JBAAR

Journal of Bioscience and Applied Research
https://jbaar.journals.ekb.eg

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Unveiling the Multifaceted Effects of Khat (*Catha edulis* Forsk.): Insights into Reproductive Health, Cardiovascular Dynamics, and Cognitive Function

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DOI:10.21608/jbaar.2024.392870

Abstract:

This study aims to analyze the influence of the water-based extract from Khat leaves, a plant frequently chewed in the Arabian Peninsula and the Horn of Africa, on the health of various vital organs. Thirty (30) albino female rats were randomized into two groups: a control group and a treatment group. The treatment group provided 1 mL of a 700 mg/kg aqueous Khat extract daily for four weeks. Khat-treated rats revealed greater malondialdehyde (MDA) levels, indicating increased oxidative stress. At the same time, their antioxidant enzymes—glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT)—declined dramatically. This shows that Khat affects the body's balance between oxidative stress and antioxidant defenses. This suggests an imbalance in the oxidative state, accompanied by heightened oxidative damage. Cytokine profiling indicated a considerable decline in the anti-inflammatory cytokine interleukin-4 (IL-4), alongside a rise in the proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta 1 (TGF-β1). The findings suggested that cardiac enzymes such as troponin, creatine kinase (CK), creatine phosphokinase (CPK), and aspartate aminotransferase (AST) were considerably elevated in the Khat treatment cohort. The hormonal testing demonstrated a considerable spike in follicle-stimulating hormone (FSH), luteinizing hormone (LH), and serum testosterone, accompanying a marked fall in estrogen levels. Histological investigations indicated significant abnormalities in the tissue architecture of the heart, brain, and ovaries in the Khat-treated patients. The findings suggest that protracted intake of Khat as a masticatory material may adversely influence the body's essential organs.

Keywords: Khat, *Catha edulis*, infertility, cardiovascular disease, brain, antioxidants, cytokines

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1. Introduction

Catha edulis, commonly called Khat, is a flowering plant from East Africa and the Arabian Peninsula. The leaves and young shoots of this plant have been used as a recreational substance by the indigenous populations of the Middle East, East Africa, and the Arabian Peninsula since the 13th century [1, 2]. The consumption of Khat leaves induces a state of euphoria and stimulation, which is analogous to, but less potent than, the effects elicited by the misuse of substances like cocaine or methamphetamine [3, 4]. The *Catha edulis* plant includes a complex chemical makeup, with its leaves and young shoots including 40 different phytochemical elements, encompassing a wide variety of alkaloids, glycosides, tannins, amino acids, vitamins, and minerals. This intricate chemical composition underscores the multifaceted

Received: August 1, 2024. Accepted: September 11, 2024. Published: November 20, 2024

