat Anti-rabbit Alexa-Fluor 488
NeuN	Mouse Monoclonal anti-NeuN	Millipore/ MAB377	Goat Anti-mouse Alexa-Fluor 546

II-8-7 Hippocampal neurogenesis

Six hippocampal sections in each rat were immunostained and visualized under Olympus FV1000D confocal microscope (Olympus, Tokyo, Japan).

Counting of DCX- and Ki67- immunopositive cells was performed at 20 x magnification along the area of DG The number were manually counted using objective along the DG area. Three rats were analyzed for each group.

Statistical analysis

Values are expressed as the mean ± standard error of the mean (SEM).

The student's *t*-test was used to analyse the data from the acute toxicity test of *Phoenix dactylifera* extract.

For the rest of experiments, one-way analysis of variance (ANOVA) was used to analyse the difference between the data of groups followed by Bonferroni's *posthoc* test using SPSS 25.

The level of significance was fixed at $p<0.05$.

Chapter III
Results and Discussion

III Result and discussion

III-1 Phytochemical screening

Phytochemical screening of our extracts identified the presence of different active compounds (Table 8) with a high abundance of phenols in the two extracts, and the absence of saponins in *Cydonia oblonga* extract

Table 8. Results of primary phytochemical screening of the extracts

(+) Presence, (-) Absence

Phytochemical classes	***Phoenix dactylifera* extract**	***Cydonia oblonga* extract**
Phenols	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(-)
Tannins	(+)	(+)
Alkaloids	(+)	(+)

III-2 Antioxidant Activity

The evaluation of the antioxidant activity of the extracts was carried out using four different methods DPPH, ABTS, CUPRAC, and metal chelating tests, which allowed us to identify an antioxidant profile of the extracts on the basis of their reactivity towards radical species, and their ability to reduce transition metals (Table 9).

As was expected, the antioxidant capacities increased in a concentration-dependent manner and the scavenging of ABTS radical was found to be higher than that of DPPH radical in the two extracts.

Table 9. The antioxidant activity of the extracts

Activity	*P. dactylifera*	*C. oblonga*	*BHA*	BHT	EDTA
DPPH IC_{50} (µg/ml)	1047.33±11.57	249,26 ± 3,75	6.14±0.41	12.99±0.41	NT
ABTS IC_{50} (µg/ml)	539.99±14.54	117,34 ± 1,41	1.29±0.30	1.81±0.10	NT
CUPRAC $A_{0.50}$ (µg/ml)	>800	167,17 ± 1,15	3,42±0,26	8.97±3.94	NT
Metal Chelating IC_{50} (µg/ml)	>800	417.98 ± 48.82	NT	NT	8.80±0.47

Values of IC_{50} and $A_{0.50}$ are expressed as means ± SEM of three parallel measurements; **BHA**: butylated hydroxy anisole; **BHT**: butylated hydroxytoluene; **EDTA**: Ethylene diamine tetra acetic acid; **NT**: Not tested

In general, fruits, vegetables, and plants contain diverse classes of phytochemicals groups. The higher diversity and quantity of these groups; the stronger the biological activities may exhibit. The primary phytochemical screening of the extracts used in our experiments indicated the presence of famous chemical classes such as phenolic, flavonoids, and tannins.

The obtained result of the antioxidant activity of Ghars extract showed a good scavenging activity which is in agreement with the study of Zineb et al. (2012) who reported that the antioxidant activities were high based on the scavenging assays of DPPH radical, 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), and potassium ferricyanide complex as reducing power assay.

Ali Haimoud et al. (2016) reported that Ghars extract exhibited scavenging of DPPH activity by (IC_{50}= 247.33±3.51 ug/ml), this level is much lower than our study.

The antioxidant activity of date fruit is due to the wide range of phenolic compounds including Gallic, ferulic, coumaric, caffeic acids, and flavonoids such as isoquercitrin, quercitrin, rutin, quercetin, and luteolin (Al Shahib and Marshall, 2002; Baliga et al.,2011; Saafi et al., 2011; Almamary et al., 2014). However, the phenolic amounts in the fruits might be affected by several factors such as growing condition, season, geographic origin, soil type, cultural methods, process and stabilization conditions, climatic conditions, use of different analytical methods, storage condition, and the choice of solvent (Besbes *et al*. 2009; Ali Haimoud et al., 2016).

As it was expected *C.oblonga* extract exhibited good antioxidant activity in all assays used with higher scavenging activity in ABTS assay than DPPH assay. The capacity of ABTS to react with hydrophilic and lipophilic compounds main explain this difference.

The extract of *C. oblonga* exhibited better scavenging of DPPH activity than that reported by Silva et al (2004) for peel and pulp extracts (IC_{50}= 0.6 mg/ml; 1.7 mg/ml, respectively). Hamauzu et al. (2005) evaluated the antioxidant properties of Chinese quince, quince, and apple fruits using DPPH method. They found that Chinese quince and quince fruit showed slightly higher activities than apple fruit, which had mainly low polymerized procyanidins as flavan-3-ol series.

Despite their beneficial roles as being required for oxygen transport, respiration, and enzyme activity, cupric ions and iron are highly reactive metals that can cause oxidative changes in proteins, lipids, and other structural components. According to the CUPRAC and metal chelating on ferrous ions data ($A_{0.5}$ = 167.17±1.15 µg/mL, 417,98 ± 48.82µg/mL, respectively, Table 8), *C.oblonga* may offer protection to the cells against oxidative damage induced by an excess of cupric ions or (Fe^{+2}) in normal physiology.

Wojdylo et al. (2013) evaluated the antioxidant properties of 13 different variety of quince fruit using the DPPH, ABTS and FRAP methods. They found a significant variation in antioxidant activity among the studied varieties which ranged between 0.9 and 2.4 µmol trolox/g dry matter for ABTS, 0.9 and 2.5 for DPPH as well as 0.4 and 1.5 for the FRAP method.

In fact, several studies reported a correlation between antioxidant activity and phenolic content in quince fruit (Silva et al.,2002; Silva et al., 2004; Legua et al., 2013), with greater activity exhibited in the peel part than the corresponding pulp part. The presence of thirteen phenolics compounds in the skin part including the six compounds identified in the pulp part (3-O-, 4-O-, and 5-O-caffeoylquinic acids, 3,5-Odicaffeoylquinic acid, quercetin 3-galactoside, and rutin) mainly explain this difference (Silva et al.,2005). Meanwhile, the presence of carotenoids, tocopherols, and vitamin C in the fruit, may also contribute to the antioxidant activity (Hamauzu et al., 2005; Magalhães et al., 2009; Legua et al., 2013; Szychowski et al.,2014). However, it was reported that the whole extract of quince fruit exhibited strong antioxidant activity comparing to those individual pure compounds (Quercetin, rutin, kaempferol) at the same concentration present in the whole extract (Fattouch et al.,2007). The antioxidant activities of the quince fruit may due to the interaction between different compounds and to possible synergic and antagonist effects (Fattouch et al.,2007; Silva et al., 2004).

Reactive oxygen species (ROS) are byproducts formed during mitochondrial electron transport and other aerobic metabolic reactions and are fundamental to several signaling processes (Forester et al., 2018). The ROS generated in humans is cleared by the presence of antioxidants in the body such as enzymes and small molecules (intracellular reduced glutathione (GSH), oxidized glutathione, bilirubin, uric acid...). However, overproduction of ROS induced an imbalance between oxidative stress and the antioxidative defense system which leads to the initiation or progression of several diseases such as cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases (Pavithra & Vadivukkarasi, 2015).

Apart from the innate defense system, it was reported that the supplementary intake of antioxidants improves the capacity of the body to counter oxidative stress and reduces the risk of these diseases (Adjimani and Asare, 2015). From this point of view, the scientific results obtained from the above experiments signify that the studied fruits are a very important source of natural antioxidants which can play a very important role in reducing oxidative stress and preventing the human body from dangerous diseases.

II-3 Anti diabetic activity

- **α-Glucosidase inhibitory activity**

The IC_{50} of *C.oblonga* extract in this experiment (IC_{50}: 326.48 ± 18.56 µg/mL), was close to the acarbose (IC_{50}: 275.98 ± 1.57 µg/mL) used as a standard.

Diabetes is a chronic metabolic disorder that has reached alarming levels. In 2019, nearly half a billion people (9.3% of adults 20–79 years) are living with diabetes worldwide. This estimated number has increased by 62% during the past 10 years; from 285 million in 2009 to 463 million, in 2019 (Saeedi et al., 2019). The primary causes in the majority of diabetic patients are thought to be an unhealthy diet and physical inactivity which lead to overweight and obesity (Fukaya et al., 2009).

The synthetic antidiabetic drugs such as acarbose, miglitol, voglibose are commonly used for treating diabetes by inhibiting α-glucosidase activity.

α-Glucosidase is the key enzyme catalysing the final step in the digestive process of carbohydrates in the intestine. Thus, the inhibition of α-glucosidase activity is a useful method to control hyperglycaemia.

The inhibition of α-glucosidase delays the liberation of D-glucose from dietary complex carbohydrates into the bloodstream and delay glucose absorption, resulting in reduced postprandial hyperglycaemia. Moreover, α-glucosidase inhibitors are expected to effectively prevent the dysfunction of β-cell insulin secretion in diabetic patients in the initial stages of diabetes because they suppress insulin secretion after meals (Fukaya et al., 2009).

It was reported that the use of α-glucosidase inhibitors drugs (AGIs) can induce many side effects, such as renal tumours, adverse gastrointestinal disturbance, and liver toxicity (Fujisawa et al., 2005). For this reason, the prevention and use of natural antidiabetic is important.

A number of studies have reported a positive relationship between polyphenol content, total flavonoid, and the ability of the extract to inhibit α-Glucosidase (Ramkumar et al., 2010). The phenolic compounds are known for their capacity to inhibit the activities of carbohydrate-hydrolyzing enzymes because of their ability to bind to proteins (Shobana et al., 2009). In addition, *in vitro*, and *in vivo* studies indicated the high inhibitory potential of flavonoids towards α-Glucosidase (Adefegha and Oboh, 2012).

The presence of antioxidant molecules such as 5- Hydroxymethyl-2-furancarboxaldehyde (5-HMF), caffeoylquinic acids, kaempferol 3- glucoside, rutin, kaempferol-3-rutinoside, and quercetin 3-galactoside (Mohebbi et al.,2019) in quince fruit mainly explain its capacity to inhibit α-glucosidase activity and justifying the traditional use of *C.oblonga* in the prevention and the treatment of diabetes.

The antidiabetic activity of quince fruit was also reported in the study of Mohebbi et al. (2019) using a rat model of diabetes.

The isolation and identification of active chemical constituents of quince fruit could be the direction of future studies.

III-4 Oral Acute toxicity test:

To authenticate the nontoxic effect of our extract, an acute toxicity experiment was conducted on female mice for *Phoenix dactylifera* extract and female albinos' rats for *C.oblonga* extract, in raison, the females were generally slightly more sensitive than male (Lipnick et al.,1995).

III-4-1 *Phoenix dactylifera* extract

The mice treated with 2000 mg/kg b.w ethanolic extract of *Phoenix dactylifera* was found to be free of any toxicity sign. In addition, all mice were survived till the end of the observation period and exhibited normal behaviour. Mice were alert with normal grooming, touch response, pain as well as body weight gain (Figure 05).

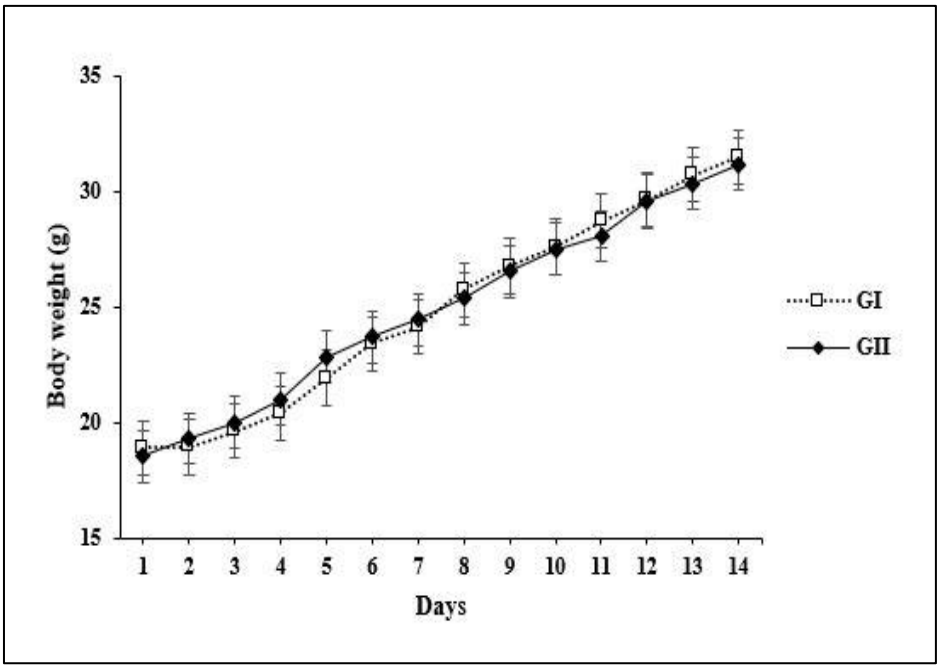

Figure 05. Body weight changes of mice in the acute toxicity study of *Phoenix dactylifera* extract. Each point represents mean ±SEM (n=5).

GI: Control group; **GII**: *Phoenix dactylifera* at the dose of 2000mg/kg

III-4-1 *Cydonia oblonga* fruit

- **Body weight**

Neither mortality either weight loss was recorded on tested rats with two doses of *C.oblonga* (2000-6000mg/kg b.w) during 14 days (Figure 06).

Physical observations indicated no signs of behavioural pattern (weakness, aggressiveness, diarrhea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotor activity, fur, lethargy, and sleep) or changes in physical appearance, injury, pain, and signs of illness of the rats during the sighting as well as in the main study.

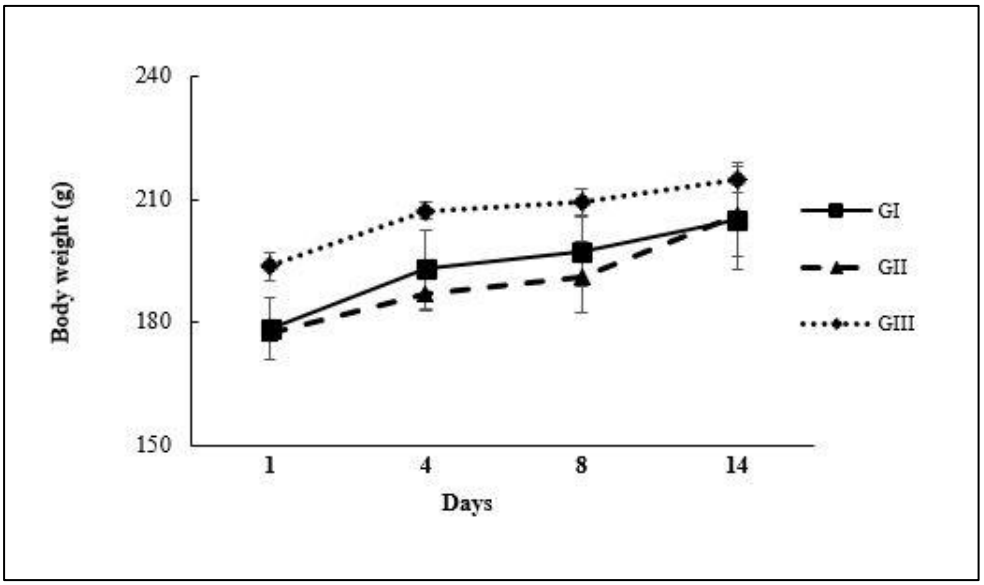

Figure 06. Body weight changes of rats in the acute toxicity study of *Cydonia oblonga* fruit extract. Each point represents mean ±SEM (n=5).

GI: Control group; **GII**: *Cydonia oblonga* extract at the dose of 2000mg/kg b.w, **GII**: *Cydonia oblonga* extract at the dose of 6000mg/kg b.w

During the experiment rats weight increased regularly. The body weight change is often used as a general guideline of the health status of animals. Meanwhile, weight gain was observed in all groups. It can be stated that the extract did not interfere with the normal metabolism of animals as corroborated by the non-significant difference from animals in the vehicle control group.

The relative weight of various organs is shown in Table 10**,** no significant differences were observed between groups. The histopathological examination was not performed, as there were no abnormalities found in the macroscopic observation of organs.

Table 10: Relative organ weight in female rats after acute oral toxicity study of *Cydonia oblonga* extract (n=5, ns: not significant)

Parameter	Control	*C. oblonga* (2000 mg/kg)	*C. oblonga* (6000 mg/kg)
Liver	3,8551 ± 0,4006	3,0578 ± 0,8302 ^{ns}	3,1819 ± 0,1608 ^{ns}
Lung	0,5717 ± 0,0470	0,5065 ± 0,0768 ^{ns}	0,4866 ± 0,0371 ^{ns}
Kidney	0,6045 ± 0,0388	0,5593 ± 0,1019 ^{ns}	0,5619 ± 0,0374 ^{ns}
Heart	0,2934 ± 0,0173	0,2893 ± 0,0357 ^{ns}	0,2863 ± 0,0220 ^{ns}
Stomach	0,6232 ± 0,0545	0,6054 ± 0,1714 ^{ns}	0,5733 ± 0,0640 ^{ns}
Spleen	0,2430 ± 0,0218	0,2905 ± 0,0428 ^{ns}	0,2545 ± 0,0181 ^{ns}
Adrenal gland	0,0454 ± 0,0083	0,0402 ± 0,0104 ^{ns}	0,0382 ± 0,0059 ^{ns}

- **Hematological and biochemical analyses:**

The hematological parameters such as white blood cells, red blood cells, platelets, haemoglobin, lymphocytes, and neutrophils didn't show any significant differences when compared to the control (Table 11) indicating that the extract has no adverse effect on haematopoiesis (Xu et al., 2015; Ji et al.,2020) and other blood cell formations.

Blood parameters that were evaluated in the present study allowed an indirect assessment of hepatic and renal function. At the hepatic level, increasing ALT and AST levels in the bloodstream are served as indicators of damage, necrosis, or degeneration of hepatic cells (Rhiouani et al., 2008; Ouyang et al., 2015; Lyoussi et al., 2018). Serum creatinine and urea levels are the most commonly used to evaluate renal function, a high

concentration of these parameters in the blood indicate the impaired glomerular filtration and kidney damage (Rhiouani et al., 2008).

In the present study, the concentration of ALT, AST, urea, and creatinine of rats treated with quince extract at different doses didn't show any significant difference compared with the control group after 14 days. Other metabolites that were measured in the present study were glucose, total cholesterol, and triglyceride and none of these parameters also showed significant values between treated and normal rats, indicating that the extract has no deleterious effect on the vital organs (Table 12) (Ji et al.,2020).

Taken together, since the tested dose failed to produce mortality and any clinical signs of toxicity, the estimated LD_{50} of the entire test extracts is more than 5000mg/kg for ghars extract (OECD, 2002) and 6000mg/kg for quince fruit, so extracts obtained from these fruits are safe for animal use.

Table 11: Effect of *Cydonia oblonga* extract on haematological parameters in acute oral toxicity test study (n=5)

Group	WBC ($x 10^3$ uL-1)	Lymphocytes (%)	Monocytes (%)	RBC ($x 10^3 uL^{-1}$)	Haematocrit (%)	MCV (fl)	Platelets ($x 10^3 uL^{-1}$)	MCH (pg)	Haemoglobin (g/dl)	MCHC (g/dl)
Control	7.55 ± 0.44	79.93 ± 7.18	2.32 ± 0.73	7.95 ± 1.11	44.00 ± 6.09	55.35 ±1.43	569.5 ±123.52	18.53 ± 054	16.02 ± 0.48	33.66 ± 047
2000 mg	9.52 ± 2.38 ns	71.56 ± 1.09 ns	2.25 ± 0.25 ns	7.64 ± 1.45 ns	42.66 ± 5.98 ns	56.74 ± 2.40 ns	559.0 ± 74.19 ns	18.30 ± 0.37 ns	15.76 ± 0.09 ns	32.36 ± 1.08 ns
6000 mg	8.22 ± 0.89 ns	78.92 ± 3.15 ns	3.00 ± 0.94 ns	8.63 ± 0.87 ns	48.08 ± 4.00 ns	55.82 ± 2.21 ns	555.8 ± 79.89 ns	18.72 ± 0.74 ns	16.1 ± 1.15 ns	33.6 ± 0.48 ns

Values are expressed as mean ± SEM
WBC: Wight blood cells; **RBC**: Red blood cells; **MCV**: Mean corpuscular volume; **MCH**: Mean corpuscular haemoglobin; **MCHC**: Mean corpuscular haemoglobin concentration, **ns:** not significant

Table 12: Effect of *Cydonia oblonga* extract on biochemical parameters in acute oral toxicity test study

Group	Glucose (g/l)	AST (U/L)	ALT (U/L)	Creatinine (mg/l)	Urea (g/l)	TG(g/l)	TCHO (g/l)	HDL-c (g/l)
Control	0.515 ±1.39	145.00 ± 15.00	53.00 ± 5.65	5.00 ± 1.58	0.36±0.01	0.507 ± .0147	0.441 ± 0.06	0.287 ± 0.067
2000 mg	0.747 ± 1.08 ns	125.33 ± 34.93 ns	46.00 ± 7.00 ns	5.25 ± 1.29 ns	0.35±0.03 ns	0.488 ± 0.027 ns	0.374 ± 0.05 ns	0.276 ± 0.143 ns
6000 mg	0.959 ± 1.40 ns	130.75 ± 22.07 ns	41.00 ± 10.00 ns	5.4 ± 0.8 ns	0.33±0.01 ns	0.488 ± 0.050 ns	0.38 ± 0.05 ns	0.244 ± 0.088 ns

Values are expressed as mean ± SEM
AST: Aspartate aminotransferase; **ALT**: Alanine aminotransferase; **TCHOL**: Total cholesterol; **TG**: Triglycerides, **ns:** not significant

III-5 Evaluation of the immunomodulatory activity of *C.oblonga* extract by carbon clearance rate test

As shown in Figure 07; Prophylactic treatment of mice with quince extract at the doses (12.5, 25, 50, 100, 200 mg/kg b.w) enhanced the rate of carbon clearance from the blood when compared with the control group.