nature of the Khat plant and the potential biological implications of its consumption, a topic that continues to intrigue researchers [5]**.** Cathinone and cathine, two psychoactive compounds found in the leaves and young shoots of the *Catha edulis* plant, stimulate the central nervous system (CNS) [6,7]**.** The observed effects associated with Khat consumption are believed to be primarily driven by the plant's ability to influence the levels of essential neurotransmitters in the brain. The complex phytochemicals in Khat, including various alkaloids, are thought to enhance the release of dopamine, norepinephrine, and serotonin while inhibiting their reuptake. This modulation of neurotransmitter systems is considered a key mechanism underlying the stimulant and psychoactive properties associated with Khat use. The prolonged use of Khat and its active compounds has been linked to various adverse effects on the CNS, including anxiety, restlessness, insomnia, and potential addiction. The effects of the Khat compounds cathinone and cathine on the central nervous system can vary significantly, depending on dosage, individual susceptibility, and other contextual variables [8]**.** Using Khat has been related to several detrimental consequences, including cardiovascular illnesses, liver damage, reproductive health difficulties, cognitive impairment, addiction, psychosis, and other mental health disorders. Chronic misuse of Khat may produce physical weariness [9]. While Khat is primarily used for its stimulant properties, there is growing interest in understanding its cytotoxic effects, both in vivo and in vitro [10]. Numerous investigations have been conducted to learn how Khat affects various physiological and immunological processes [11,12]. In vitro studies examine the cytotoxic effects of khat extracts on isolated cells or cell lines cultured in a laboratory. These studies provide insights into the direct impact of Khat on cellular processes and mechanisms. A study by Abou-Elhamd et al. examined the effects of *Catha edulis* extract on the KOV3 human ovarian adenocarcinoma cell line. The study uncovered various cellular abnormalities, including reduced cell size, compromised cell membrane integrity, and the induction of apoptosis [13]. These findings indicate that Khat may exert detrimental effects on ovarian cancer cells, potentially altering their size, structure, and overall viability. El-Setouhy and Hasasn [14] focused on the genotoxicity effects of Khat in animal studies, revealing its potential to cause DNA damage in various tissues, including bone marrow and buccal mucosa. The findings suggest a potential link between Khat and cancer, but the direct correlation in humans remains unproven. However, certain studies have indicated a statistical association between Khat use and an increased risk of cancer, highlighting the need for further investigation into the potential carcinogenic effects of Khat. The research by Al-Qadhi et al. revealed that Khat exposure can lead to concerning cellular changes, such as reduced cell viability, increased apoptosis, heightened ROS production, altered cell characteristics, and disrupted cell cycle progression. These findings suggest Khat may have significant detrimental impacts on cellular function and health, warranting further investigation into the broader implications of Khat consumption. Within this context, cells exposed to Khat exhibited increased concentrations of the pro-apoptotic protein Bax and reduced levels of the anti-apoptotic protein Bcl-2 [15]. Additionally, there was an upregulation of proteins such as p38, p53, p16, and p21 and premature expression of differentiation markers. Others showed that Chewing Khat has various adverse effects, such as increased alertness, anxiety, stress, and depression [16]. Khat can lead to detrimental physical effects such as oral lesions, gastric cancers, duodenal ulcers, liver toxicity, hypertension, cardiovascular issues, and stroke. Furthermore, extended and excessive khat use can result in psychological dependence, depression, and potentially even psychotic disorders [17]. As per the World Health Organization, Khat acts as a stimulant similar to amphetamine, inducing feelings of euphoria and a loss of appetite [17]. Using Khat for

a long time can lead to severe problems like cardiovascular disease, gastrointestinal disorders, and dental decay [18]. Several studies have examined the impact of a water-based extract from *Catha edulis*, also known as Khat, on rat ovaries [19]. Our study focused on gaining a deeper understanding of the impact of Khat chewing on vital organs, specifically the heart, ovary, and brain. By investigating these organs, we aimed to provide additional insights and clarify previous research findings, advancing a more thorough comprehension of the consequences of khat usage.

2. Materials and Methods

2.1. Animals and Experimental Design

Thirty female Wistar rats, each weighing between 190 and 210 grams, were brought from the Medical Research Center, Jazan University, Jazan, Saudi Arabia. The rats were maintained in a controlled environment with a 12-hour light/dark cycle, a temperature of 20 ± 4 °C, and a relative humidity of 60% ± 10%. They had access to standard rodent food and water. After a week of acclimatization, the rats were randomly divided into two groups of 15: a control group and a treatment group. The treatment group received 1 mL of Khat extract (700 mg/kg body weight) daily via gastric cannula for four weeks. In contrast, the control group was given 1 mL of saline solution daily for the same period. Upon completion of the study, the rats were fasted overnight and then humanely euthanized using diethyl ether. Blood samples were collected in gel separator vacutainers containing clot activators. These samples were allowed to clot at room temperature for 30 to 60 minutes before being centrifuged at 794 g for 20 minutes. The serum obtained was stored at -40°C. Additionally, tissue samples from various organs were collected and preserved in neutral formalin for subsequent histological analysis.

2.2. Preparation method of Khat extract

The source of the Khat extract utilized in this study was the Health Research Center, Jazan University, and prepared according to a method adapted from Mwenda et al. [3]. The process began with separating khat leaves from the shoots, which were then thoroughly washed with distilled water, diced on metal plates, and pulverized using a blender. The pulverized material was immersed in distilled water and placed in a rotary shaker for several hours. It underwent two stages of filtration: first through gauze rolls to remove larger particles, and then through an 11 mm Grade 1 Whatman filter. Fresh distilled water was used to remove any plant material that was still present but did not make it past the filter. To create a dry powder-like substance, the filtrate was gathered in a beaker and set on a magnetic stirrer to evaporate overnight at 45°C. This powder was stored at 4°C until further use, and fresh khat extract was prepared by dissolving the powdered extract in distilled water immediately before daily oral administration to the rats. This standardized preparation method ensured consistent dosing of the khat extract throughout the study.

2.3. Evaluation of Antioxidant Activity

- MDA: Malondialdehyde levels in rat serum were quantified using the Rat Malondialdehyde ELISA Kit (Catalog No. MBS268427, MyBioSource).
- GSH: Glutathione concentrations were measured with the Rat Glutathione ELISA Kit (Catalog No. MBS265966, MyBioSource).
- SOD: Superoxide Dismutase activity was assessed using the Rat Superoxide Dismutase ELISA Kit (Catalog No. MBS036924, MyBioSource).
- CAT: Catalase levels were determined with the Rat Catalase ELISA Kit (Catalog No. abx155309, Abbexa).

2.4. Cytokines Assay

- IL-4: Interleukin 4 levels were measured using the Rat Interleukin 4 ELISA Kit (Catalog No. abx050121, Abbexa).
- TNF-α: Tumor Necrosis Factor-alpha was quantified with the Rat TNF-α ELISA Kit (Catalog No. abx585237, Abbexa).

- TGF-β1: TGF beta-1 levels were assessed using the Rat TGF-β1 ELISA Kit (Catalog No. BMS623-3, Invitrogen).

2.5. Cardiac Enzymes

- CK MB: Rat CKMB (Creatine Kinase MB Isoenzyme) was measured using an ELISA Kit from ELabscience (Catalog No. E-EL-R1327).
- Troponin: Rat cardiac Troponin I was quantified with the ELabscience Rat Troponin I ELISA Kit (Catalog No. E-EL-R1253).
- CPK: Creatine phosphokinase levels were determined using the MyBioSource Rat Creatine phosphokinase ELISA Kit (Catalog No. MBS744369).
- AST: Aspartate aminotransferase (AST) was measured with the MyBioSource Rat AST ELISA Kit (Catalog No. MBS264975).

2.6. Hormonal Assay

- LH: Luteinizing Hormone levels were assessed using the MyBioSource Rat Luteinizing Hormone ELISA Kit (Catalog No. MBS764675).
- FSH: Follicle stimulating hormone was quantified with the MyBioSource Rat Follicle stimulating hormone ELISA Kit (Catalog No. MBS2502190).
- Estrogen: Rat estrogen hormone levels were measured using the MyBioSource Rat Estrogen ELISA Kit (Catalog No. MBS2607338).
- Testosterone: Testosterone concentrations were determined with the MyBioSource Rat Testosterone ELISA Kit (Catalog No. MBS282195).

2.7. Histological investigation:

To examine the tissues, samples from the heart, brain, and ovary were collected from the different experimental groups. These tissue samples were fixed in a 10% formalin solution for preservation. The fixed tissues were then embedded in paraffin wax to maintain their structural integrity. Thin sections were cut from the paraffin-embedded tissues using a rotary microtome. The tissue sections were then stained with hematoxylin and eosin, a common histological staining technique, to enable visualization under a light compound microscope.

2.8. Statistical analysis:

SPSS version 19 was employed to perform the statistical analysis for this study. The data was organized and presented as mean values accompanied by their respective standard deviations (S.D.), offering a transparent overview. To detect significant differences among the groups, we utilized the Student's t-test. We maintained a rigorous threshold of $p < 0.05$ to ensure the statistical significance and reliability of our findings.