Pre-treated groups with 50 and 100mg had the highest phagocytic index comparing to other groups. No significant difference has been reported between the two doses (100 and 200 mg). *C.oblonga* extract was found to stimulate the phagocytic activity of the macrophages in a dose dependent manner.

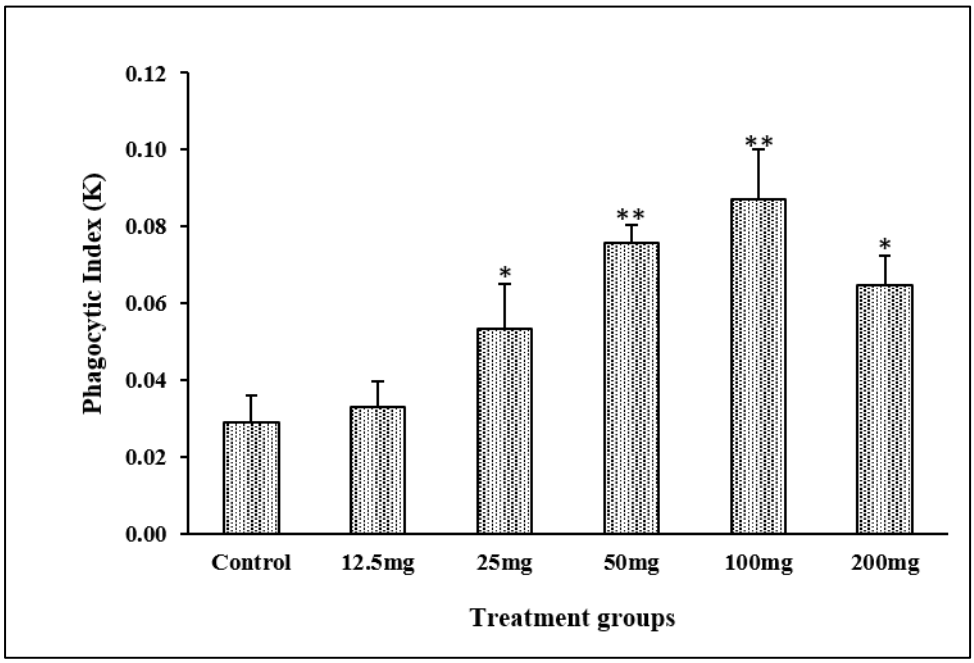

Figure 07. Effect of *Cydonia oblonga* fruit extract on phagocytic index. n = 5, values are the mean ± SEM, *$p < 0.05$; **$p < 0.01$ vs control group

The clearance rate (Figure 08) of carbon ink from the bloodstream was decreased highly and significantly in all groups pre-treated with the fruit extract, compared to the control group.

At the doses of 50 and 100 mg/kg mice showed the fattest clearance rate of carbon particles from the bloodstream.

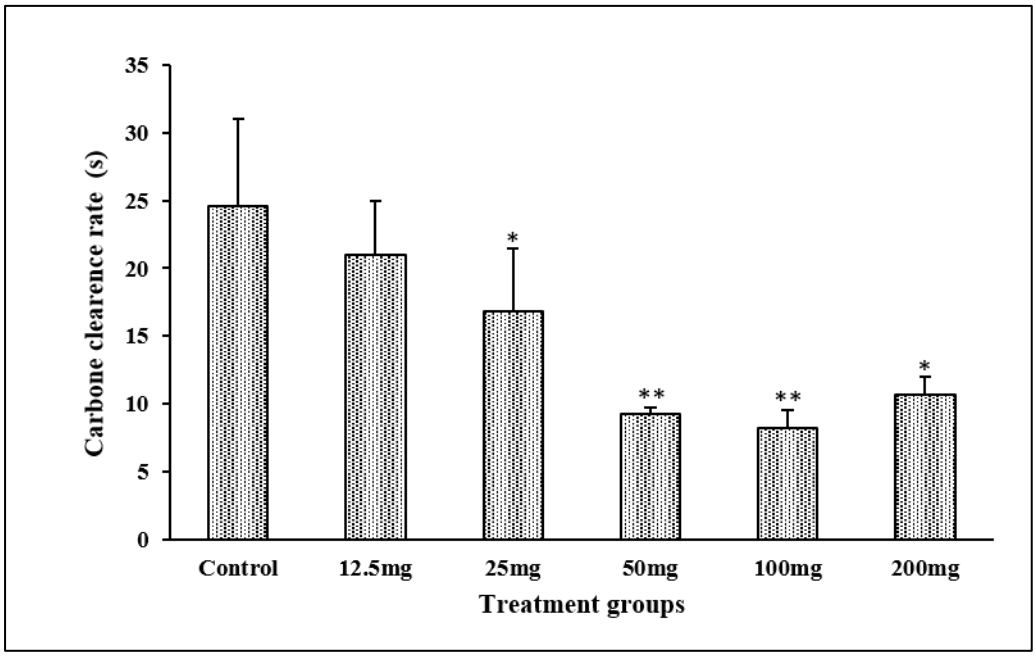

Figure 08. Effect of *Cydonia oblonga* on the half time of carbon in the blood stream. n = 5, values are the mean ± SEM, *$p < 0.05$, **$p < 0.01$ vs control group

- **Reduced glutathione**

Figure 09 shows the GSH levels in the liver of mice after 10min of the carbon ink injection

Liver GSH contents in the *C.oblonga* extract pre-treated mice with (25,50,100 and 200 mg) were significantly decreased, compared to the control group.

No difference was noted between groups pre-treated with the extract at the doses (50,100,200 mg, ($p>0.05$)).

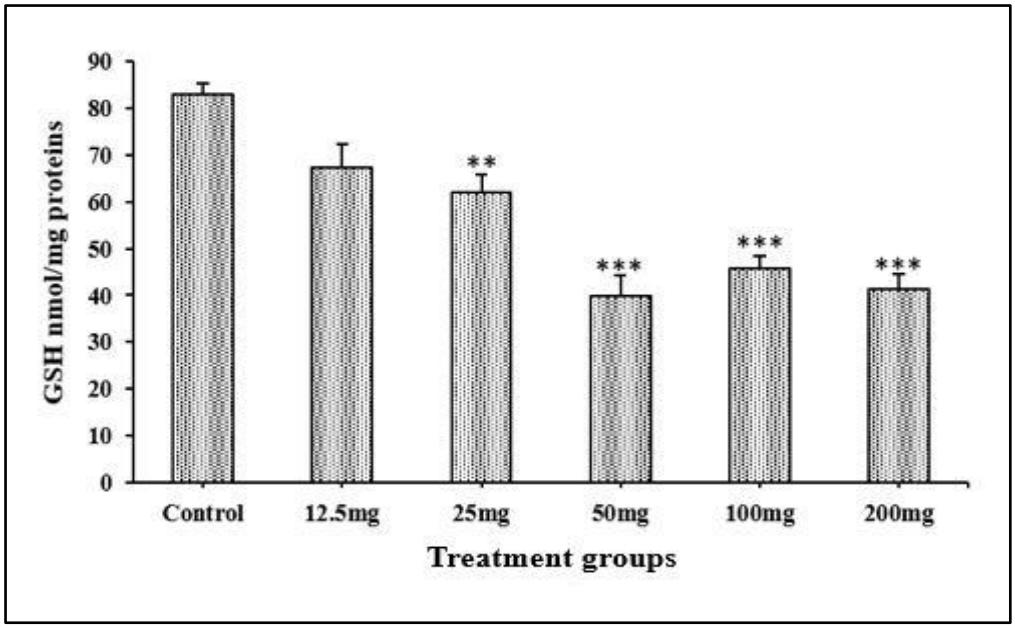

Figure 09. Reduced glutathione levels in the liver of control and mice pre-treated with *Cydonia oblonga* fruit extract after carbon ink injection. n = 5, values are the mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$ vs control group.

Our results of phagocytic activity are in agreement with several studies reported that some fruits (Alves et al.,2020) and plants extract (Bin-Hafeez et al., 2003; Benmebarek et al.,2013; Chan-Zapata et al., 2018) enhanced the nonspecific immunity mechanism by increasing the phagocytic activity of macrophages.

The process of phagocytosis by macrophages includes opsonisation of the carbon particles of the ink with antibodies and complement C3b, leading to a more rapid clearance of foreign particulate matter from the blood.

Numerous studies have shown the immunostimulant effect of plants extract, Gao et al. (2004) have reported that the extract of Curcumin from *Curcuma longa* inhibited the IL-2 induced proliferation of spleen cells completely at concentrations of 12.5-30 mmol/L. The study of Hajra et al. (2012) confirmed that the methanolic extract of *Swietenia mahagoni* seeds has therapeutic potential and could be served as an effective immunomodulatory candidate without any side effects.

Glutathione exists in reduced (GSH) and oxidized (GSSG; gluthathione disulphide) forms in cells and tissues with 90-95% exist in GSH form in healthy cells. GSH itself is synthesized in all cells from glutamate, cysteine, and glycine. It is an essential co-factor for some antioxidant enzymes and a powerful non-enzymatic antioxidant which considered as a therapeutic agent for several diseases. Dysfunction of the glutathione system has been implicated in a number of neurodegenerative diseases and is a potential contributor to oxidative damage following temporary ischemia. The decrease of reduced glutathione levels in our experiment might be due to the high utilisation of reduced glutathione to stimulate phagocytic activity through nuclear factor kappa B activation (Kwon et al., 2019). This finding suggest that *C.oblonga* extract increase the capacity of the detoxification ability of the organism.

It has also been reported that diabetics patients tended to have decreased phagocytic and bactericidal ability of neutrophils during active infection which results in an increased risk of infectious complication and poor prognosis (Top et al., 2007). From our data and other studies (Magalhães et al., 2009, Aslam et al., 2014; Zhou et al., 2014; Mirmohammadlu et al.,2015) we hypothesized that *Cydonia oblonga* fruit may play an important natural source for diabetic patients to prevent the progression of immune dysfunction.

In the first step of our study, we showed that the *Phoenix dactylifera* and *Cydonia oblonga* fruit extracts are effective in scavenging free radicals and can serve as potential antioxidants with safety. However, the phytochemicals responsible for the biological activities of these fruits still need to be identified. In addition, our data support folk medicine, which uses *Cydonia oblonga* fruits for the treatment of diabetes.

III- Effects of chronic stress and *Phoenix dactylifera* on a rat model of depression

III-6-1 Body weight change

Figure 10 shows losing body weight gain in all groups comparing to the control group through the treatment period. Corticosterone injection had significantly affected the animal body weight. No other group differences were significant.

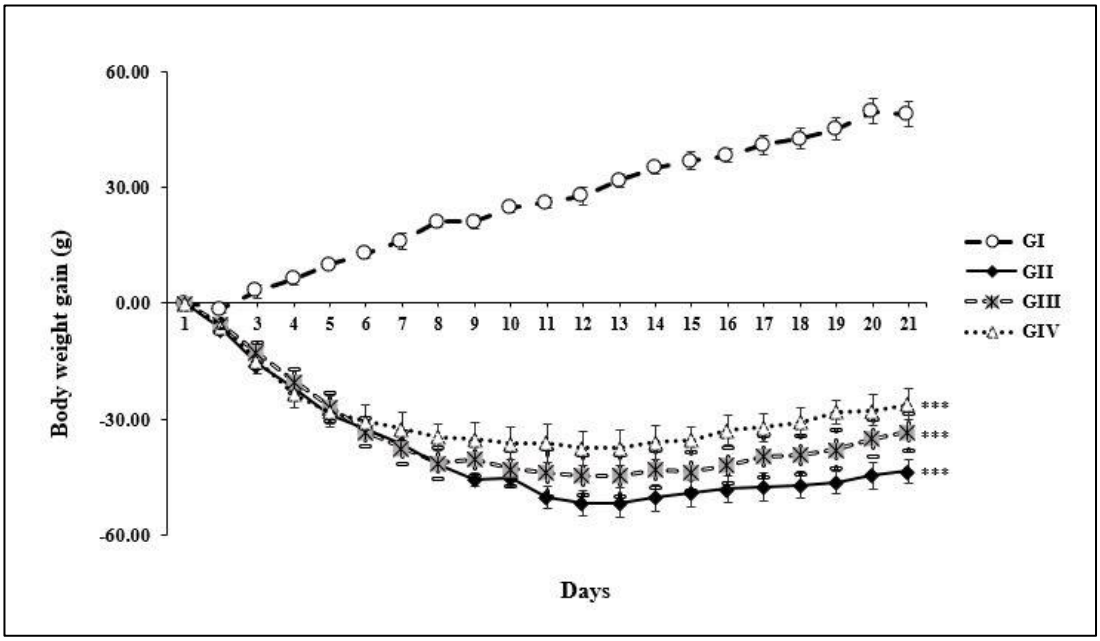

Figure 10. Effects of *Phoenix dactylifera* extract on the corticosterone injection-induced changes in body weight gain during the experiment period. n=5, values are mean ± SEM, ***$p < 0.001$ versus the control group

GI: Control group; **GII**: Corticosterone group; **GIII**: Corticosterone + 600mg/kg *Phoenix dactylifera* extract, **GIV**: Corticosterone+ 1000mg/kg *Phoenix dactylifera* extract

III-6-2 Forced swimming test

The results in figure 11 concerning the effect of chronic corticosterone injection and *Phoenix dactylifera* extract treatment on the forced swimming test showed a significant increase in immobility time in GII.

The increase in immobility time was suppressed close to the control group by daily administration of *Phoenix dactylifera* extract at both doses.

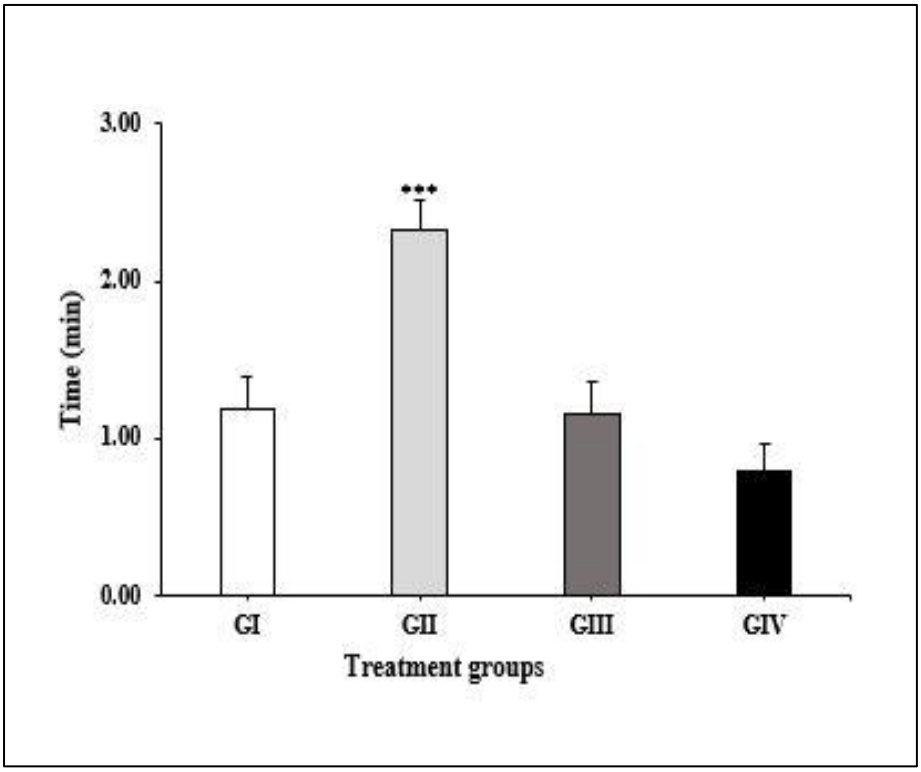

Figure 11. Effect of *Phoenix dactylifera* extract and the chronic corticosterone injection on immobility time in the forced swimming test. n=5. The bars represent the mean ± SEM, ***$p < 0.001$ versus the control group.

GI: Control group, **GII:** Chronic corticosterone injection group, **GIII:** Chronic corticosterone injection /*Phoenix datylifera* extract (600mg/kg) group, **GIV:** Chronic corticosterone injection / *Phoenix datylifera* extract (1000mg/kg) group

The main finding of the present experiment is that *P.dactylifera* can reverse depressive-like behaviours induced by chronic corticosterone injection.

The repeated corticosterone injection induces a significant decrease in the animal bodyweight. This result is in agreement with previous studies (Lee et al., 2013; Yu et al., 2014). In addition, the study of Li et al. (2017) reported that 21 daily injections of 40 mg/kg corticosterone significantly decreased bodyweight for both adult and adolescent rats.

However, it should be noted that other studies were reported an increase in body weight in animals who received chronic glucocorticoid treatment (Karatsoreos et al., 2010 ; van Donkelaar et al., 2014 ; Li et al., 2016). This discrepancy may explain by the different routes of administration.

In this study, treatment with *Phoenix dactylifera* at the two doses (600-1000mg/kg b.w) failed to reverse the alteration of bodyweight.

After repeated corticosterone injection, the depressive-like behaviour of mice was assessed by using FST. In general, FST is commonly considered as one of the most behavioural screens for antidepressant treatments.

Several previous studies have indicated that repeated corticosterone treatments can influence rat behaviour in the forced-swim test. For example, the study of Kott et al. (2016) reported that 21 daily injections of 40 mg/kg corticosterone increased rat forced-swim test immobility. In addition, 20 consecutive daily injections of 20 mg/kg corticosterone were also found to increase rat immobility time in FST (Hill et al., 2003). These results suggest that repeated corticosterone injections can reliably prolong immobility and increase depression-like behaviour in a dose-dependent manner in male rats. In this regard, the 21-day schedule of daily corticosterone injection significantly induced depression-like behaviours, as revealed by the significant increase of immobility time in FST.

Treatment with *Phoenix dactylifera* at the two doses (600-1000mg/kg b.w) dramatically suppressed the increase in immobility time in FST, which indicates the antidepressant activity of the extract.

This result is in accordance with the study of Agbon et al (2017) who reported that the neurotoxicity induced by the administration of mercuric chloride ($HgCl_2$, 5 mg/kg) in rats was reversed by the oral administration of aqueous fruit extract of *P. dactylifera*, and ethanol fruit extract of *P. dactylifera* at the dose of (500, 1000mg/kg). In addition, groups treated with these two extracts ameliorated the neurobehavioural deficits (anxiety-related responses, short- and long-term memory impairments, and motor deficits) observed in $HgCl_2$-treated rats relative to the control group.

Results from the study of Pujari et al. (2013) reported that 15 days pre-treatment with extract of *P. dactylifera* at the doses of (100-300 mg/kg) protect cortical neuronal damage in the cerebral ischemia-rat model.

Our finding showed that the rat model of depression had been successfully established by chronic corticosterone injection and suggests a potential neuroprotective activity of the *Phoenix dactylifera* extract which may be due to the polyphenolic compounds present in the fruit. The compounds which can reverse these behavioural changes will be further studied as potent antidepressants.

III-7 Effects of chronic stress and *Cydonia oblonga* on the rat model of depression

III-7-1 Effects of chronic stress and *Cydonia oblonga* extract on physiological parameters

- **Bodyweight**

The body weight gain of rats exposed to chronic immobilisation stress for 21 days was significantly impaired in comparison to other groups.

The results summarized in Figure 12, show a significant weight loss during the first four days in stressed rats. After this period, a slight tendency to recover normal body weight gain was observed but the weight gain was significantly lower ($p<0.01$) than control group rats.

The comparison between rats treated with *C. oblonga* extract and non-treated rats (stress group) showed statistically significant improvement of bodyweight gain impaired by immobilisation stress ($p < 0.05$) (15.2% for the GII and 60.0% for the GIII in comparison with the control group).

The administration of *C.oblonga* extract to the rats without immobilisation stress accelerated weight gain compared to the control rats.

- **Daily food intake**

The daily food intake was strongly influenced by chronic stress ($p < 0.01$), especially during the first four days. The rats submitted to chronic immobilisation stress (GII and GIII) consumed less commercial chow than did the unstressed rats (Figure 13).

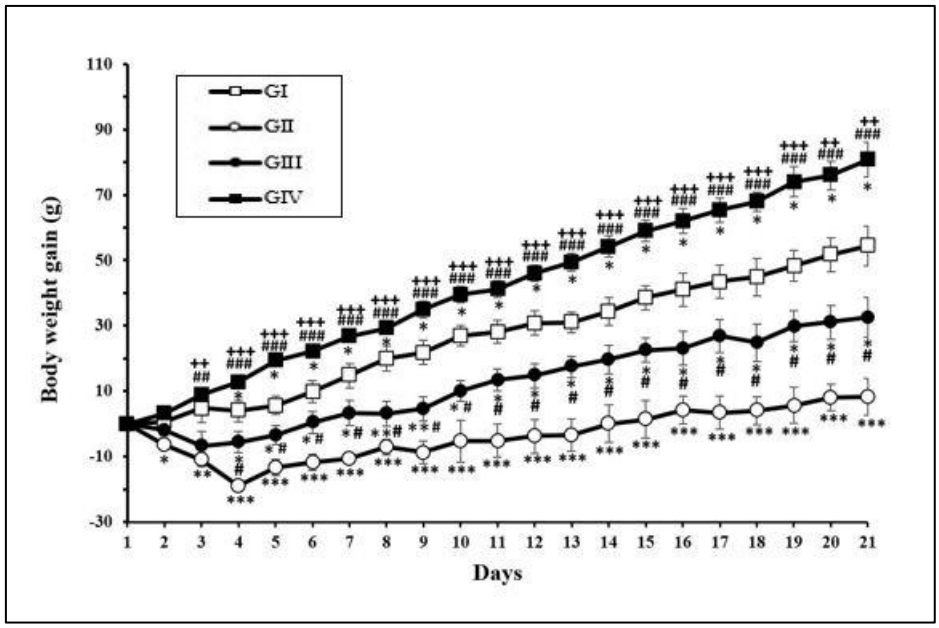

Figure 12. Effect of *Cydonia oblonga* extract on the chronic immobilisation stress-induced changes in body weight gain during the experiment period. n=5, values are mean ± SEM, (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) versus the control group; ($^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$) versus the GII; ($^{++}p < 0.01$, $^{+++}p < 0.001$) versus the GIII.

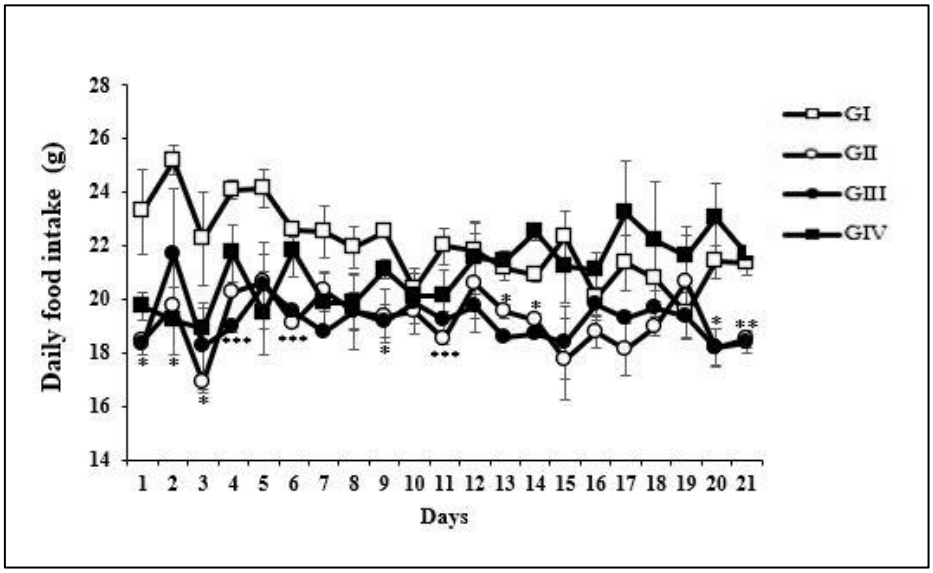

Figure 13. Effect of *Cydonia oblonga* extract on the chronic immobilisation stress on daily food intake. Values are mean ± SEM, (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) versus the control group.

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w).

- **Daily Water intake**

As shown in figure 14, there was no difference in average daily water intake between rats in GI, GII, and GIV.

Stressed rats treated with 300mg/kg of *C. oblonga* extract showed decreased water consumption during the stress period compared to other groups.

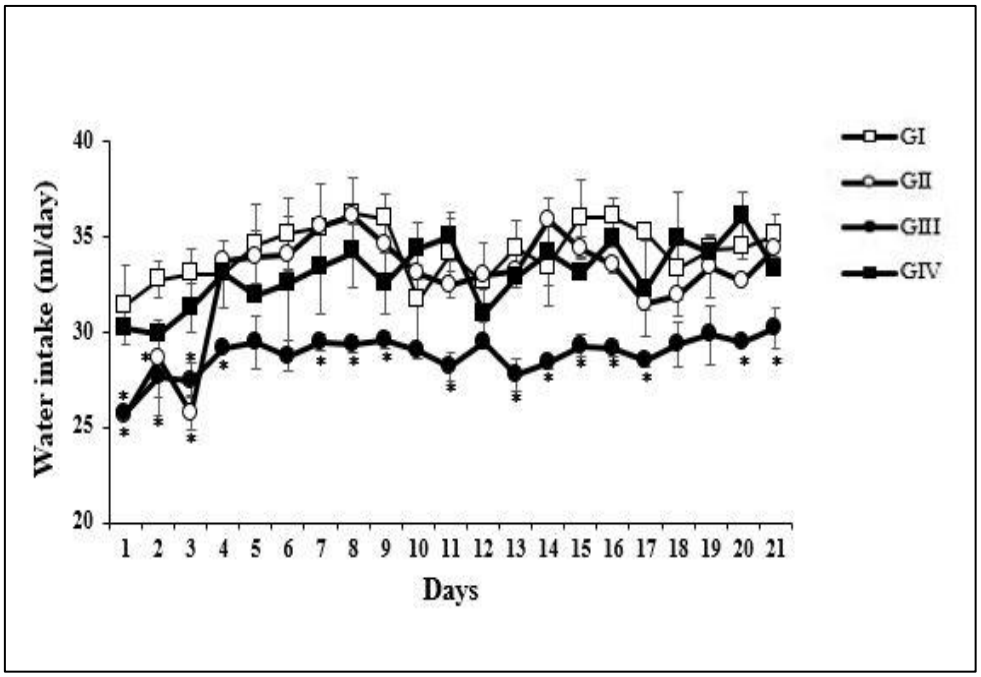

Figure 14. Effect of *Cydonia oblonga* extract on the chronic immobilisation stress on daily water intake. Values are mean ± SEM, significant difference from the control group is shown $*p < 0.05$.

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

- **Adrenal gland weight and plasma corticosterone levels**

Organ masse of adrenal glands was determined on the day of sacrifice. Repeated exposure to the immobilisation stress resulted in hypertrophy of the adrenals as shown by significant enlargement of the adrenal glands (Figure 15).

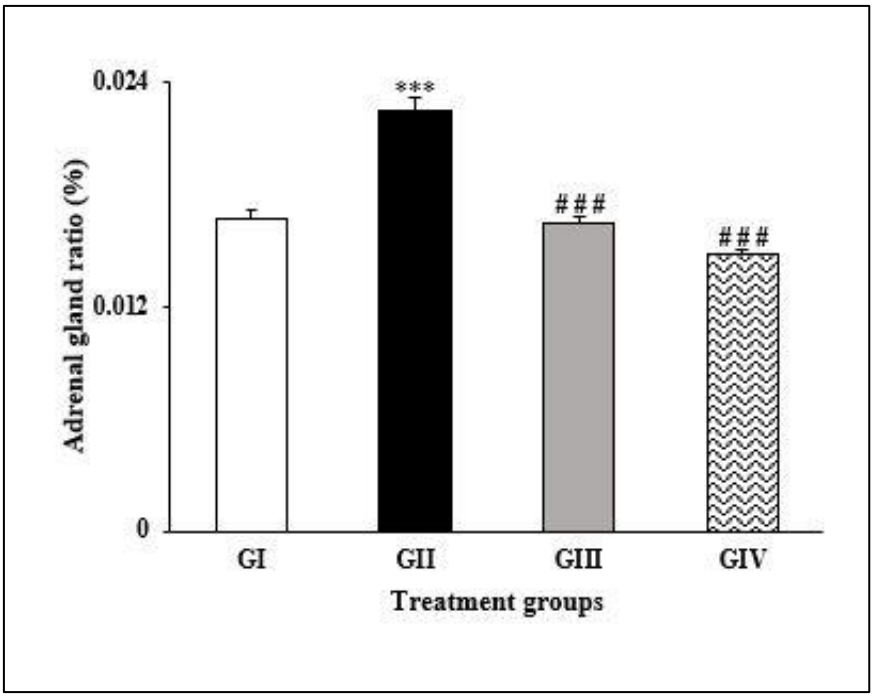

Figure 15. Effect of *Cydonia oblonga* extract and the chronic immobilisation stress on adrenal gland ratio (g organ/ g body weight). Values are mean ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the control group.

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

Adrenal hypertrophy is the consequence of frequent or persistent adrenocortical activation and it is a classical indicator of chronic stress.

The adrenal hypertrophy was concomitant with increased plasma corticosterone levels ($p < 0.001$) in stressed rats (Figure 16).

It can be seen in figures 15 and 16 that the treatment with *C. oblonga* extract suppressed the chronic stress-induced adrenal hypertrophy and high corticosterone levels, while the administration of *C. oblonga* extract itself had no significant effect on both corticosterone levels and the adrenal gland/body weight ratio.

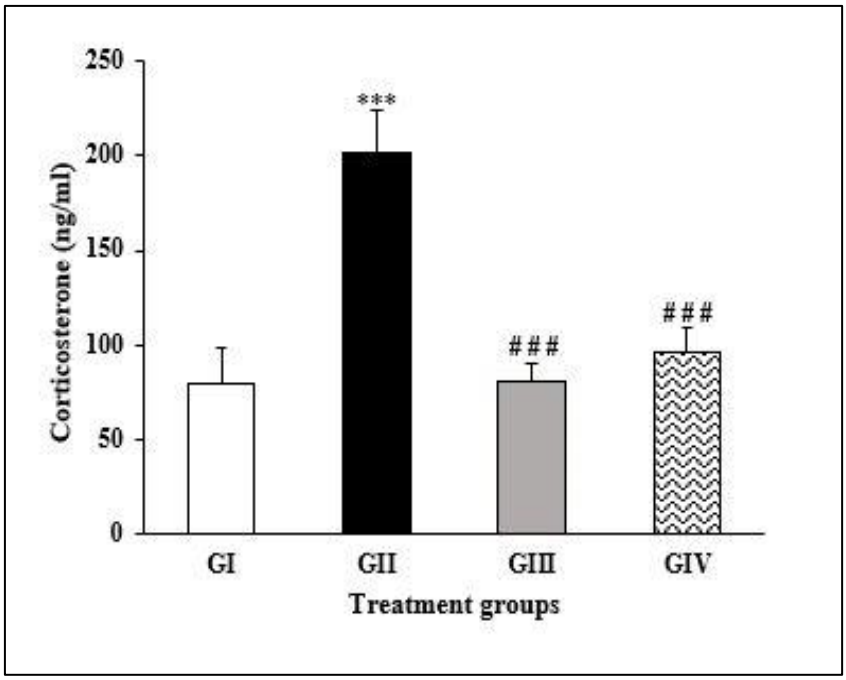

Figure 16. Change in corticosterone levels induced by chronic immobilisation stress and *Cydonia oblonga* extract treatment. n=5, data expressed mean ± S.E.M $^{***}p<0.001$ *vs* the GI, $^{###}p<0.001$ *vs* the GII (Bonferroni's *post-hoc* test).

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

Chronic immobilisation stress is a workable rodent model with high-intensity stressors incorporating prolonged, uncontainable, and unpredictable factors. It has been reported that the lack of physical activity is associated with psychiatric disorders (Chigr et al., 2009) and neurological abnormalities (Ampuero et al., 2015) which can be reversed only partially and with difficulty.

The decreased rate of body weight gain and hypertrophy of the adrenal gland has previously been shown to be reliable physiologic markers for chronic stress (Chigr et al., 2009; García-Iglesias et al., 2013; Ampuero et al., 2015). The reduction in weight gain in the stressed rats seems to be correlated with the reduction in food intake during the entire period of the experiment. Despite no significant difference in food intake between the

stressed rats, the treatment with *C.oblonga* extract completely reversed the chronic immobilisation stress-induced less weight gain. So, the decrease in body weight gain in the stressed group may be explained by the higher corticosterone levels in these rats. Kim et al 2013, reported that changes in plasma corticosterone levels following restraint stress in mice were negatively correlated with changes in the body weight.

Liu et al. (2011) reported that high circulatory corticosterone reduced appetite and body weight by downregulating hypothalamic neuropeptide Y/agouti gene-related protein and amphetamine-regulated transcript peptide mRNA expression levels resulting in a reduction in food intake and body weight gain. In addition, the elevated corticosterone levels may stimulate the catabolism of skeletal muscle proteins or the expression of other genes involved in bodyweight control (Jeong et al., 2013).

Although it is difficult to give an explanation for the decrease in water intake by stressed rats treated with *C.oblonga* extract at the dose of 300mg/kg, it appears that the reduced water intake in this group did not affect basal metabolic activity because these rats showed similar appearance as the control rats and kept their plasma corticosterone levels at the baseline.

In accordance with several animal studies (Zhu et al., 2008; Yun et al., 2010; García-Iglesias et al., 2013; Thakur et al., 2014) that have demonstrated increased basal corticosterone levels in rodent after a prolonged period of stress, it seems therefore reasonable to assume that *C.oblonga* fruit could suppress the chronic immobilisation stress-induced elevation of basal corticosterone secretion through regulating the HPA axis although further molecular studies are required to fully validate this proposal.

III-7-2 Effects of chronic stress and *Cydonia oblonga* extract on rat behavioural

- **Open Field test:**

As shown in figure 17, the total distance travelled in the field was affected neither by chronic stress nor the extract treatment.

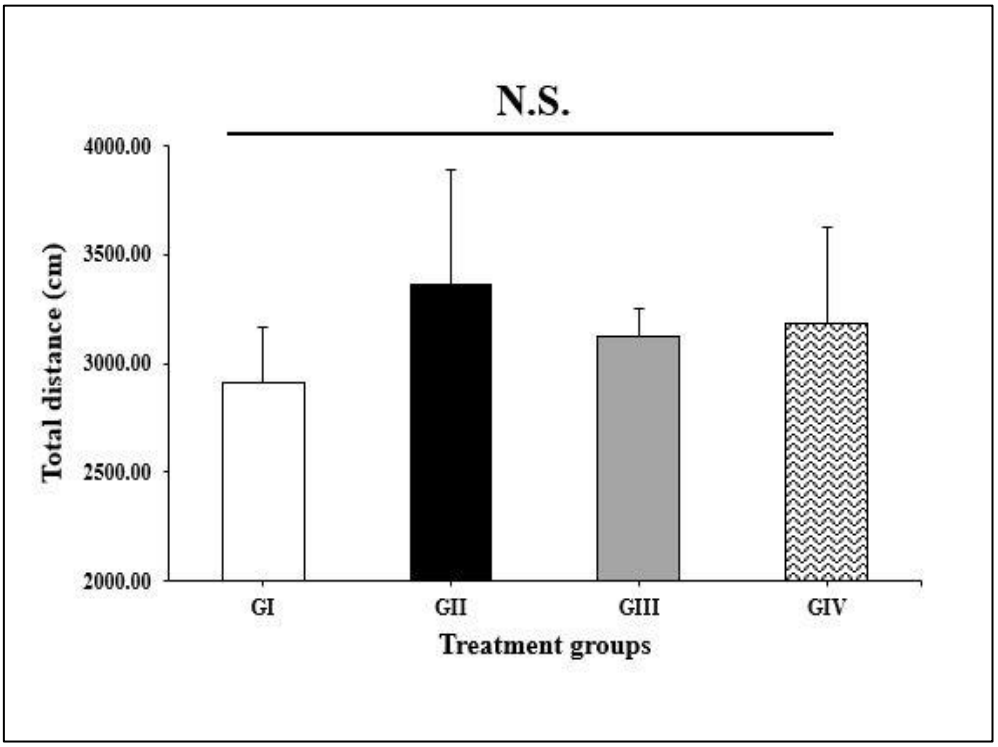

Figure 17. Effect of *Cydonia oblonga* extract and the chronic immobilisation stress in the total distance travelled in open field during the test. n=5, **N.S.**; not significant

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

The distance travelled in the peripheral zone and the time spent in this zone were significantly higher in stressed rats comparing to other groups ($p < 0.05$, Figure 18 a, b).

This increase was in parallel with the decrease in the distance travelled and time spent in the central zone ($p < 0.05$ and $p < 0.001$, respectively, figure 19 a, b).

No significant difference was observed between stressed rats treated with *C.oblonga* and control rats in all measured parameters in peripheral and central zones.

The time spent in the central zone was higher in the GIV compared to other groups (Figure 18 (a, b), figure 19 (a, b)).

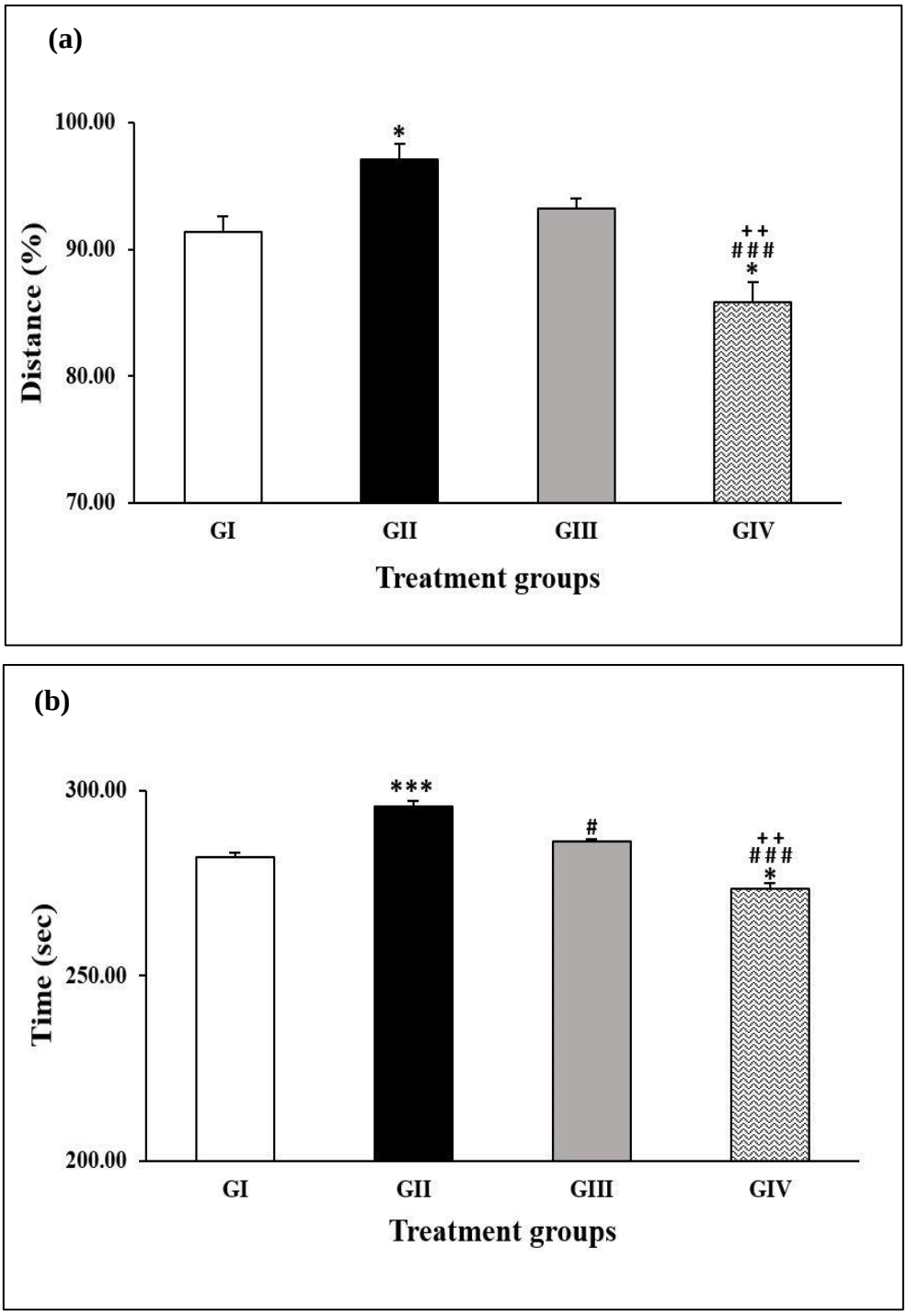

Figure 18: Effect of *Cydonia oblonga* extract and the chronic immobilisation stress in (**a)** Percentage of distance travelled and (**b)**time spent in the peripheral zone, **n=5.** The bars represent the mean ± SEM, $*p < 0.05$, $***p < 0.001$ versus the GI; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ versus the GII; $^{++}\ p < 0.01$ versus the GIII group. **GI:** Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

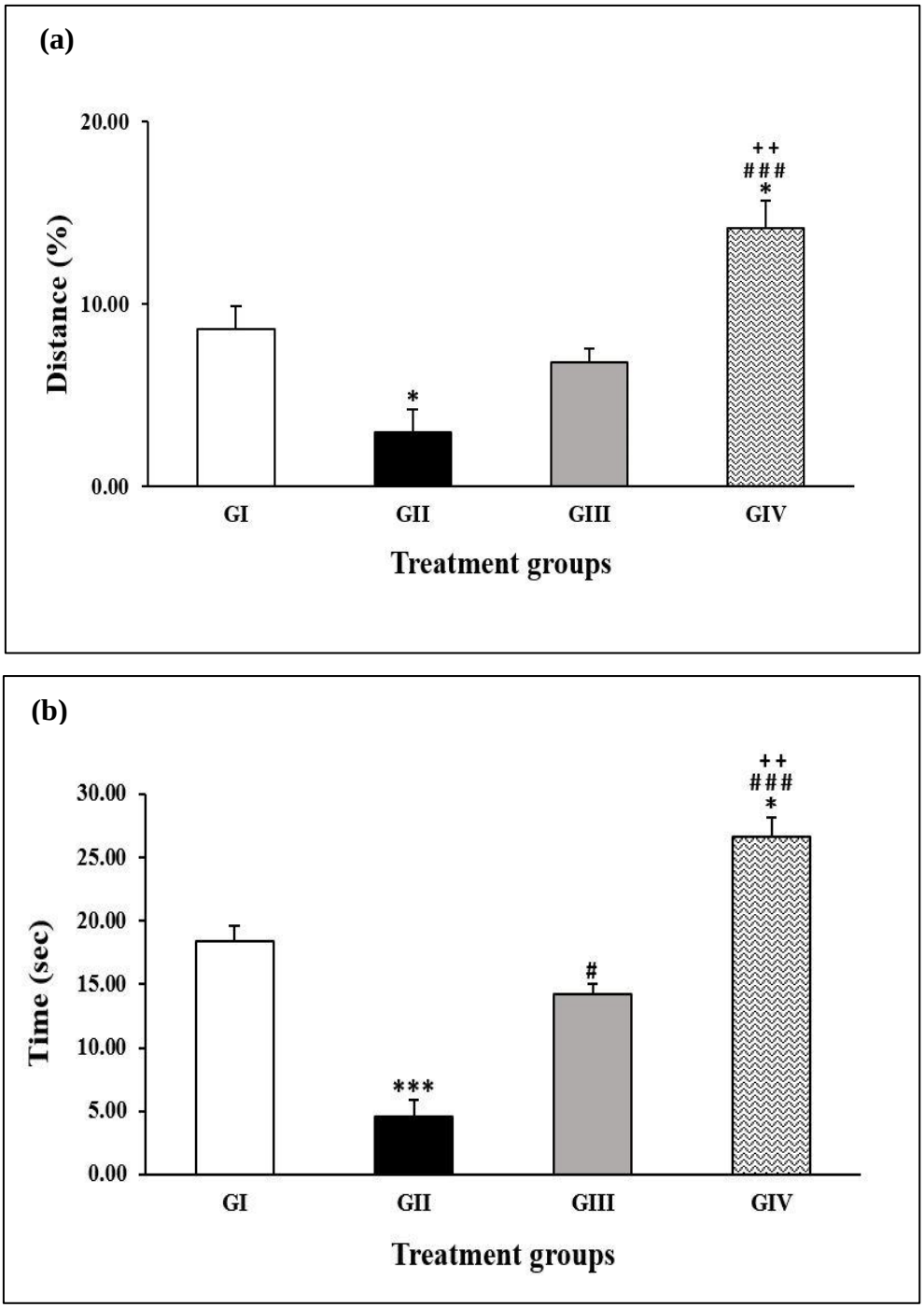

Figure 19: Effect of *Cydonia oblonga* extract and the chronic immobilisation stress in (**a)** Percentage of distance travelled and (**b)**time spent in the central zone, **n=5.** The bars represent the mean ± SEM, $^{*}p < 0.05$, $^{***}p < 0.001$ versus the GI; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ versus the GII; $^{++}p < 0.01$ versus the GIII group. **GI:** Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

The OFT was used to evaluate both locomotor activity and anxiety-like behaviour. During exposure to an open field, rats showed a pronounced behavioural activity to the sudden change from a familiar environment to an open area.