3. Results

3.1. Antioxidant Criteria

The study demonstrated substantial changes in oxidative stress markers between the treatment and control groups. Serum MDA (malondialdehyde) levels in the treatment group rose considerably by 87.03% relative to the control group, signifying greater oxidative damage. Conversely, serum SOD (superoxide dismutase) levels decreased significantly by 51.96%, while serum CAT (catalase) levels were also markedly lower by 34.95% in the treatment group. Additionally, serum GSH (glutathione) levels showed a significant reduction of 45.37%. These results imply that the therapy group had increased oxidative stress, elevated lipid peroxidation, and a compromised antioxidant defense system.

3.2. Immunological Criteria

Table 2 and Figure 2 provide a visual representation of the data, which indicates a notable decrease of approximately 31.71% in serum IL-4 levels within the treated group when compared to the control group. In contrast, serum TNF-α levels increased by 43.15%, and serum TGF-β1 levels rose by 48.97% in the treatment group relative to the control group.

3.3. Cardiac Enzymes

Table 3 and Figure 3 show significant increases in several markers in the treated group compared to the control group: serum Troponin levels increased by 290.16%, creatine kinase (CK-MB) levels rose by 184.16%, creatine phosphokinase (CPK) levels increased by 67.91%, and aspartate aminotransferase (AST) levels rose by 182.16%.

3.4. Hormonal assay

Table 4 and Figure 4 highlight the substantial variations in hormone levels observed in the treated group, which were notably different from those in the control group: serum Luteinizing Hormone (LH) increased by 173.95%, Follicle-Stimulating Hormone (FSH) rose by 54.95%, and Testosterone levels surged by 722.45%. In contrast, serum Estrogen levels decreased by 23.49% in the treated group.

3.5. Histopathological Criteria

Figures 5a, b, and c show a control heart with a normal pericardium, healthy cardiac muscle fibers, and intermediate blood vessels. In contrast, (Figures 5d, e, and f) depict the treated group with thickened pericardium, and cellular degeneration in the heart muscle, characterized by the presence of apoptotic fibers with condensed nuclei, along with mild congestion in the blood capillaries. Figures (6a, b, and c) of control brain sections reveal average meninges, cerebral cortex, intracranial blood vessels, neurons, and glial cells. However, (Figures 6d, e, and f) of treated brain sections display thickened meninges were observed, accompanied by an inflammatory infiltrate, astrogliosis, degenerated neurons, and abscess formation. Control hippocampus sections in (Figures 7a, b, c, d, e, and f) exhibit typical Cornu Amonis regions and standard blood vessels, whereas treated hippocampus sections in (Figures 7g, h, i, j, k, and l) show edema, degenerated pyramidal neurons, and dilated congested blood vessels. Control ovary sections in Figures 8a, b, and c showcase various standard follicle types and healthy cells, while treated ovary sections in (Figures 8d, e, f, and g) display a combination of cystic follicular dilation, atrophic linings, multiple corpora lutea in an edematous stroma, apoptotic stromal cells, and dilated, congested blood vessels.

Table (1): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some antioxidant enzymes for the treated group compared to the control.

Parameters/ % of change	Control	Treatment	Sig (2-tailed)	t value
MDA nm/mL	22.52 ± 1.81	42.12 ± 4.35	0.000	-10.186
% of change		**87.03**		
GSH ug/mL	10.25 ± 0.86	5.60 ± 0.61	0.000	10.824
% of change		**-45.37**		
SOD U/mL	16.57 ± 1.26	7.96 ± 1	0.000	13.105
% of change		**-51.96**		
CAT ng/mL	102.03 ± 5.3	66.37 ± 4.85	0.000	12.163
% of change		**-34.95**		

Table (2): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some cytokines for the treated group compared to the control.

Parameters/ % of change	Control	Treatment	Sig (2-tailed)	t value
IL-4 pg/mL	165.24 ± 5.78	112.84 ± 7.61	0.000	13.439
% of change		**-31.71**		
TNF α pg/mL	222.25 ± 4.43	318.16 ± 25.27	0.000	-9.159
% of change		**43.15**		
TGF β1 pg/mL	192.7 ± 10.27	287.06 ± 20.3	0.000	-10.159
% of change		**48.97**		

Table (3): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some cardiac enzymes for the treated group compared to the control.

Parameters/ % of change	Control	Treatment	Sig (2-tailed)	t value
Troponin pg/mL	0.61 ± 0.11	2.38 ± 0.76	0.000	-5.666
% of change		**290.16**		
CK-MB pg/mL	142.21 ± 4.87	404.1 ± 27.84	0.000	-22.697
% of change		**184.16**		
CPK pg/mL	215.06 ± 16.12	361.11 ± 39.47	0.000	-8.390
% of change		**67.91**		
(AST) U/L	101.81 ± 5.25	287.27 ± 26.17	0.000	-17.023
% of change		**182.16**		

Table (4): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some sexual hormones for the treated group compared to the control.

Parameters/ % of change	Control	Treatment	Sig (2-tailed)	t value
LH mIU/mL	2.15 ± 0.29	5.89 ± 1.08	0.000	-8.162
% of change		**173.95**		
FSH ng/mL	2.73 ± 0.29	4.23 ± 0.87	0.002	-4.011
% of change		**54.95**		
Estrogen pg/mL	63.55 ± 3.21	48.62 ± 4.35	0.000	6.762
% of change		**-23.49**		
Testosterone ng/mL	0.49 ± 0.03	4.03 ± 0.68	0.000	-12.674
% of change		**722.45**		

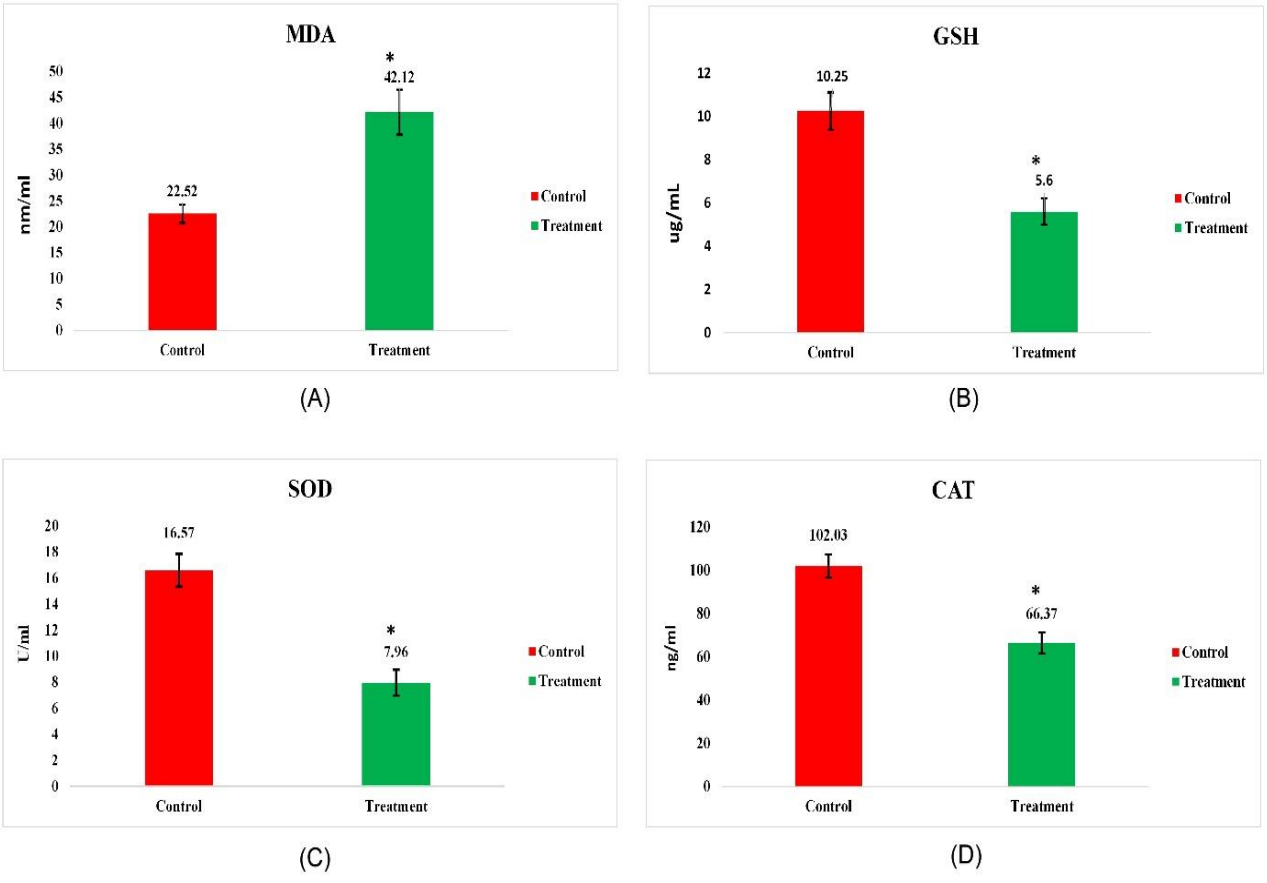

Figure (1): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some antioxidant enzymes for the treated group compared to the control.