Stressed rats spent less time in the central zone and more time in the peripheral zone which is considered to reflect the fear of entering and exploring a novel environment and is associated with increased anxiety levels of the animal. Our results are in agreement with previous studies (Chen et al., 2008; Ampuero et al., 2015; Liu et al., 2019) confirming the anxiogenic effects induced by chronic immobilisation stress.

The treatment with *C.oblonga* extract at the dose of 300mg/kg b.w reversed chronic immobilisation stress-induced changes in behaviours, indicating an anxiolytic effect of the extract.

Normal rats treated with quince extract showed longer distance and time spent in the central zone compared to other groups, suggesting that the daily consumption of the fruit extract can improve the mood function of animals.

No difference in the total distance travelled was seen among all groups, suggesting that locomotor activity has not been affected either by chronic stress or the extract treatment. These results are consistent with behavioural data frPom the study of Dhamija et al. (2017) and Jiang et al. (2018) where the use of *Gardinia indica* fruit and Dammarane sapogenins respectively have shown anxiolytic effect without affecting the locomotive activity by chronic stress or the treatment used. On the contrary, some studies using Naringenin (Tayyab et al., 2019), Quercetin (Mehta et al., 2017); reported anxiety-like behaviour concomitant with a decrease in locomotor activity in stressed animals comparing to treated one. The reason for this discrepancy may relate to the use of different strains of animals, the open field arena dimensions, chronic stress procedures, and drugs, and so on.

Therefore, the OFT results suggest that *Cydonia oblonga* had no effect on the locomotor activity in rats but exerted an anxiolytic effect against chronic immobilisation stress.

- **Sucrose preference test**;

In the immobilisation group, rats showed a significant reduction in the percentage of sucrose solution intake ($p < 0.01$, **figure 20**), suggesting anhedonic behaviour.

Rats in other groups (GI, GIV) showed undistinguishable sucrose solution intake compared to the vehicle-treated group.

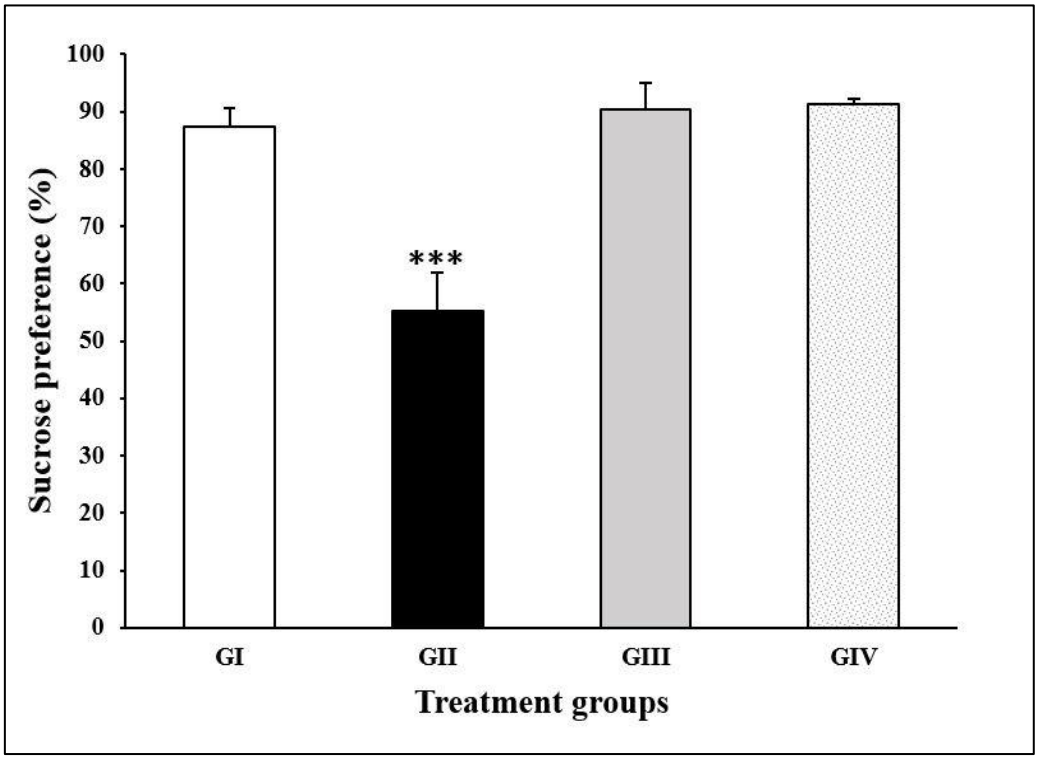

Figure 20. Effect of *Cydonia oblonga* extract and the chronic immobilisation stress on the sucrose preference test and **n=5**. The bars represent the mean ± SEM, ***$p < 0.001$ versus the control group.

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

- **Forced swimming test**

The FST showed a significant increase in immobility time in the chronic stress group an index of behavioural despair (Figure 21a) (Thakur et al., 2014; Tayyab et al., 2019), coupled with a significant decrease in climbing (high mobility) behaviour compared to the control group ($p < 0.001$) (Figure 21b).

Treated animals (GIII) were observed to be more active and vigorously attempted to rescue themselves, as indicated by the significantly higher frequency of climbing behaviour. However, no difference was noted between rats in swimming behaviour (**Figure 21c**)

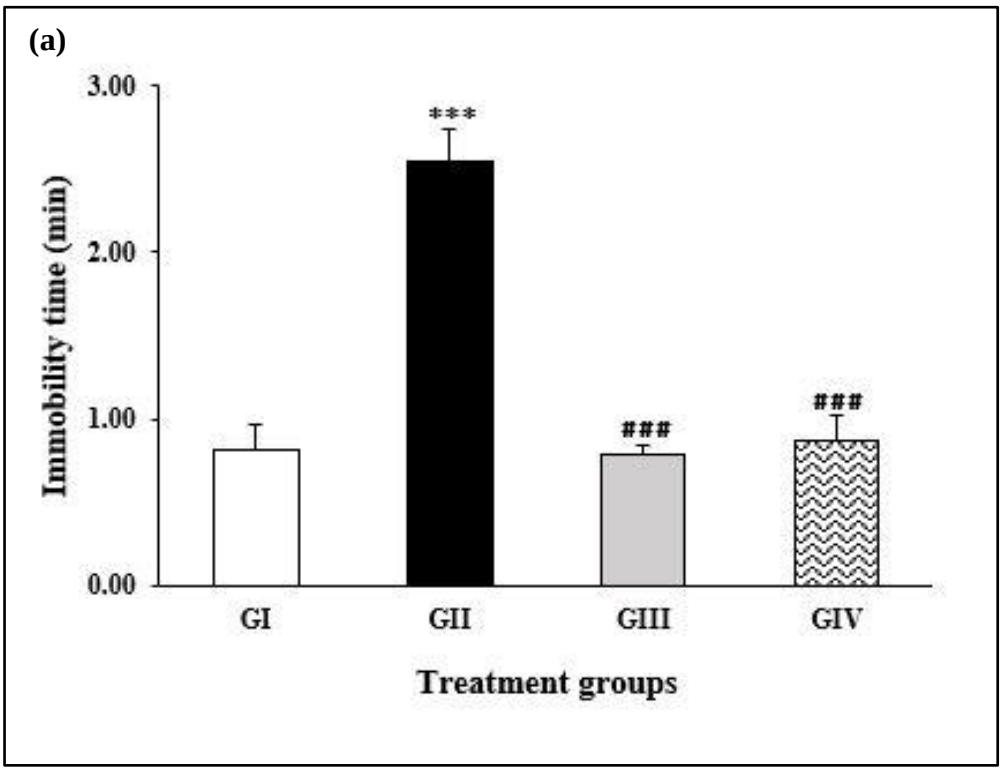

Figure 21(a). Effect of *Cydonia oblonga* extract and the chronic immobilisation stress on immobility time in the forced swimming test. n=5. The bars represent the mean ± SEM, ***$p < 0.001$ versus the CNTR group. N.S., not significant

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

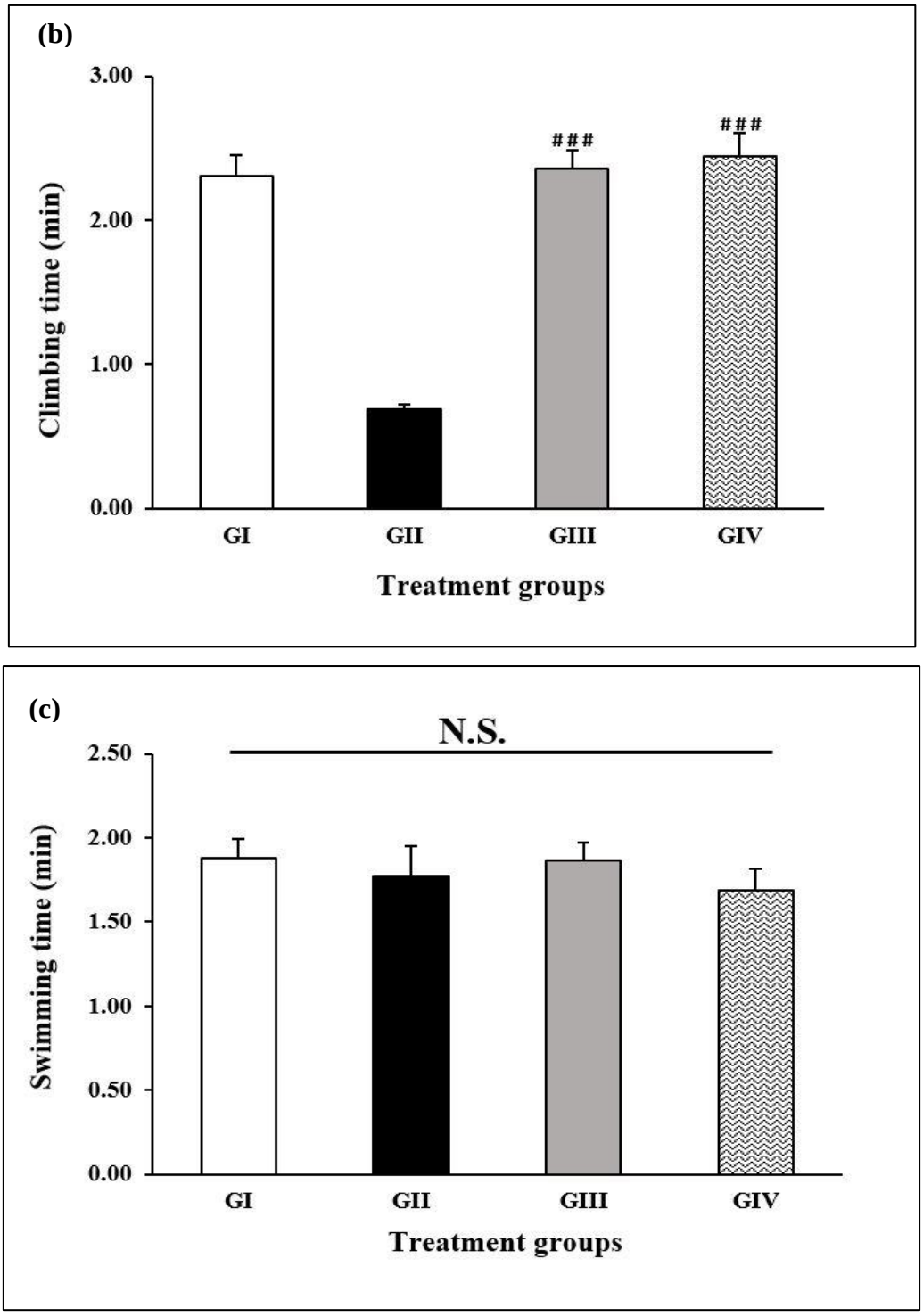

Figure 21. Effect of *Cydonia oblonga* extract and the chronic immobilisation stress on **(b)** climbing and **(c)** swimming time in the forced swimming test. n=5. The bars represent the mean ± SEM, ***$p < 0.001$ versus the CNTR group. N.S., not significant. **GI:** Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

The SPT and FST are classical tests useful for evaluating anhedonia and behavioural despair, respectively. This is consistent with the fact that the animals that consumed more sucrose were those which showed greater levels of immobility.

In our experiment, the decrease in sucrose preference and the increase in immobility time in FST demonstrated that the rat model of depression has been successfully established by chronic immobilisation stress.

Both the anhedonic behaviour in the SPT and the depressive-like behaviour in the FST were rescued by the administration of the extract.

It was reported that antidepressant drugs which inhibit norepinephrine reuptake (desipramine or reboxetine) effectively reduced immobility and selectively increased climbing behaviour without affecting swimming, whereas the selective serotonin reuptake inhibitor (fluoxetine), which works through the serotonin system, reduced immobility and selectively increased swimming, without affecting climbing (Cryan et al.,2002; Thompson et al.,2004). In the present study, climbing time was inversely related to immobility in all groups, even in the absence of treatment (control group), but was significantly lower only in the chronic stress group.

Taken together, our results confirmed that the 6 hours of immobilisation stress for 21 days caused depression-like behaviours in rats, which could be recovered to the control levels by *C.oblonga* extract administration via its antidepressant-like effect.

The main findings of the present study are that the chronic stress-induced behavioural changes can be normalized by chronic administration of *C.oblonga* fruit extract.

III-7-3 Effect of chronic stress and *Cydonia oblonga* extract on neurogenesis

Immunohistochemical staining was performed to assess the cell proliferation (Ki67 protein), and the neurogenesis (immature new-born neurons with doublecortin (DCX) staining) in the DG of the hippocampus.

Immunohistochemical assessment of rat hippocampus revealed that chronic immobilisation stress modulated hippocampal structure, cell proliferation, and neurogenesis.

It can be seen clearly that there is a remarkable alteration in the structure of the CA3 region of the hippocampus in GII compared to the control group (Figures 22-23).

No effect of immobilisation stress was observed in stressed rats treated with quince extract regarding the CA3 structure (Figure 24).

Normal rats received 300mg/kg of *C.oblonga* extract showed a similar structure to that observed in control rats (Figure 25).

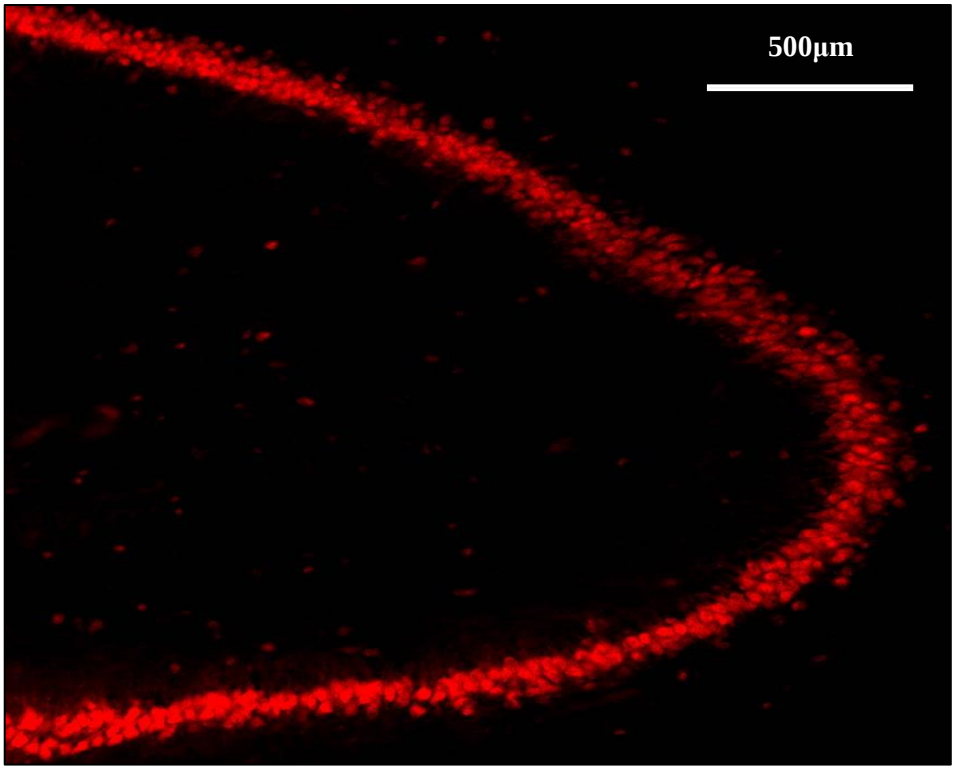

Figure22. representative image of NeuN staining in CA3 region in vehicle-treated rats. Scale bar = 500 µm.

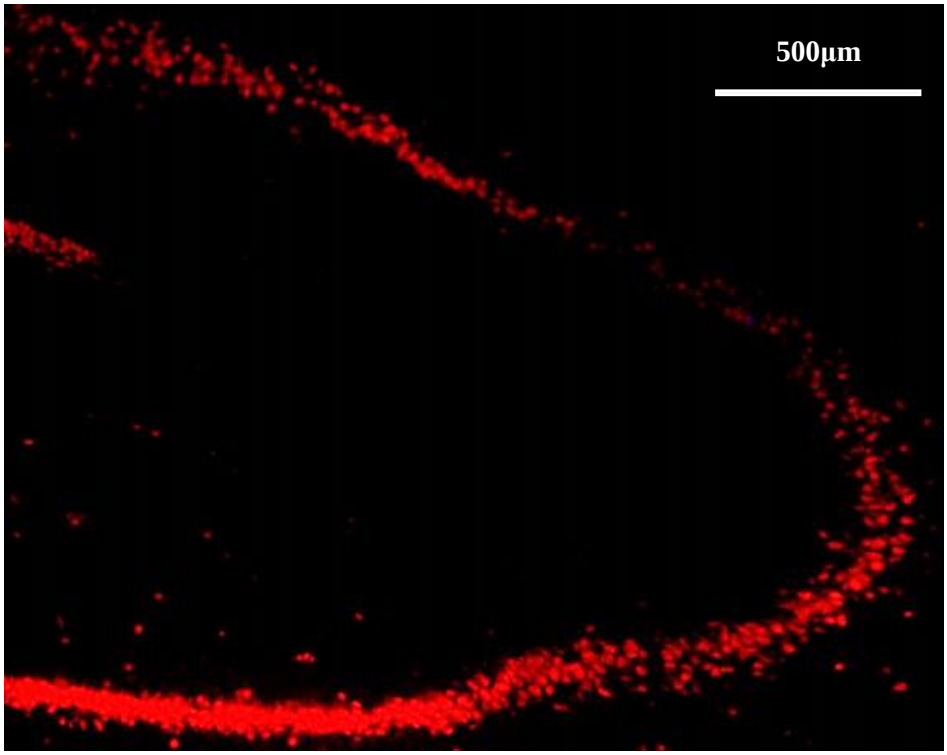

Figure 23. Representative image showed a specific abnormal structure of the CA3 region in chronic immobilisation stress rats. Scale bar = 500 µm.