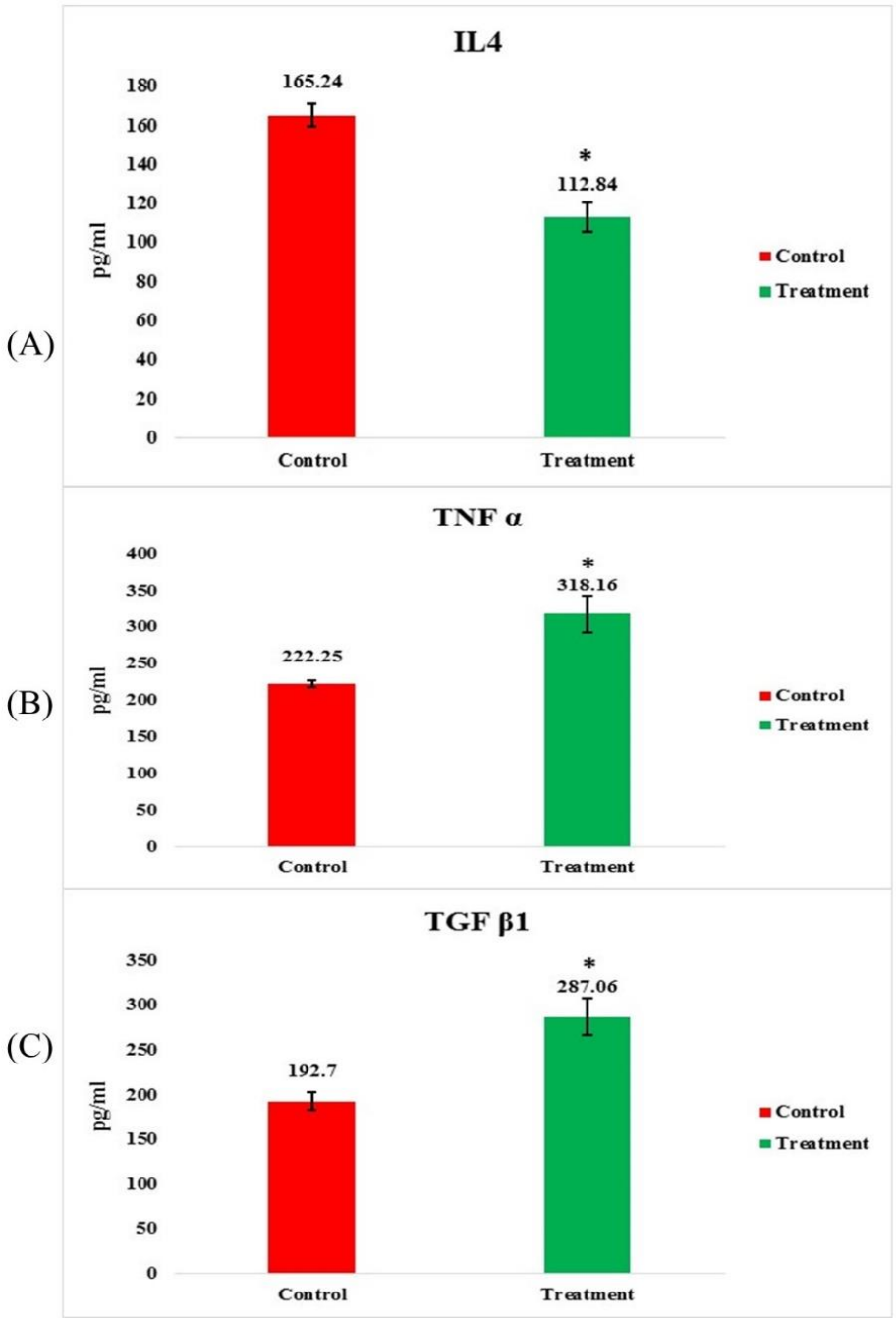

Figure (2): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some cytokines for the treated group compared to the control.