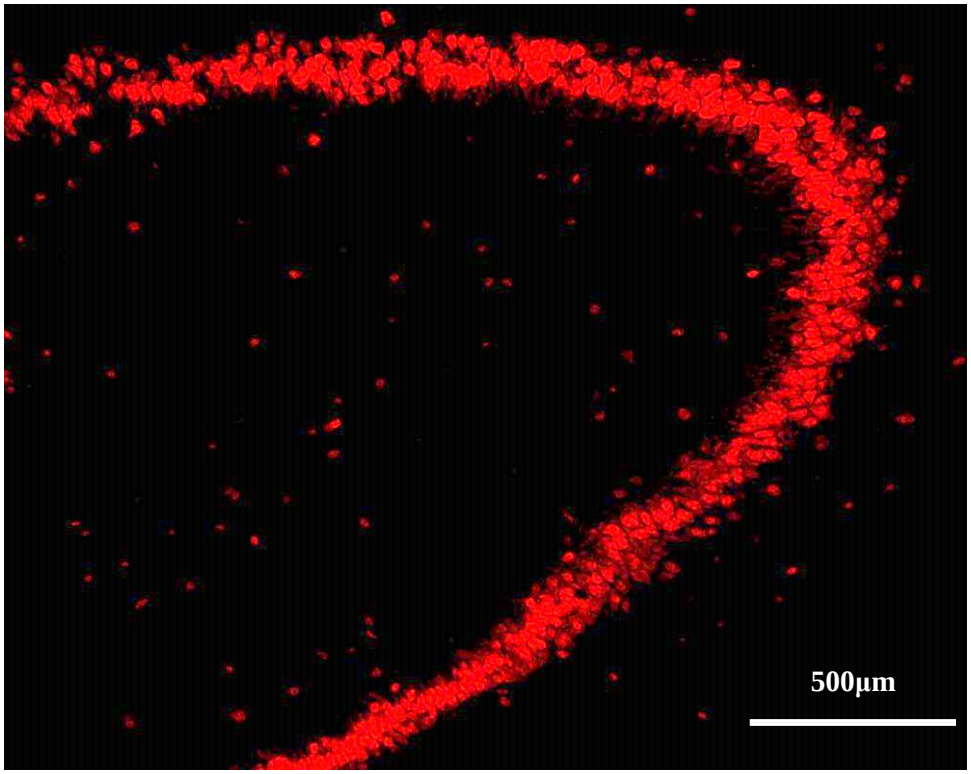

Figure 24. Representative image of NeuN staining in CA3 region in stressed rats treated with *Cydonia oblonga* extract. Scale bar = 500 µm.

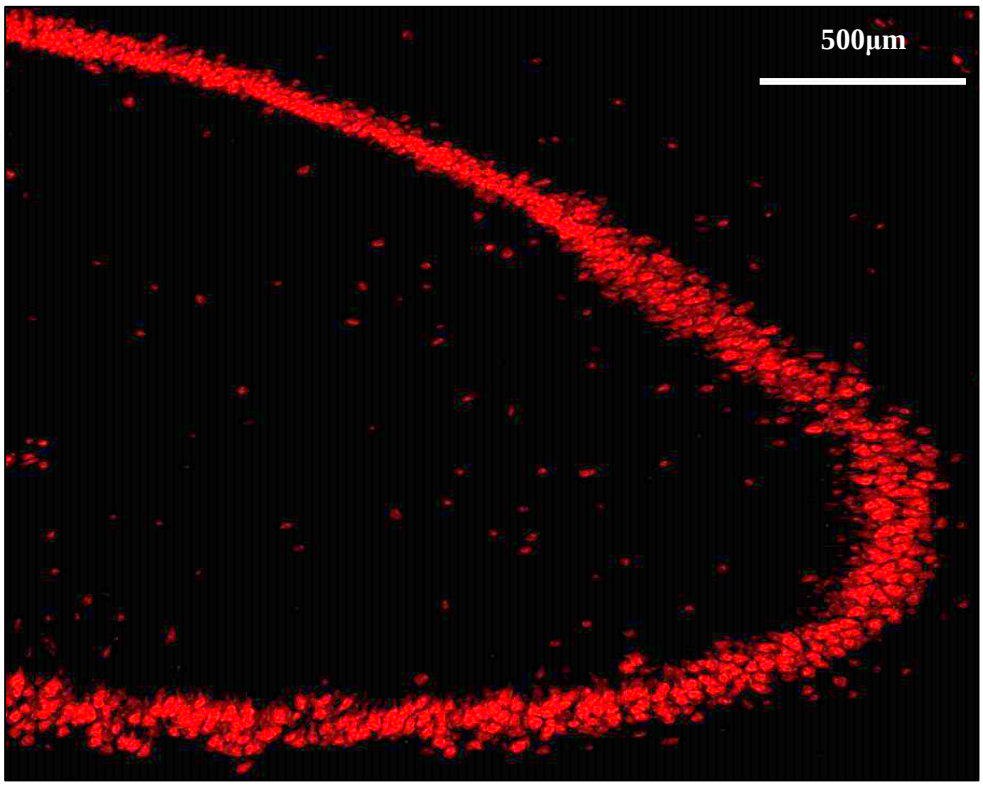

Figure 25. Representative image of NeuN staining in CA3 region in rats treated with *Cydonia oblonga* extract. Scale bar = 500 µm.

Figure 26 showed the normal arborisation of new-born neurons in the DG which was less and worse in the stressed rats (Figure 27). This effect was completely reversed by the treatment with *C.oblonga* extract (Figure 28).

No structural difference of new-born neurons was observed between GI and GIV (figure 29).

In addition, the quantitative assessment showed changes in the number of DCX-positive cells among the groups. The Rats in the second group had significantly fewer DCX-positive immature neurons compared to the first third and the fourth groups ($p < 0.001$) (Figure 30).

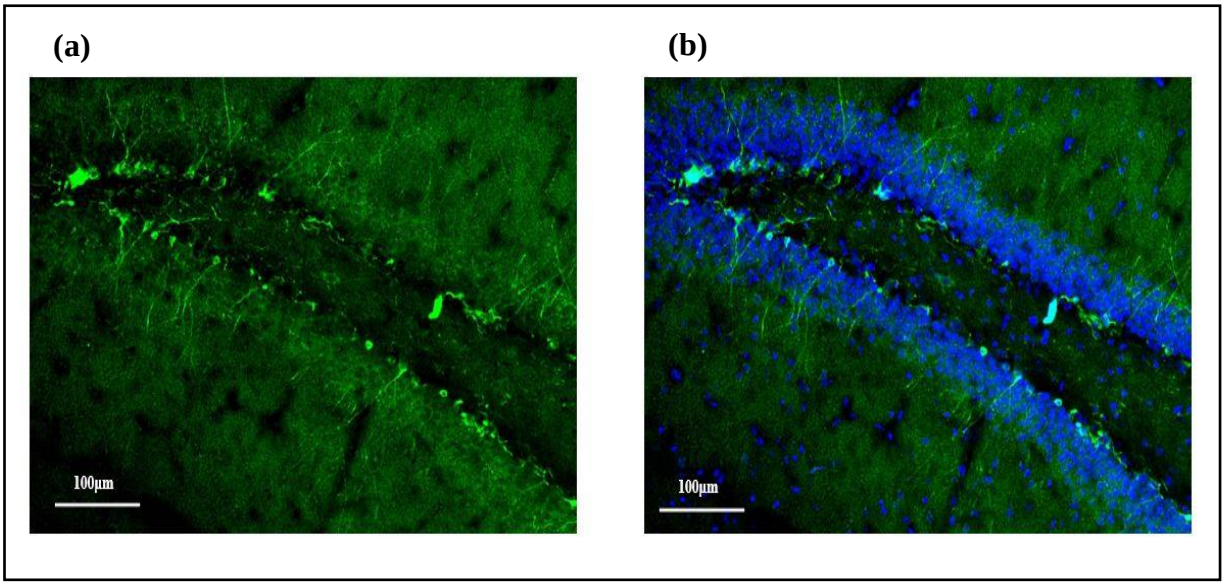

Figure 26 (a, b). Representative image of DCX-positive immature neuron (green) stanning in the dentate gyrus in vehicle- treated rats. DAPI (blue) was used to label nucleus. Scale bar = 100 µm.

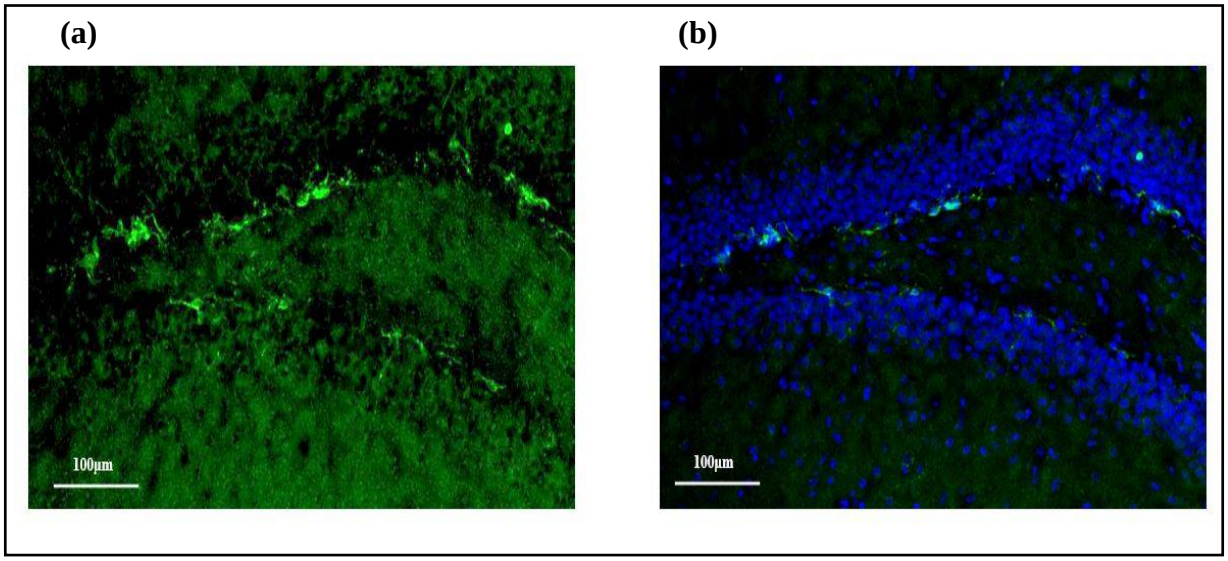

Figure 27 (a, b). Representative image of DCX-positive immature neuron (green) stanning in the dentate gyrus in chronic immobilisation stress rats. DAPI (blue) was used to label nucleus. Scale bar = 100 µm.

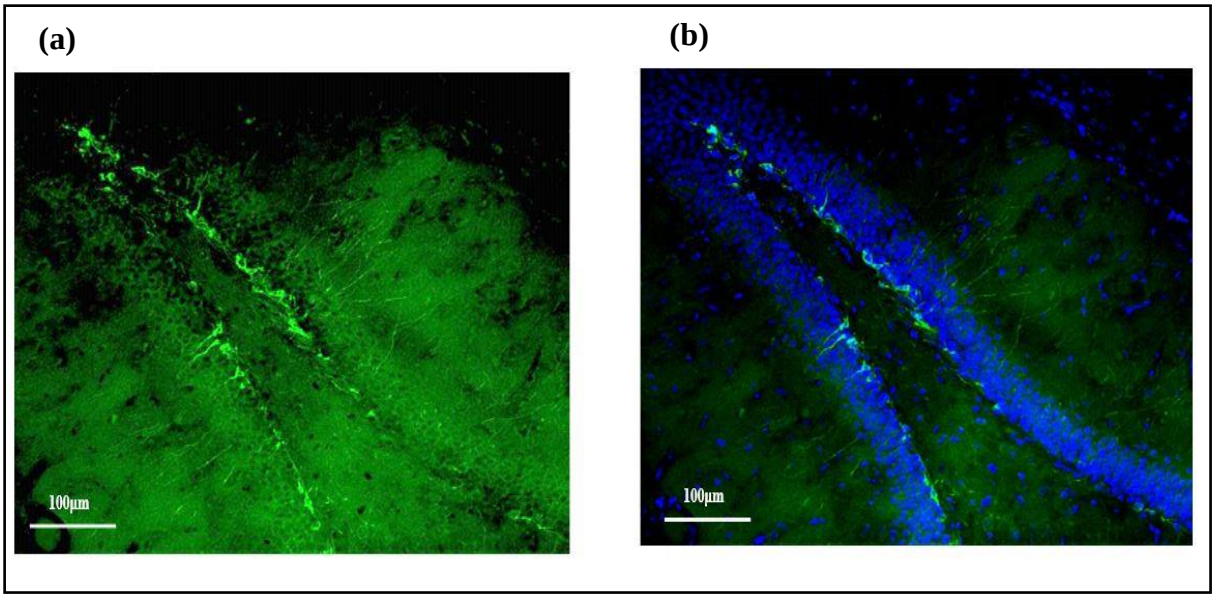

Figure 28 (a, b). Representative image of DCX-positive immature neuron (green) stanning in the dentate gyrus in stressed rats treated with *C.oblonga* extract. DAPI (blue) was used to label nucleus. Scale bar = 100 µm.

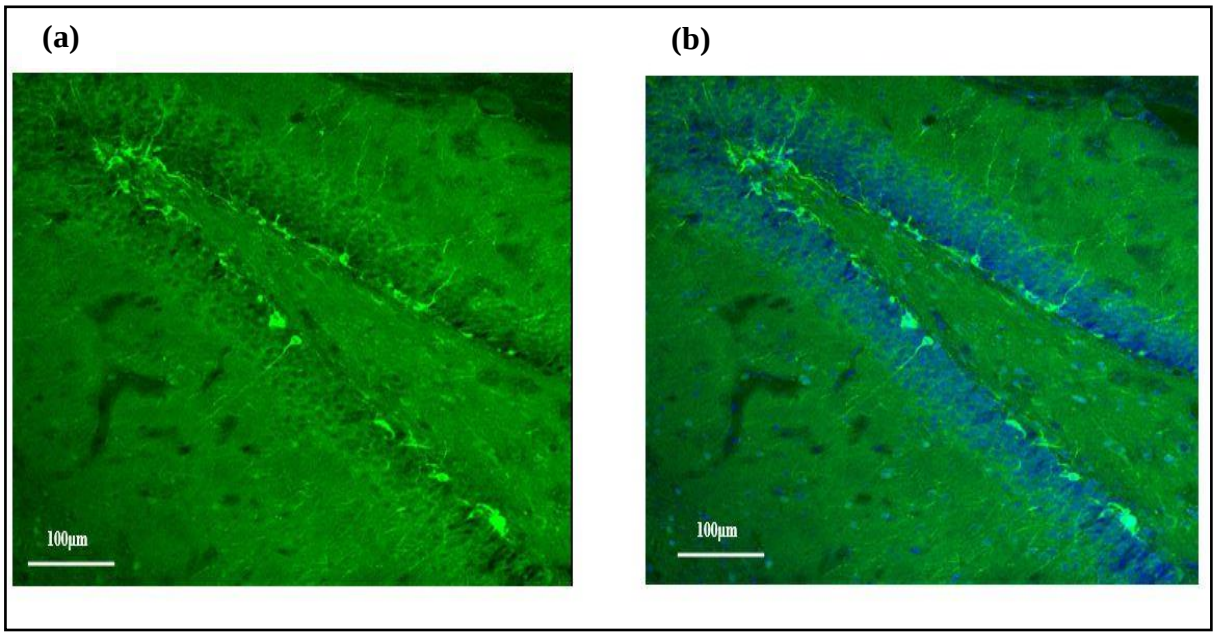

Figure 29 (a,b). Representative image of DCX-positive immature neuron (green) stanning in the dentate gyrus in rats treated with *C.oblonga* extract. DAPI (blue) was used to label nucleus. Scale bar = 100 µm.

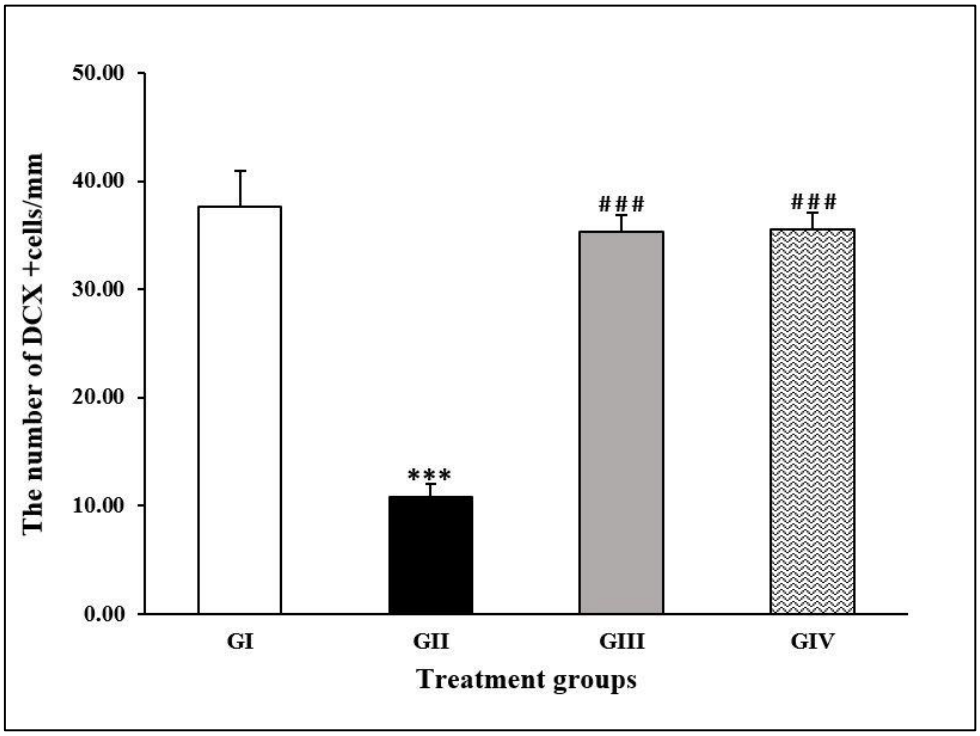

Figure 30. Effect of *Cydonia oblonga* extract and the chronic immobilisation stress on the number of DCX-positive cells in the DG. n=5. The bars represent the mean ± SEM, ***$p < 0.001$ versus the CNTR group, $^{###}p < 0.001$ versus the CIS group. N.S., not significant. White bar = 500 µm.

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

The normal distribution of proliferative cells showed in figure 31 (control group) was less in the dentate gyrus of stressed rats (Figure 32).

Undistinguished distribution of new cells was observed between control rats and rats in GIII (Figure 33) and GIV (Figure 34).

Quantitative assessment of Ki67- positive cells showed changes in the number among the groups. The Rats in the GI had significantly fewer Ki67-positive cells compared to the other groups ($p < 0.001$). The number of Ki67-positive cells in the GIII, GIV was almost the same as those of the control group (Figure 35).

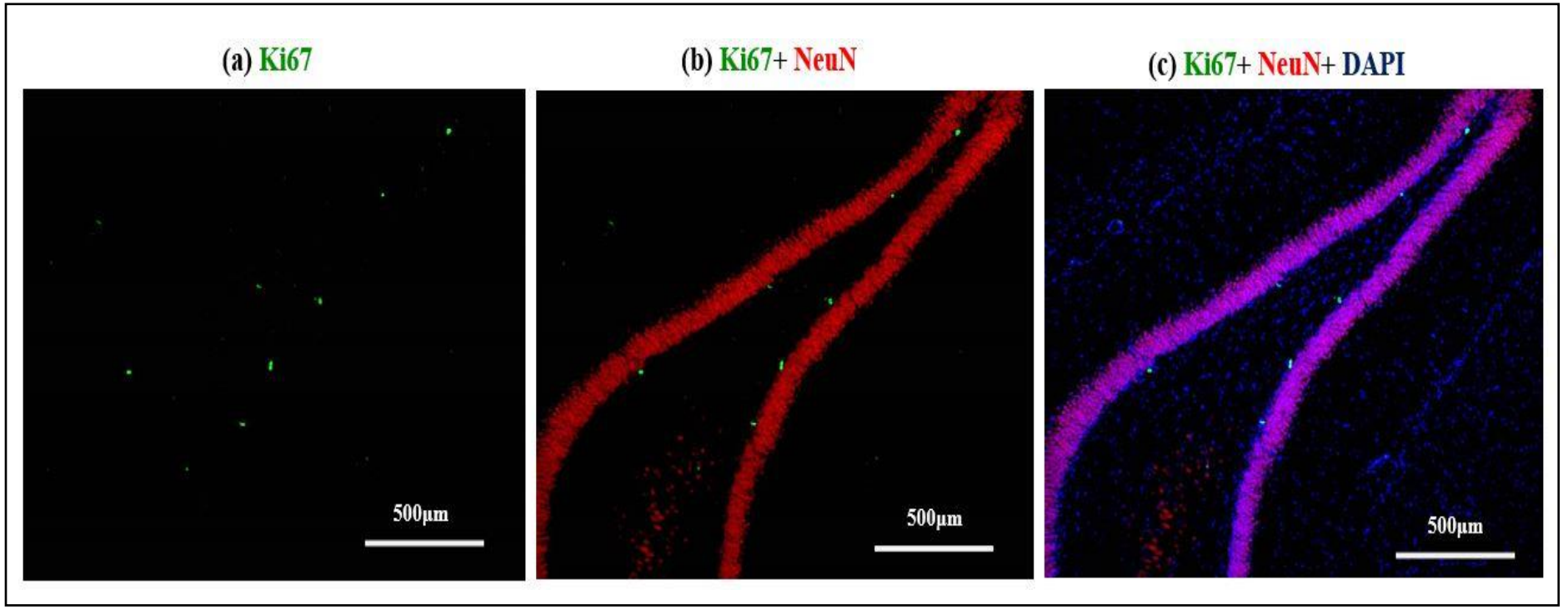

Figure 31 (a, b, c). Representative image of Ki67-positive cells (green) stanning in the dentate gyrus in vehicle- treated rats. DAPI (blue) was used to label nucleus. Scale bar = 100 µm

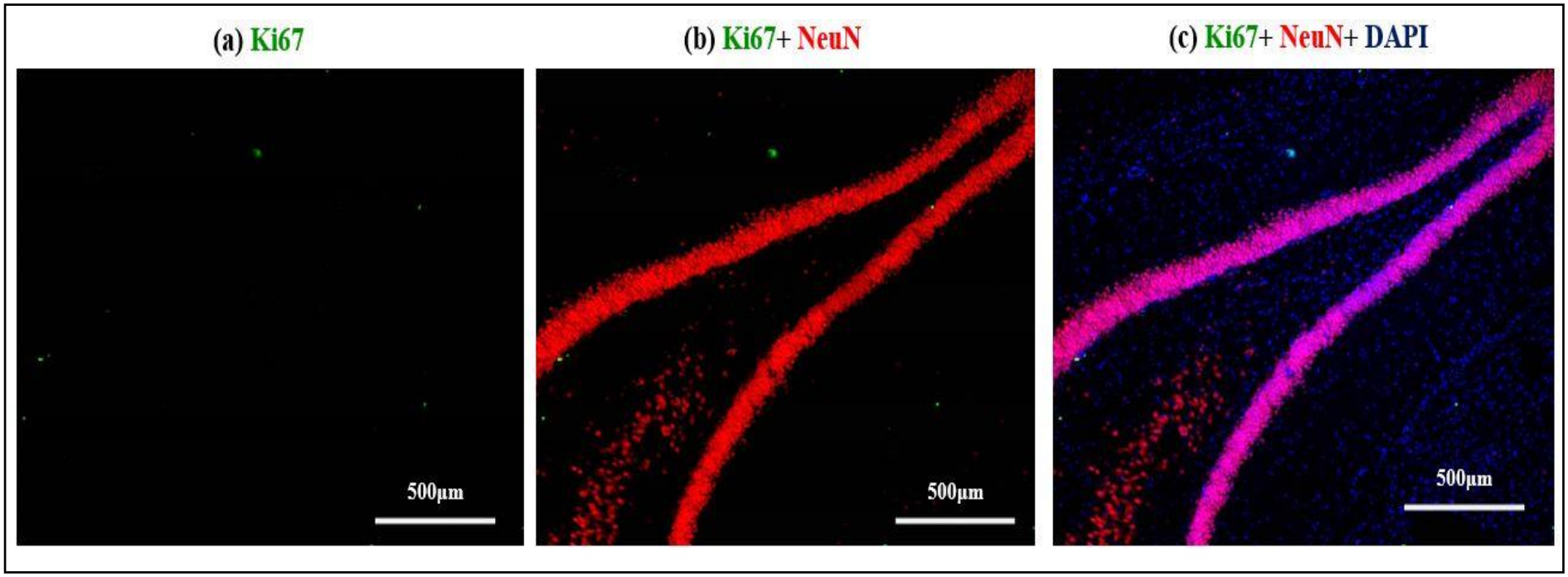

Figure 32 (a, b, c). Representative image of Ki67-positive cells (green) stanning in the dentate gyrus in chronic immobilisation stress rats. DAPI (blue) was used to label nucleus. Scale bar = 100 µm

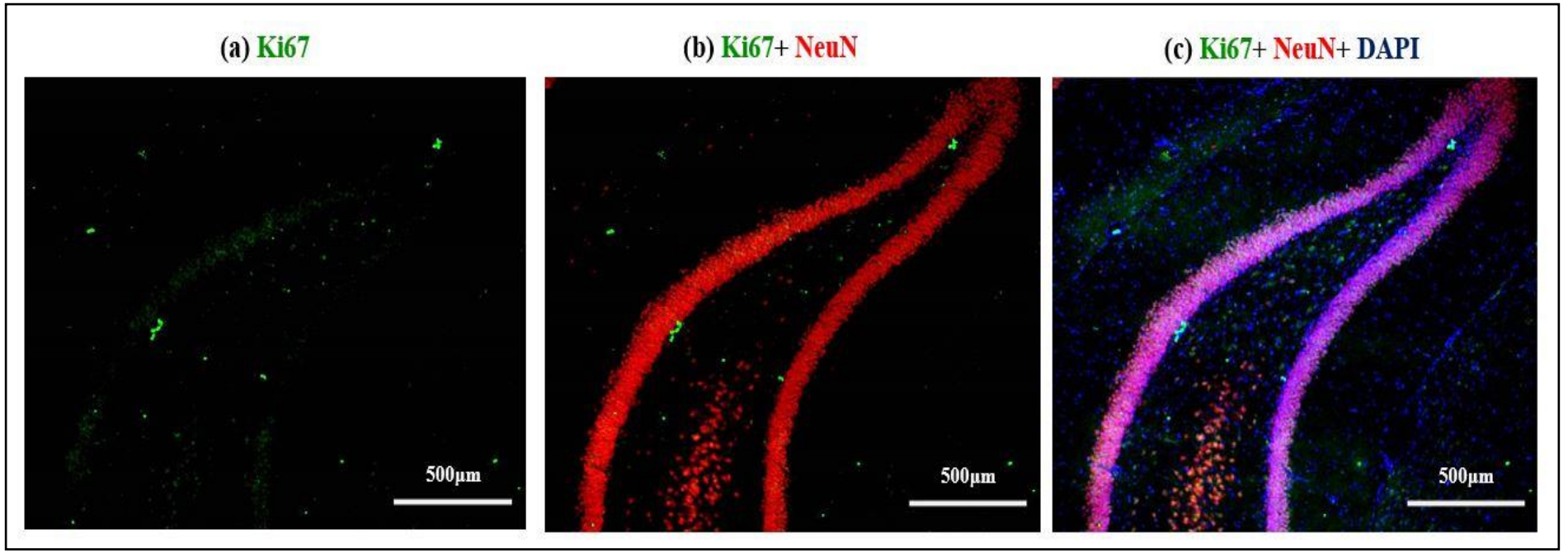

Figure 33 (a, b, c). Representative image of Ki67-positive cells (green) stanning in the dentate gyrus in stressed rats treated with *C.oblonga* extract. DAPI (blue) was used to label nucleus. Scale bar = 100 µm

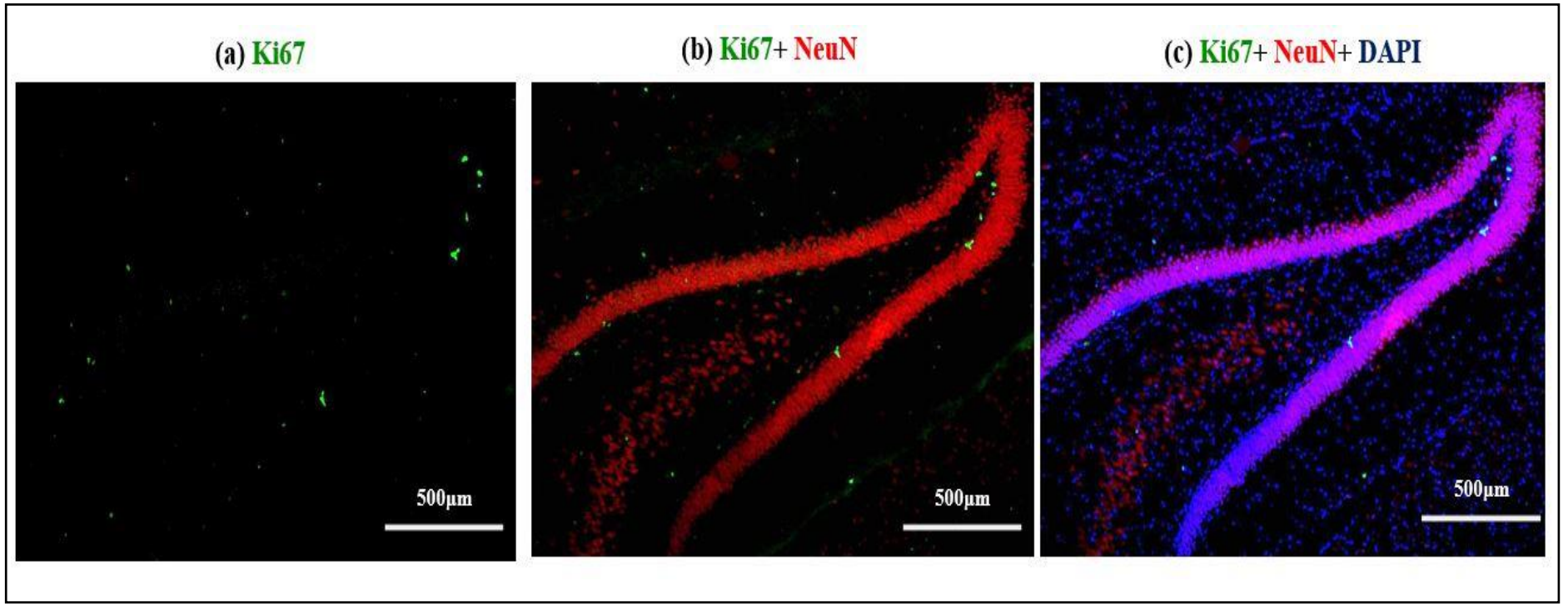

Figure 34 (a, b, c). Representative image of Ki67-positive cells (green) stanning in the dentate gyrus in rats treated with *C.oblonga* extract. DAPI (blue) was used to label nucleus. Scale bar = 100 µm

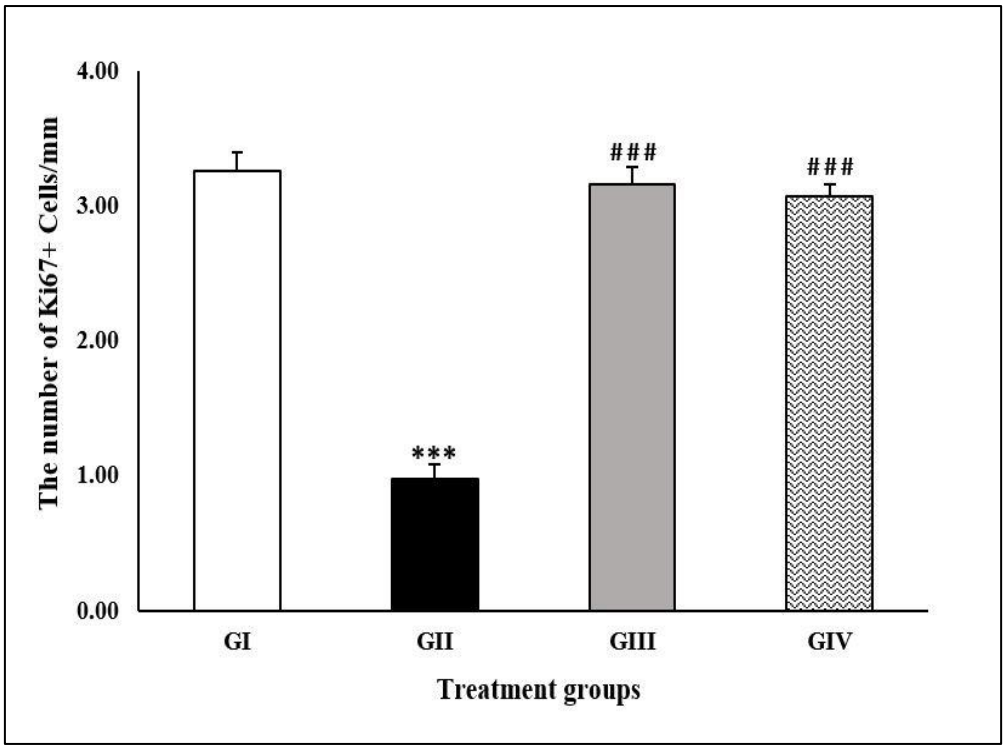

Figure 35. Effect of *Cydonia oblonga* extract and the chronic immobilisation stress on the number of Ki67-positive cells in the DG. n=5. The bars represent the mean ± SEM, ***$p < 0.001$ versus the CNTR group, $^{###}p < 0.001$ versus the CIS group. N.S., not significant. White bar = 500 μm.

GI: Control group, **GII:** Chronic immobilisation stress group, **GIII:** Chronic immobilisation stress/ *Cydonia oblonga* extract (300mg/kg b.w) group, **GIV:** group treated with *Cydonia oblonga* extract (300mg/kg b.w)

The results of our study are in line with previous reports, where the chronic stress impaired the neurogenesis and induce behavioural dysfunction in rodent which considered an important novel therapy for psychiatric disorders such as anxiety and depression (Zhu et al., 2008; Yun et al., 2010; Castilla-Ortega et al., 2011; Hueston et al., 2017).

The arborization of DCX-positive cells in the DG of the stressed rats was less and worse than that of the control group, indicating that chronic immobilisation stress not only reduced the number of DCX-positive cells but also delayed neuronal maturation. In contrast, the stressed rats pre-treated with *C.oblonga* (300 mg/kg b.w) reversed the decrease in hippocampal neurogenesis and alleviated the morphological changes of the CA3 region, suggesting that the treatment with the extract could protect the impairments in the cell proliferation, generation of new neurons, and cytoarchitectural alteration caused by chronic immobilisation stress.

Our findings suggest a correlation between the rate of hippocampal neurogenesis and depression levels although further studies should be performed to provide a detailed mechanism of how the protection of adult neurogenesis is involved in the antidepressant-like effect of *Cydonia oblonga* fruit.

It is known that hormonal changes that occur during acute and chronic stress situations can affect glucose homeostasis in both healthy people and in those with diabetes. In fact, there is no clear evidence that stressful life events promote the development of diabetes in children or in adults (Marcovecchio and Chiarelli, 2012). However, clinical studies suggest a bidirectional relationship between diabetes and depression, where diabetes may increase the risk for depressive symptoms and depression may increase the risk for diabetes.

Ho et al. (2012) reported that streptozotocin-induced diabetes caused depressive-like behaviour and decreased hippocampal cell proliferation in mouse model of diabetes.

It is also noteworthy that exposure to stress for long period can increase ROS (reactive oxygen species) in the hippocampus, which causes a severe reduction of neuronal production (Yuan et al., 2015).

As mentioned in the first part of this thesis, *Cydonia oblonga* and *Phoenix dactylifera* expressed good antioxidant activity which can modulate the intracellular signalling cascade

proteins and lipid kinases. Therefore, the extract of these fruits might have protected NSPCs and newborn neurons from the ROS-dependent adverse effects on neurogenesis.

The data of our study suggests that *Cydonia oblonga* fruit could successfully prevent and/or reverse the detrimental effects of diabetes on the brain and behaviour.

Conclusion and Perspectives

Conclusion and perspective

In conclusion, our study extends our knowledge of the health benefit of *Phoenix dactylifera* and *Cydonia oblonga* fruits, showing for the first time the potential ability of their extracts to prevent neurobehavioural changes induced by chronic stress.

The antioxidant properties reported in the first part of our study indicated that these fruits contain compounds that can act as reducing agents and free radical scavengers in cellular reactions. Similarly, the result of quince extract from the inhibitory effects on an α-glucosidase level could be used as a monotherapy along with an appropriate diabetic diet and exercise or might be in conjunction with antidiabetic therapy to manage and prevent progression of diabetes. In addition, the quince fruit provides acts as an immunostimulant agent in the reticuloendothelial system.

The second part of our research study was designed to investigate the effect of fruits extracts on neurobehavioural damages induced by stressful life events. The results from both experiments confirm previous studies reported that chronic stress induced reduce body weight concomitant with depressive like-behaviour in rats.

On the basis of our experiment and data from the literature, we can conclude that *Phoenix dactylifera* can express antidepressant activity.

We have also shown that daily exposure to six hours immobilisation stress for 3 weeks resulted in transient suppression of cell proliferation and a significant decrease of the new born cell survival and doublecortin expression in the dentate gyrus. We demonstrated, to the best of our knowledge, for the first time that *Cydonia oblonga* fruit can prevent the onset of depression-like behaviours in chronically stressed rats by enhancing hippocampal neurogenesis.

These studies shed light on what effects stress has on our brain health and how could we reduce and suppress these effects by the dietary food in the context of a healthy life.

Based on the present results, future work can evaluate many topics:

- Purification of the bioactive molecules presented in the fruit's extracts.
- Evaluation of the molecular mechanism by which *Phoenix dactylifera* and *Cydonia oblonga* extracts can provide their antidepressive effect.
- Evaluate the mechanism by which the antidepressive activity of *Cydonia oblonga* extract can prevent hippocampal neurogenesis.
- Evaluation of the effect of fruits extract on other diseases induced by chronic stress

References

Adefegha S.A.A., Oboh G. (2012). *in vitro* inhibition activity of polyphenolrich extracts from *Syzygium aromaticum* (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe^{2+} -induced lipid peroxidation in rat pancreas. *Asian Pacific Journal of Tropical Biomedicine*. 2(10): 774-781.

Adjimani J. P., Asare P. (2015). Antioxidant and free radical scavenging activity of iron chelators. *Toxicology Reports*. 2: 721-728.

Agbon A. N., Abubakar M. G., Enemeli F. U., Mahdi O., Bobb K. A., Sule H., et al. (2017). Assessment of Ethanol Fruit extract of *Phoenix dactylifera* L. (Date Palm) on mecury chloride–induced cerebral and cerebellar alterations in Wistar rats. *Journal of Anatomy Science*. *8*(1): 188-201.

Ahmed S., Alam Khan R., Jamil S. (2016). Anti- hyperlipidemic and hepatoprotective effects of native date fruit variety" Aseel"(*Phoenix dactylifera*). *Pakistan Journal of Pharmaceutical Sciences*. *29*(6).

Aiyegoro O.A., koh A.I. (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine*. *10*(1).

Al-Alawi R. A., Al-Mashiqri J. H., Al-Nadabi J. S., Al-Shihi B. I., Baqi Y. (2017). Date palm tree (*Phoenix dactylifera* L.): natural products and therapeutic options. *Frontiers in Plant Science*. *8*(845).

Al-Farsi M., Alasalvar C., Morris A., Baron M., Shahidi F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry*. *53*(19): 7592-7599.

Ali Haimoud S., Allem R., Merouane A. (2016). Antioxidant and anti-inflammatory properties of widely consumed date palm (*Phoenix dactylifera L*.) Fruit varieties in Algerian oases. *Journal of Food Biochemistry*. *40*(4): 463-471.

Ali S. H., Madhana R. M., Athira K. V., Kasala E. R., Bodduluru L. N., Pitta S., et al. (2015). Resveratrol ameliorates depressive-like behavior in repeated corticosterone-induced depression in mice. *Steroids*. *101*: 37-42.

Al-Mamary M., Al-Habori M., Al-Zubairi A. S. (2014). The *in vitro* antioxidant activity of different types of palm dates (*Phoenix dactylifera*) syrups. *Arabian Journal of Chemistry*. *7*(6): 964-971.

Al-Qarawi A. A., Ali B. H., Al-Mougy S. A., Mousa H. M. (2003). Gastrointestinal transit in mice treated with various extracts of date (*Phoenix dactylifera* L.). *Food and Chemical Toxicology*. *41*(1): 37-39.

Al-Shahib W., Marshall R. J. (2002). Dietary fibre content of dates from 13 varieties of date palm *Phoenix dactylifera* L. *International Journal of Food Science & Technology*. *37*(6): 719-721.

Al-Shwyeh H. A. (2019). Date palm (*Phoenix dactylifera* L.) fruit as potential antioxidant and antimicrobial agents. *Journal of Pharmacy & Bioallied Sciences*. *11*(1).

Alves N. D., Patrício P., Correia J. S., Mateus-Pinheiro A., Machado-Santos A. R., Loureiro-Campos E., et al. (2018). Chronic stress targets adult neurogenesis preferentially in the suprapyramidal blade of the rat dorsal dentate gyrus. *Brain Structure and Function*. *223*(1): 415-428.

Alves S. F., Gomes C. M., de Oliveira M. G., de Andrade W. M., Moreira L. C., Borges, L. L., et al. (2020). Cytotoxicity, phagocytic activity, and leishmanicidal potential of extract standardized in geranylgeraniol obtained from the fruit of *Pterodon emarginatus* vogel. *Pharmacognosy Magazine*. *16*(67): 140.

Ampuero E., Luarte A., Santibañez M., Varas-Godoy M., Toledo,J., Diaz-Veliz G., et al. (2015). Two chronic stress models based on movement restriction in rats respond selectively to antidepressant drugs: aldolase C as a potential biomarker. *International Journal of Neuropsychopharmacology*. *18*(10).

Andreu Z., Khan M. A., González-Gómez P., Negueruela S., Hortigüela R., San Emeterio J., et al. (2015). The Cyclin-Dependent Kinase Inhibitor p27kip1 Regulates Radial Stem Cell Quiescence and Neurogenesis in the Adult Hippocampus. *Stem Cells*. *33*(1): 219-229.

Apak R., Güçlü K., Özyürek M., Karademir S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*. *52*(26): 7970-7981.

Ashraf M. U., Muhammad G., Hussain M. A., Bukhari S. N. (2016). *Cydonia oblonga* M., a medicinal plant rich in phytonutrients for pharmaceuticals. *Frontiers in Pharmacology*. *7* (163).

Aslam M., Sial A. A. (2014). Effect of hydroalcoholic extract of *Cydonia oblonga* Miller (Quince) on sexual behaviour of wistar rats. *Advances in Pharmacological Sciences, 2014*.

Baliga M. S., Baliga B. R. V., Kandathil S. M., Bhat H. P., Vayalil P. K. (2011). A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). *Food Research International*. *44*(7): 1812-1822.

Banasr M., Dwyer J. M., Duman, R. S. (2011). Cell atrophy and loss in depression: reversal by antidepressant treatment. *Current Opinion in Cell Biology*. *23*(6): 730-737.

Benmebarek A., Zerizer S., Laggoune S., Kabouche, Z. (2013). Immunostimulatory activity of *Stachys mialhesi* de Noé. *Allergy, Asthma & Clinical Immunology*. *9*(1): 1-4.

Besbes S., Drira L., Blecker C., Deroanne C., Attia H. (2009). Adding value to hard date (*Phoenix dactylifera L*.): Compositional, functional and sensory characteristics of date jam. *Food chemistry*. *112*(2): 406-411.

Biglari F., AlKarkhi A. F., Easa A. M. (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food chemistry*. *107*(4): 1636-1641.

Bin-Hafeez B., Haque R., Parvez S., Pandey S., Sayeed I., Raisuddin, S. (2003). Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice. *International Immunopharmacology*. *3*(2): 257-265.

Biozzi G., Benacerraf B., Halpern B. N. (1953). Quantitative study of the granulopoetic activity of the reticuloendothelial system II. A study of the kinetics of the granulopoetic activity of the RES in relation to the dose of carbon injected. Relationship between the weight of the organs and their activity. *Britich Journal of Experimental Pathology*. *34*: 441-448.

Blois MS. (1958). Antioxidant determinations by the use of a stable free radical. Nature. *181*(4617): 1199-1200.

Boulenouar N., Marouf A., Cheriti A. (2011). Antifungal activity and phytochemical screening of extracts from *Phoenix dactylifera* L. cultivars. *Natural Product Research*. *25*(20): 1999-2002.

Bowley M. P., Drevets W. C., Öngür D., Price J. L. (2002). Low glial numbers in the amygdala in major depressive disorder. *Biological psychiatry*. *52*(5): 404-412.

Bradford M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. *72*(1-2): 248-254.

Casadesus G., Shukitt-Hale B., Stellwagen H. M., Zhu X., Lee H. G., Smith M. A., Joseph J. A. (2004). Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. *Nutritional Neuroscience*. *7*(5-6) : 309-316.

Castilla-Ortega E., Hoyo-Becerra C., Pedraza C., Chun J., De Fonseca F. R., Estivill-Torrús G., Santín L. J. (2011). Aggravation of chronic stress effects on hippocampal neurogenesis and spatial memory in LPA 1 receptor knockout mice. *Plos One*. *6*(9): e25522.

Chan J. N. M., Lee J. C. D., Lee S. S., Hui K. K., Chan A. H., Fung T. K. H., et al. (2017). Interaction effect of social isolation and high dose corticosteroid on neurogenesis and emotional behavior. *Frontiers in Behavioral Neuroscience*. *11*(18).

Chan-Zapata I., Canul-Canche J., Fernández-Martín K., Martín-Quintal Z., Torres-Romero J. C., Lara-Riegos J. C., et al. (2018). Immunomodulatory effects of the methanolic extract from *Pouteria campechiana* leaves in macrophage functions. *Food and Agricultural Immunology*. *29*(1): 386-399.

Chatzigeorgiou S., Thai Q. D., Tchoumtchoua J., Tallas K., Tsakiri E. N., Papassideri I., et al. (2017). Isolation of natural products with anti-ageing activity from the fruits of *Platanus orientalis*. *Phytomedicine*. *33*: 53-61.

Chen J. X., Li W., Zhao X., Yang J. X. (2008). Effects of the Chinese traditional prescription Xiaoyaosan decoction on chronic immobilization stress-induced changes in behavior and brain BDNF, TrkB, and NT-3 in rats. *Cellular and Molecular Neurobiology*. *28*(5): 745-755.

Chigr F., Rachidi F., Segura S., Mahaut S., Tardivel C., Jean A., et al. (2009). Neurogenesis inhibition in the dorsal vagal complex by chronic immobilization stress in the adult rat. *Neuroscience*. *158*(2): 524-536.

Christie K., Turnley A. (2013). Regulation of endogenous neural stem/progenitor cells for neural repair-factors that promote neurogenesis and gliogenesis in the normal and damaged brain. *Frontiers in Cellular Neuroscience*. *6* (70).

Commenges D., Scotet V., Renaud S., Jacqmin-Gadda H., Barberger-Gateau P., Dartigues J. F. (2000). Intake of flavonoids and risk of dementia. *European Journal of Epidemiology*. *16*(4): 357-363.

Cryan J. F., Markou A., Lucki I. (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in Pharmacological Sciences*. *23*(5): 238-245.

Daskalova E., Delchev S., Topolov M., Dimitrova S., Uzunova Y., Valcheva-Kuzmanova S., et al. (2019). *Aronia melanocarpa* (Michx.) Elliot fruit juice reveals neuroprotective effect and improves cognitive and locomotor functions of aged rats. *Food and Chemical Toxicology*. *132*.

de Almeida Lopes M. M., Sanches A. G., de Souza K. O., de Oliveira Silva E. (2018). Quince *Cydonia oblonga*. In *Exotic Fruits* (pp. 363-368). Academic Press.

Decker E. A., Welch B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry*. *38*(3): 674-677.

Dhamija I., Parle M., Kumar S. (2017). Antidepressant and anxiolytic effects of *Garcinia indica* fruit rind via monoaminergic pathway. *3 Biotech*. *7*(2): 1-12.

Doungue H. T., Kengne A. P. N., Kuate D. (2018). Neuroprotective effect and antioxidant activity of *Passiflora edulis* fruit flavonoid fraction, aqueous extract, and juice in aluminum chloride-induced Alzheimer's disease rats. *Nutrire*. *43*(1).

El Hilaly J., Ennassir J., Benlyas M., Alem C., Amarouch M. Y., Filali-Zegzouti Y. (2018). Anti-inflammatory properties and phenolic profile of six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. *Journal of King Saud University-Science*. *30*(4): 519-526.

FAOSTAT, 2018. Agro-Statistics Database. Food and Agriculture Organization of the United Nations. <http://www.fao.org/corp/copyright/en/> (accessed 18 August 2020).

Farioli-Vecchioli S., Mattera A., Micheli L., Ceccarelli M., Leonardi L., Saraulli D., et al. (2014). Running rescues defective adult neurogenesis by shortening the length of the cell cycle of neural stem and progenitor cells. *Stem Cells*. *32*(7): 1968-1982.

Fattouch S., Caboni P., Coroneo V., Tuberoso C. I., Angioni A., Dessi S., Marzouki N., Cabras P. (2007). Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *Journal of Agricultural and Food Chemistry*. *55*(3): 963–969.

Flores N. P., Bona N. P., Luduvico K. P., Cardoso J. D. S., Soares M. S. P., Gamaro G. D., et al. (2020). *Eugenia uniflora* fruit extract exerts neuroprotective effect on chronic unpredictable stress-induced behavioral and neurochemical changes. *Journal of Food Biochemistry*. *44*(10).

Forrester S. J., Kikuchi D. S., Hernandes M. S., Xu Q., Griendling K. K. (2018). Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Research*. 122: 877-902.

Fujisawa T., Ikegami H., Inoue K., Kawabata Y., Ogihara T. (2005). Effect of two α-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. *Metabolism*. 54(3): 387-390.

Fukaya N., Mochizuki K., Tanaka Y., Kumazawa T., Jiuxin Z., Fuchigami M., Goda, T. (2009). The α-glucosidase inhibitor miglitol delays the development of diabetes and dysfunctional insulin secretion in pancreatic β-cells in OLETF rats. *European journal of pharmacology*. *624*(1-3): 51-57.

Gao X., Kuo J., Jiang H., Deeb D., Liu Y., Divine G., et al. (2004). Immunomodulatory activity of curcumin: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production *in vitro*. *Biochemical Pharmacology*. *68*(1): 51-61.

García-Iglesias B. B., Mendoza-Garrido M. E., Gutiérrez-Ospina G., Rangel-Barajas C., Noyola-Díaz M., Terrón, J. A. (2013). Sensitization of restraint-induced

corticosterone secretion after chronic restraint in rats: involvement of 5-HT7 receptors. *Neuropharmacology*. *71*: 216-227.

Garthe A., Behr J., Kempermann G. (2009). Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *Plos One*. *4*(5).

Gjerstad J. K., Lightman S. L., Spiga F. (2018). Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. *Stress*. *21*(5): 403-416.

Golovatscka V., Ennes H., Mayer E. A., Bradesi S. (2012). Chronic stress-induced changes in pro-inflammatory cytokines and spinal glia markers in the rat: a time course study. *Neuroimmunomodulation*. *19*(6): 367-376.

Gregus A., Wintink A. J., Davis A. C., Kalynchuk L. E. (2005). Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behavioural Brain Research*. *156*(1): 105-114.

Hajra S., Mehta A., Pandey P. (2012). Immunostimulating activity of methanolic extract of *Swietenia mahagoni* seeds. *International Journal of Pharmacie and Pharmeurical Sciences*. *4*(1): 442-445.

Hamauzu Y., Inno T., Kume C., Irie M., Hiramatsu, K. (2006). Antioxidant and antiulcerative properties of phenolics from Chinese quince, quince, and apple fruits. *Journal of Agricultural and Food Chemistry*. *54*(3): 765-772.

Hamauzu Y., Yasui H., Inno T., Kume C., Omanyuda M. (2005). Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis Schneid*), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *Journal of Agricultural and Food Chemistry*. *53*(4): 928-934.

Heine V. M., Maslam S., Joels M., Lucassen P. J. (2004). Increased P27 KIP1 protein expression in the dentate gyrus of chronically stressed rats indicates G1 arrest involvement. *Neuroscience*. *129*(3): 593-601.

Herman J. P., McKlveen J. M., Ghosal S., Kopp B., Wulsin A., Makinson R., Scheimann J., Myers B. (2016). Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Comprehensive Physiology*. *6*(2): 603–621.

Hill M.N., Brotto L.A., Lee T.T., Gorzalka B.B. (2003). Corticosterone attenuates the antidepressant-like effects elicited by melatonin in the forced swim test in both male

and female rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. *27*(6): 905-911.

Ho N., Balu D. T., Hilario M. R., Blendy J. A., Lucki I. (2012). Depressive phenotypes evoked by experimental diabetes are reversed by insulin. *Physiology & Behavior*. *105*(3): 702-708.

Hueston C. M., Cryan J. F., Nolan Y. M. (2017). Stress and adolescent hippocampal neurogenesis: diet and exercise as cognitive modulators. *Translational Psychiatry*. *7*(4): e1081-e1081.

Ishurd O., Zgheel F., Kermagi A., Flefla M., Elmabruk M. (2004). Antitumor activity of β-d-glucan from Libyan dates. *Journal of Medicinal Food*. *7*(2): 252-255.

Ismail A. M., Mohamed E. A., Marghany M. R., Abdel-Motaal F. F., Abdel-Farid I. B., El-Sayed M. A. (2016). Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts. *Journal of the Saudi Society of Agricultural Sciences*. *15*(2): 112-117.

Jacobson L. (2005). Hypothalamic-pituitary-adrenocortical axis regulation. *Endocrinology and Metabolism Clinics*. 34 (2): 271-292

Jassim S. A., Naji M. A. (2010). *in vitro* evaluation of the antiviral activity of an extract of date palm (*Phoenix dactylifera* L.) pits on a Pseudomonas phage. *Evidence-Based Complementary and Alternative Medicine, 7*.

Jeon S. G., Kim K. A., Chung H., Choi J., Song E. J., Han S. Y., et al. (2016). Impaired memory in OT-II transgenic mice is associated with decreased adult hippocampal neurogenesis possibly induced by alteration in Th2 cytokine levels. *Molecules and Cells*. *39*(8).

Jeong J. Y., Lee D. H., Kang S. S. (2013). Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice. *Endocrinology and Metabolism*. *28*(4): 288-296.

Ji K. Y., Kim K. M., Oh J. J., Kim J. W., Lee W. J., Cho H., et al. (2020). Assessment of the 4-week repeated-dose oral toxicity and genotoxicity of GHX02. *Journal of Applied Toxicology*. *40*(2): 270-284.

Jiang N., Zhang B. Y., Dong L. M., Lv J. W., Lu C., Wang Q., et al. (2018). Antidepressant effects of dammarane sapogenins in chronic unpredictable mild stress-induced depressive mice. *Phytotherapy Research*. *32*(6): 1023-1029.

Johnson J. D., Barnard D. F., Kulp A. C., Mehta D. M. (2019). Neuroendocrine regulation of brain cytokines after psychological stress. *Journal of the Endocrine Society*. *3*(7): 1302-1320.

Karatsoreos I. N., Bhagat S. M., Bowles N. P., Weil Z. M., Pfaff D. W., McEwen B. S. (2010). Endocrine and physiological changes in response to chronic corticosterone: a potential model of the metabolic syndrome in mouse. *Endocrinology*. *151*(5): 2117-2127.

Kim J. G., Jung H. S., Kim K. J., Min S. S., Yoon, B. J. (2013). Basal blood corticosterone level is correlated with susceptibility to chronic restraint stress in mice. *Neuroscience letters*. *555*: 137-142.

Kott J. M., Mooney-Leber S. M., Shoubah F. A., Brummelte S. (2016). Effectiveness of different corticosterone administration methods to elevate corticosterone serum levels, induce depressive-like behavior, and affect neurogenesis levels in female rats. *Neuroscience*. *312*: 201-214.

Krugers H. J., Lucassen P., Karst H., Joëls M. (2010). Chronic stress effects on hippocampal structure and synaptic function: relevance for depression and normalization by anti-glucocorticoid treatment. *Frontiers in Synaptic Neuroscience*. *2* (24).

Kwon D. H., Lee H., Park C., Hong S. H., Hong S. H., Kim G. Y., et al. (2019). Glutathione induced immune-stimulatory activity by promoting M1-like macrophages polarization via potential ROS scavenging capacity. *Antioxidants*. *8*(9).

Lee B., Sur B., Kwon S., Yeom M., Shim I., Lee H., Hahm D. H. (2013). Chronic administration of catechin decreases depression and anxiety-like behaviors in a rat model using chronic corticosterone injections. *Biomolecules & Therapeutics*. *21*(4).

Lee B., Sur B., Shim I., Lee H., Hahm D. H. (2015). *Angelica gigas* ameliorate depression-like symptoms in rats following chronic corticosterone injection. *BMC Complementary and Alternative Medicine*. *15*(1): 210.

Legua P., Serrano M., Melgarejo P., Valero D., Martínez J. J., Martínez R., Hernández F. (2013). Quality parameters, biocompounds and antioxidant activity in fruits of nine quince (*Cydonia oblonga* Miller) accessions. *Scientia Horticulturae*. *154* : 61-65.

Li J., Xie X., Li Y., Liu X., Liao X., Su Y. A., Si T. (2017). Differential behavioral and neurobiological effects of chronic corticosterone treatment in adolescent and adult rats. *Frontiers in Molecular Neuroscience*. *10 (*25).

Li Y. C., Liu Y. M., Shen J. D., Chen J. J., Pei Y. Y., Fang X. Y. (2016). Resveratrol ameliorates the depressive-like behaviors and metabolic abnormalities induced by chronic corticosterone injection. *Molecules*. *21*(10): 1341.

Lipnick R. L., Cotruvo J. A., Hill R. N., Bruce R. D., Stitzel K. A., Walker A. P., et al. (1995). Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. *Food and Chemical Toxicology*. *33*(3): 223-231.

Liu L., Zhang Q., Cai Y., Sun D., He X., Wang L., et al. (2016). Resveratrol counteracts lipopolysaccharide-induced depressive-like behaviors via enhanced hippocampal neurogenesis. *Oncotarget*. *7*(35).

Liu X. Y., Shi J. H., Du W. H., Fan Y. P., Hu X. L., Zhang C. C., Chen F. L. (2011). Glucocorticoids decrease body weight and food intake and inhibit appetite regulatory peptide expression in the hypothalamus of rats. *Experimental and Therapeutic Medicine*. *2*(5): 977-984.

Locatelli V., Bresciani E., Tamiazzo L., Torsello A. (2010). Central nervous system-acting drugs influencing hypothalamic-pituitary-adrenal axis function. In *Pediatric Neuroendocrinology* (Vol. 17, pp. 108-120). Karger Publishers.

Lordan S., Smyth T. J., Soler-Vila A., Stanton C., Ross R. P. (2013). The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. *Food chemistry*. *141*(3): 2170-2176.

Lyoussi B., Tangi K. C., Morel N., Haddad M., Quetin-Leclercq, J. (2018). Evaluation of cytotoxic effects and acute and chronic toxicity of aqueous extract of the seeds of *Calycotome villosa* (Poiret) Link (subsp. intermedia) in rodents. *Avicenna Journal of Phytomedicine*. *8*(2): 122.

MADR, (2015). Ministère de l'agriculture et du développement rural, Algérie.Statistiques agricoles, série B.

Magalhães A. S., Silva B. M., Pereira J. A., Andrade P. B., Valentão P., Carvalho M. (2009). Protective effect of quince (*Cydonia oblonga* Miller) fruit against oxidative hemolysis of human erythrocytes. *Food and Chemical Toxicology*. *47*(6): 1372-1377.

Mansouri A., Embarek G., Kokkalou E., Kefalas P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chemistry*. *89*(3): 411-420.

Marcovecchio M. L., Chiarelli F. (2012). The effects of acute and chronic stress on diabetes control. *Science signaling*. *5*(247): pt10-pt10.

Mehta V., Singh T. R., Udayabanu M. (2017). Quercetin ameliorates chronic unpredicted stress-induced behavioral dysfunction in male Swiss albino mice by modulating hippocampal insulin signaling pathway. *Physiology & Behavior*. *182*: 10-16.

Micheli L., Ceccarelli M., D'Andrea G., Tirone F. (2018). Depression and adult neurogenesis: positive effects of the antidepressant fluoxetine and of physical exercise. *Brain Research Bulletin*. *143*: 181-193.

Miller C. J., Dunn E. V., Hashim I. B. (2003). The glycaemic index of dates and date/yoghurt mixed meals. Are dates 'the candy that grows on trees'? *European Journal of Clinical Nutrition*. *57*(3): 427-430.

Mirmohammadlu M., Hosseini S. H., Kamalinejad M., Gavgani M. E., Noubarani M., Eskandari, M. R. (2015). Hypolipidemic, hepatoprotective and renoprotective effects of *Cydonia oblonga* Mill. fruit in streptozotocin-induced diabetic rats. *Iranian Journal of Pharmaceutical Research: IJPR*. *14*(4).

Mohamed A. E. (2000). Trace element levels in some kinds of dates. *Food Chemistry*. *70*(1): 9-12

Mohebbi S., Naserkheil M., Kamalinejad M., Hosseini S. H., Noubarani M., Mirmohammadlu M., Eskandari M. R. (2019). Antihyperglycemic activity of quince (*Cydonia oblonga* Mill.) fruit extract and its fractions in the rat model of diabetes. *International Pharmacy Acta*. *2*(1): 2-7.

Murray F., Smith D. W., Hutson P. H. (2008). Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *European Journal of Pharmacology*. *583*(1): 115-127.

Nollet M., Gaillard P., Tanti A., Girault V., Belzung C., Leman S. (2012). Neurogenesis-independent antidepressant-like effects on behavior and stress axis response of a dual orexin receptor antagonist in a rodent model of depression. *Neuropsychopharmacology*. *37*(10): 2210-2221.

OECD (2002), Test No. 423: Acute oral toxicity - acute toxic class method, OECD guidelines for the testing of chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071001-en.

Olff M., Güzelcan Y., de Vries G. J., Assies J., Gersons B. P. (2006). HPA-and HPT-axis alterations in chronic posttraumatic stress disorder. *Psychoneuroendocrinology*. *31*(10) : 1220-1230.

Ouyang C. B., Liu X. M., Liu Q., Bai J., Li H. Y., Li Y., et al. (2015). Toxicity assessment of cadinene sesquiterpenes from Eupatorium adenophorum in mice. *Natural Products and Bioprospecting*. *5*(1): 29-36.

Parihar V. K., Hattiangady B., Kuruba R., Shuai B., Shetty A. K. (2011). Predictable chronic mild stress improves mood, hippocampal neurogenesis and memory. *Molecular Psychiatry*. *16*(2): 171-183.

Pavithra K., Vadivukkarasi S. (2015). Evaluation of free radical scavenging activity of various extracts of leaves from Kedrostis foetidissima (Jacq.) Cogn. *Food Science and Human Wellness*. 4: 42-46.

Pujari R. R., Vyawahare N. S., Thakurdesai P. A. (2013). Protective effects of *Phoenix dactylifera* against oxidative stress and neuronal damage induced by global cerebral ischemia in rats. *Biomedicine & Aging Pathology*. *3*(2): 75-81.

Ramkumar K.M., Thayumanavan B., Palvannan T., Rajaguru P. (2010). Inhibitory effect of Gymnema montanum leaves on α-glucosidase activity and α-amylase activity and their relationship with polyphenolic content. *Medicinal Chemistry Research*. *19*(8): 948-961.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. *26*(9-10): 1231-1237.

Rhiouani H., El-Hilaly J., Israili Z. H., Lyoussi B. (2008). Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *Journal of Ethnopharmacology*. *118*(3): 378-386.

Rop O., Balik J., Řezníček V., Juríková T., Škardová P., Salaš P., et al. (2011). Chemical characteristics of fruits of some selected quince (*Cydonia oblonga* Mill.) cultivars. *Czech Journal of Food Sciences*.

Rosa P. B., Ribeiro C. M., Bettio L. E., Colla A., Lieberknecht V., Moretti M., Rodrigues A.L.S. (2014). Folic acid prevents depressive-like behavior induced by chronic corticosterone treatment in mice. *Pharmacology Biochemistry and Behavior*. *127*: 1-6.

Saafi E. B., Louedi M., Elfeki A., Zakhama A., Najjar M. F., Hammami M., Achour L. (2011). Protective effect of date palm fruit extract (*Phoenix dactylifera* L.) on dimethoate induced-oxidative stress in rat liver. *Experimental and Toxicologic Pathology*. *63*(5): 433-441.

Saeedi P., Petersohn I., Salpea P., Malanda B., Karuranga S., Unwin, N., et al. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes Research and Clinical Practice*. *157*.

Santarelli L., Saxe M., Gross C., Surget A., Battaglia F., Dulawa S., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*. *301*(5634): 805-809.

Selye H. (1976). *Stress in health and disease*. Butterworth-Heinemann.

Shobana S., Sreerama Y.N., Malleshi N.G. (2009). Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of α- glucosidase and pancreatic amylase. *Food Chemistry*. 115(4): 1268-1273.

Silva B. M., Andrade P. B., Ferreres F., Domingues A. L., Seabra R. M., Ferreira M. A. (2002). Phenolic profile of quince fruit (*Cydonia oblonga* Miller) (pulp and peel). *Journal of Agricultural and Food Chemistry*. *50*(16): 4615-4618.

Silva B. M., Andrade P. B., Martins R. C., Valentão P., Ferreres F., Seabra R. M., Ferreira M. A. (2005). Quince (*Cydonia oblonga* Miller) fruit characterization using principal component analysis. *Journal of Agricultural and Food Chemistry*. *53*(1): 111-122.

Silva B. M., Andrade P. B., Valentão P., Ferreres F., Seabra R. M., Ferreira M. A. (2004). Quince (*Cydonia oblonga* Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. *Journal of Agricultural and Food Chemistry*. *52*(15): 4705-4712.

Smith S.M., Vale W.W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*. *8*(4).

Surget A., Saxe M., Leman S., Ibarguen-Vargas Y., Chalon S., Griebel G., et al. (2008). Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biological Psychiatry*. *64*(4): 293-301.

Szychowski P. J., Munera-Picazo S., Szumny A., Carbonell-Barrachina Á. A., Hernández, F. (2014). Quality parameters, bio-compounds, antioxidant activity and sensory attributes of Spanish quinces (*Cydonia oblonga* Miller). *Scientia Horticulturae*. *165*: 163-170.

Tayyab M., Farheen S., Khanam N., Hossain M.M., Shahi M.H. (2019). Antidepressant and neuroprotective effects of naringenin via sonic hedgehog-GLI1 cell signaling pathway in a rat model of chronic unpredictable mild stress. *Neuromolecular Medicine*. *21*(3): 250-261.

Thakur A.K., Soni U.K., Rai G., Chatterjee S.S., Kumar V. (2014). Protective effects of *Andrographis paniculata* extract and pure andrographolide against chronic stress-triggered pathologies in rats. *Cellular and Molecular Neurobiology*. *34*(8): 1111-1121.

Thompson M.R., Li K. M., Clemens K.J., Gurtman C.G., Hunt G.E., Cornish J.L., McGregor I.S. (2004). Chronic fluoxetine treatment partly attenuates the long-term

anxiety and depressive symptoms induced by MDMA ('Ecstasy') in rats. *Neuropsychopharmacology*. *29*(4): 694-704.

Toda T., Parylak S.L., Linker S.B., Gage F.H. (2019). The role of adult hippocampal neurogenesis in brain health and disease. *Molecular Psychiatry*. *24*(1): 67-87.

Top C., Yildiz S., Öncül O., Qydedi T., Çevikbaş A., Soyogul U. G., Çavuşlu Ş. (2007). Phagocytic activity of neutrophils improves over the course of therapy of diabetic foot infections. *Journal of Infection*. *55*(4): 369-373.

Umar A., Iskandar G., Aikemu A., Yiming W., Zhou W., Berké B., et al. (2015). Effects of *Cydonia oblonga* Miller leaf and fruit flavonoids on blood lipids and anti-oxydant potential in hyperlipidemia rats. *Journal of Ethnopharmacology*. *169*: 239-243.

van Donkelaar E. L., Vaessen K. R., Pawluski J. L., Sierksma A. S., Blokland A., Cañete R., Steinbusch H. W. (2014). Long-term corticosterone exposure decreases insulin sensitivity and induces depressive-like behaviour in the C57BL/6NCrl mouse. *Plos One*. *9*(10).

Vayalil P. K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaceae). *Journal of Agricultural and Food Chemistry*. *50*(3): 610-617.

Wang Y., Chao X., Shi H., Mehboob H., Hassan W. (2019). *Phoenix dactylifera* Protects against Doxorubicin-Induced Cardiotoxicity and Nephrotoxicity. *Cardiology Research and Practice*. *2019*.

World Health Organization. (2011). Mental health atlas-2011 country profiles. *Department of Mental Health and Substance Abuse, WHO*.

World Health Organization. (2017). Depression and other common mental disorders: global health estimates (No. WHO/MSD/MER/2017.2). World Health Organization.

Xu K., Lin Y., Zhang R., Lan M., Chen C., Li S., et al. (2015). Evaluation of safety of iridoids rich fraction from *Valeriana jatamansi* Jones: Acute and sub-chronic toxicity study in mice and rats. *Journal of Ethnopharmacology*. *172*: 386-394.

Xu Y., Ku B., Cui L., Li X., Barish P. A., Foster T. C., Ogle W. O. (2007). Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A

mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. *Brain Research*. *1162*: 9-18.

Xu Y., Li S., Chen R., Li G., Barish P. A., You W., et al. (2010). Antidepressant-like effect of low molecular proanthocyanidin in mice: involvement of monoaminergic system. *Pharmacology Biochemistry and Behavior*. *94*(3): 447-453.

Yu J., Yu B., He J., Zheng P., Mao X., Han G., Chen D. (2014). Chronic glucocorticoid exposure-induced epididymal adiposity is associated with mitochondrial dysfunction in white adipose tissue of male C57BL/6J mice. *Plos One*. *9*(11).

Yuan T.F., Gu S., Shan C., Marchado S., Arias-Carrión O. (2015). Oxidative stress and adult neurogenesis. *Stem Cell Reviews and Reports*. 11(5): 706-709.

Yun J., Koike H., Ibi D., Toth E., Mizoguchi H., Nitta A., et al. (2010). Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: possible involvement of a brain-specific transcription factor Npas4. *Journal of Neurochemistry*. *114*(6): 1840-1851.

Zaid A., De Wet P. F. (1999). Pollination and bunch management. Date palm cultivation. FAO, Roma (Italia).

Zhao C., Deng W., Gage F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell*. *132*(4): 645-660.

Zhou W., Abdusalam E., Abliz P., Reyim N., Tian S., Aji Q., et al. (2014). Effect of *Cydonia oblonga* Mill. fruit and leaf extracts on blood pressure and blood rheology in renal hypertensive rats. *Journal of Ethnopharmacology*. *152*(3): 464-469.

Zhu M. Y., Wang W. P., Huang J., Feng Y. Z., Regunathan S., Bissette G. (2008). Repeated immobilization stress alters rat hippocampal and prefrontal cortical morphology in parallel with endogenous agmatine and arginine decarboxylase levels. *Neurochemistry International*. *53*(6-8): 346-354.

Zineb G., Boukouada M., Djeridane A., Saidi M., Yousfi M. (2012). Screening of antioxidant activity and phenolic compounds of various date palm (*Phoenix dactylifera*) fruits from Algeria. *Mediterranean journal of nutrition and metabolism*. *5*(2): 119-126.

Web site

- https://basicmedicalkey.com/stress-and-disease

Appendix

Appendix

Table. Instrument tools for brain dissection and extraction

Surgery Instrument	Details
	Blunt/ Blunt scissor, standard pattern
	Blunt Scissor, fine pattern
	Crile Serrated Clamp Scissor, Straight Serrated, Length 16cm
	Forceps Length 12cm
	Large Forceps, Length 14 cm
	Rodent Brain matrix

Appendix

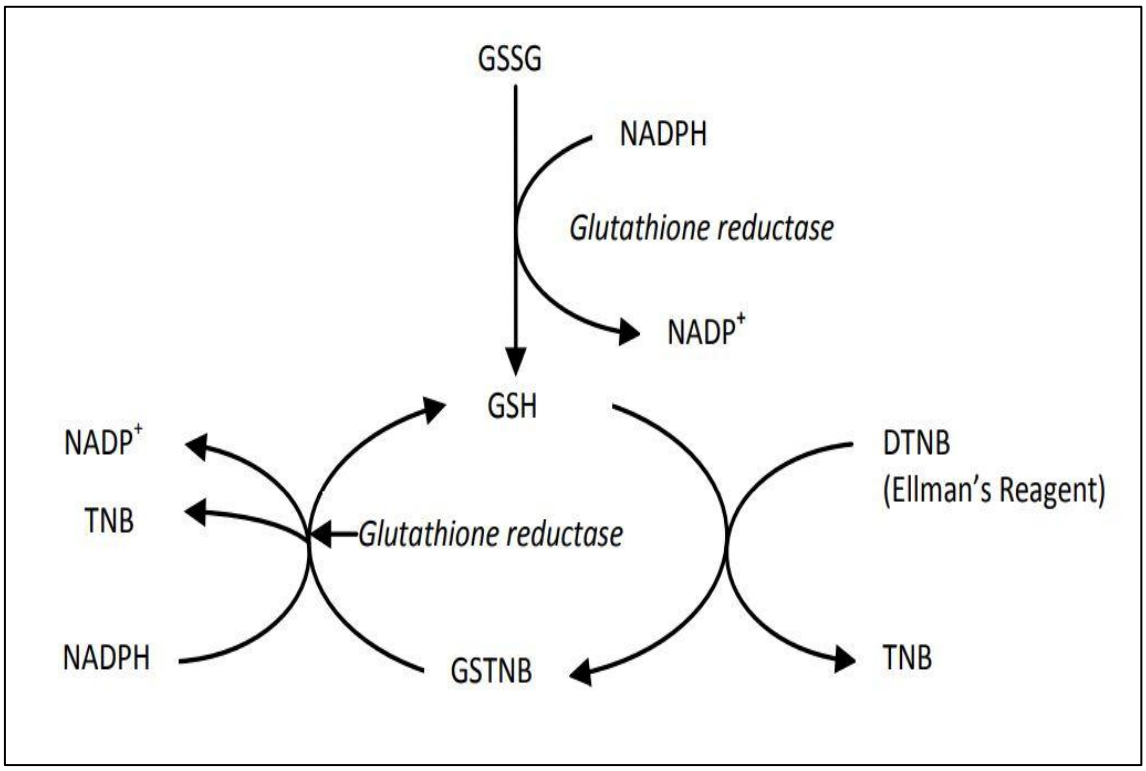

Figure: enzymatic recycling of reduced glutathione (GSH)

Appendix

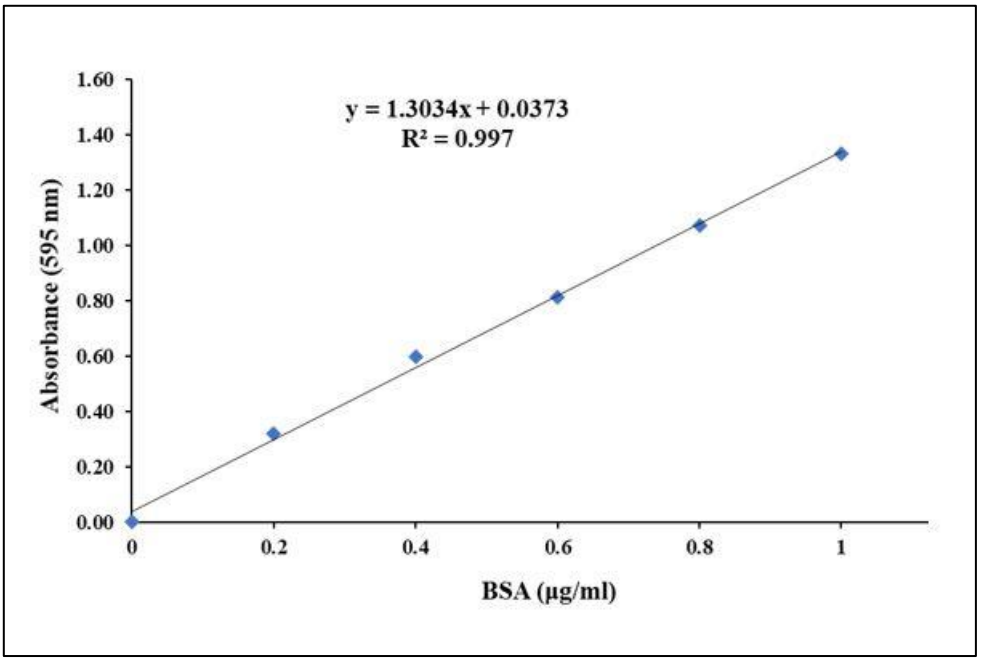

Figure: Bradford assay standard curve of concentration versus absorbance.

The concentration of protein (in mg/ml) was determined using the equation $y=1.3034x+0.0373$ with an R^2 value of 0.997, where y is the absorbance and x is the sample concentration.

Abstract

Abstract

Stressful life events promote alterations in the brain which may lead to several physical and mental diseases, such as depression, one of the major prevalent mental diseases worldwide. This thesis aims to examine whether the hydroethanolic extract of *Phoenix dactylifera* and *Cydonia oblonga* fruits can prevent the onset of depression-like behaviours in chronically stressed rats.

In the present study, we first evaluated the antioxidant activity and the toxicity of the extracts. The *in vitro* antidiabetic activity, and *in vivo* immunomodulatory activity were evaluated for *C. oblonga* extract. The obtained results showed the antioxidant capacity of our extracts and their safety up to 2000mg. In addition, *C. oblonga* extract showed antidiabetic activity as evidenced by its capacity to inhibit the α-glucosidase enzyme. Moreover, at the concentration of 50 and 100 mg/kg *C. oblonga* extracts significantly increased the clearance rate of carbon from the bloodstream concomitant with increased liberation of GSH from liver cells.

In the second part, we carried *in vivo* experiments to establish the neurobehavioural protective effect of our extracts using two models of chronic stress. For *P.dactylifera* experiment; an animal model of chronic administration (21 days) of high dose corticosterone was used to induce depression-like behaviour. Results showed that the treatment with *P. dactylifera* extract ameliorated the depression-like behaviour as observed in the reduced time of immobility in the forced swimming test (FST). For *C. oblonga* experiment, chronic immobilisation stress was used as a rat model of depression. We found that the treatment with *C. oblonga* ameliorated stress-induced depressive behaviour, and prevented the decrease of neurogenesis in the hippocampus. Moreover, the daily administration of *C. oblonga* extract improved the mood function in normal rats.

To our knowledge, this is the first study that demonstrated the antidepressant-like effect of *P. dactylifera* and *C. oblonga* fruits in a rat model of depression. This study also demonstrated for the first time the antidepressant effect of *C. oblonga* fruit by enhancing hippocampal neurogenesis in a rat model of depression.

Key words: Antioxidant activity, antidiabetic activity, chronic stress, *Cydonia oblonga*, depression, hippocampal neurogenesis, immunomodulatory activity, *Phoenix dactylifera*, toxicity.

Résumé

Les événements stressants de la vie provoquent l'apparition des altérations au niveau du cerveau et peuvent conduire à plusieurs maladies mentales comme la dépression qui est l'une des pathologies mentales prévalentes dans le monde.

L'objectif visé de cette thèse est de tester la capacité des extraits hydro-éthanoliques des fruits *Phoenix dactylifera* et *Cydonia oblonga* pour prévenir l'apparition de comportements dépressifs chez des rats après l'application du stress.

Dans la première partie, nous avons évalué l'activité antioxydante, ainsi que la toxicité des extraits. Les activités antidiabétique et immunomodulatrice, de l'extrait de *C.oblonga* ont été étudiées. Les résultats ont montré la capacité antioxydante des extraits avec l'absence d'effet toxique à une dose de 2000 mg/kg. L'extrait de *C. oblonga* a révélé un effet inhibiteur sur l'enzyme α-glucosidase, signifiant une activité antidiabétique. En outre, cet extrait a augmenté l'activité phagocytaire du système réticulo-endothélial à des doses de 50 et 100 mg/kg en stimulant la libération de GSH par les hépatocytes.

Dans la deuxième partie, nous avons étudié l'effet protecteur des extraits sur les changements neurocomportementaux en utilisant deux modèles de stress chronique.

Pour le *P. dactylifera*, un modèle animal de la dépression a été élaboré par l'administration chronique de la corticostérone à forte dose pendant 21 jours. Les résultats ont montré que l'extrait de *P. dactylifera* a amélioré le comportement dépressif induit par l'injection quotidienne de la corticostérone en diminuant la durée d'immobilité dans la nage forcée.

Pour le *C. oblonga*, un protocole de stress d'immobilisation chronique a été conçu afin d'induire un comportement dépressif chez les rats. Le traitement par l'extrait de *C. oblonga* (300mg/kg) élimine totalement les marqueurs physiologiques et comportementaux du stress chronique, et prévient l'altération de la neurogenèse dans l'hippocampe. De plus, l'administration quotidienne de l'extrait de cognassier a amélioré la fonction de l'humeur chez les rats normaux.

À notre connaissance, cette étude originale a montré l'effet antidépresseur de *P. dactylifera* et *C. oblonga* chez les rats modèles de la dépression et d'autre part, *C. oblonga* a révélé l'effet antidépresseur en améliorant la neurogenèse de l'hippocampe chez les rats après l'application du stress.

Mots clés. Activité antioxydante, activité antidiabétique, activité immunomodulatrice, dépression, *Cydonia oblonga, Phoenix dactylifera*, stress chronique, toxicité.

ملخص

ملخص

إن أحداث الحياة المجهدة تتسبب في احداث تغييرات على الدماغ قد تؤدي الى عدة امراض بدنية وعقلية، مثل الاكتئاب، الذي يعتبر أحد الأمراض العقلية الشائعة في مختلف أنحاء العالم. تهدف هذه الاطروحة الى تقييم إمكانية المستخلصات الايثانولية لفاكهتي التمر والسفرجل من منع ظهور السلوكيات الشبيهة بالاكتئاب في الجرذان التي تعاني من الاجهاد المزمن.

في الجزء الأول من هذه الدراسة، قمنا بتقييم النشاط المضاد للأكسدة باستخدام أربع طرق بالإضافة الى تقييم سمية المستخلصات، التمر (نوعية غرسِ) والسفرجل. كذلك تم تقييم النشاط المضاد للسكري، النشاط المناعي باستخدام طريقة معدل إزالة الكربون الغروي من الدم.

أظهرت النتائج التي تم الحصول عليها قدرة المستخلصات على مقاومة الأكسدة و غياب اثار السمية عند الجرعة 2000مغ/كغ, كما أظهر مستخلص فاكهة السفرجل نشاطاً مضاداً للسكري بقدرته على تثبيط إنزيم ألفا جلوكسيداز .بالاظافة الى ذلك، فإن هذا المستخلص عند تركيز 50 و100 مغ/كغ يزيد بشكل كبير من معدل إزالة الكربون من مجرى الدم بالتزامن مع زيادة تحرير الغلوتاثيون من خلايا الكبد.

في الجزء الثاني، قمنا بإجراء دراسات داخل خلوية لتحديد التأثير الوقائي لهذه المستخلصات على السلوك العصبي باستخدام نموذجين من الإجهاد المزمن.

بالنسبة لتجربة مستخلص التمر، تم حقن الجرذان بجرعة عالية من الكرتكوسترون لمدة 21 يوم لتطوير الاكتئاب كسلوك واستكشاف التغيرات البيولوجية التي يحدثها العلاج بمستخلص التمر. أظهرت النتائج أن العلاج بهذا المستخلص أدى إلى تخفيف سلوك الاكتئاب من خلال قدرته على تخفيض وقت ايقاف الحركة في اختبار السباحة الاجبارية .

في تجربة مستخلص السفرجل، استُخدَم بروتوكول عدم الحركة للجرذان لتطوير الاكتئاب. حيث بينت النتائج ان المعالجة بالمستخلص نجحت في قمع العلامات الفسيولوجية والسلوكية للإجهاد المزمن، والتشوهات البنيوية في قرن آمون. فضلاً عن ذلك فإن المعالجة بمستخلص السفرجل أدت الى تحسين المزاج في الجرذان العادية .

حسب علمنا، هذه أول دراسة بينت تأثير مستخلصات فاكهتي التمر والسفرجل كمضادات للاكتئاب عند الجرذان نموذج الاكتئاب. كما أظهرت هذه الدراسة للمرة الأولى ان النشاط المضاد للاكتئاب لفاكهة السفرجل يرجع على الأرجح لقدرته على تعزيز تكوين الخلايا العصبية في عصاب قرن آمون في الجرذ نموذج الاكتئاب.

الكلمات المفتاحية

النشاط المضاد للأكسدة، النشاط المضاد للسكري، الإجهاد المزمن، التمر، السفرجل، الاكتئاب، النشاط المناعي، السمية، قرن آمون .

Année universitaire : 2020/2021	Présentée par : SAKHRI Fatma Zahra

Intitulé

Effect of *Phoenix dactylifera* and *Cydonia oblonga* fruits on some diseases induced by stress

Thèse en vue de l'obtention du diplôme de doctorat en sciences

Résumé

Les événements stressants de la vie provoquent l'apparition des altérations au niveau du cerveau et peuvent conduire à plusieurs maladies mentales comme la dépression qui est l'une des pathologies mentales prévalentes dans le monde.

L'objectif visé de cette thèse est de tester la capacité des extraits hydro-éthanoliques des fruits *Phoenix dactylifera* et *Cydonia oblonga* pour prévenir l'apparition de comportements dépressifs chez des rats après l'application du stress.

Dans la première partie, nous avons évalué l'activité antioxydante, ainsi que la toxicité des extraits. Les activités antidiabétique et immunomodulatrice, de l'extrait de *C.oblonga* ont été étudiées. Les résultats ont montré la capacité antioxydante des extraits avec l'absence d'effet toxique à une dose de 2000 mg/kg. L'extrait de *C. oblonga* a révélé un effet inhibiteur sur l'enzyme α-glucosidase, signifiant une activité antidiabétique. En outre, cet extrait a augmenté l'activité phagocytaire du système réticulo-endothélial à des doses de 50 et 100 mg/kg en stimulant la libération de GSH par les hépatocytes.

Dans la deuxième partie, nous avons étudié l'effet protecteur des extraits sur les changements neurocomportementaux en utilisant deux modèles de stress chronique.

Pour le *P. dactylifera*, un modèle animal de la dépression a été élaboré par l'administration chronique de la corticostérone à forte dose pendant 21 jours. Les résultats ont montré que l'extrait de *P. dactylifera* a amélioré le comportement dépressif induit par l'injection quotidienne de la corticostérone en diminuant la durée *d'immobilité dans la* nage forcée.

Pour le *C. oblonga*, un protocole de stress d'immobilisation chronique a été conçu afin d'induire un comportement dépressif chez les rats. Le traitement par l'extrait de *C. oblonga* (300mg/kg) élimine totalement les marqueurs physiologiques et comportementaux du stress chronique, et prévient l'altération de la neurogenèse dans l'hippocampe. De plus, l'administration quotidienne de l'extrait de cognassier a amélioré la fonction de l'humeur chez les rats normaux.

À notre connaissance, cette étude originale a montré l'effet antidépresseur de *P. dactylifera* et *C. oblonga* chez les rats modèles de la dépression et d'autre part, *C. oblonga* a révélé l'effet antidépresseur en améliorant la neurogenèse de l'hippocampe chez les rats après l'application du stress.

.

Mots clés : Activité antioxydante, Activité antidiabétique, Activité immunomodulatrice, *Cydonia oblonga*, Dépression, Neurogenèse de l'hippocampe, *Phoenix dactylifera*, Stress chronique, Toxicité.