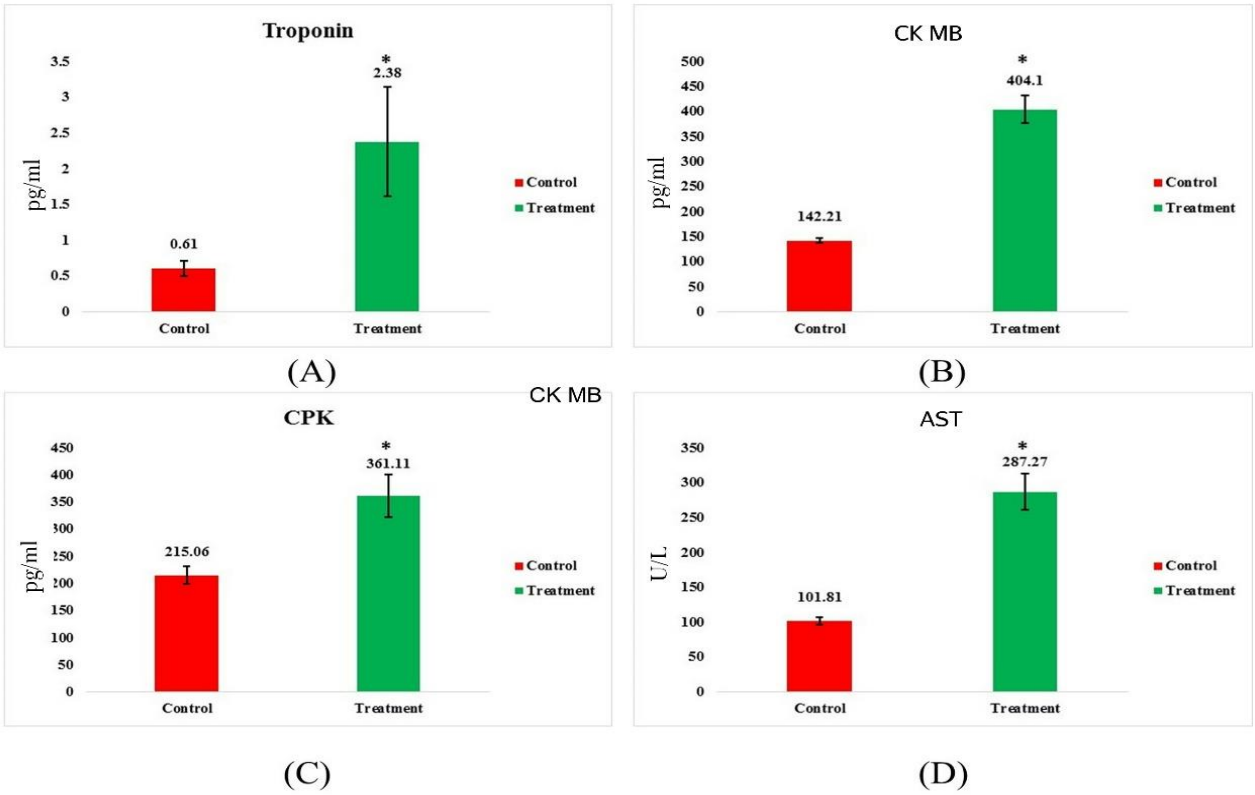

Figure (3): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some cardiac enzymes for the treated group compared to the control.

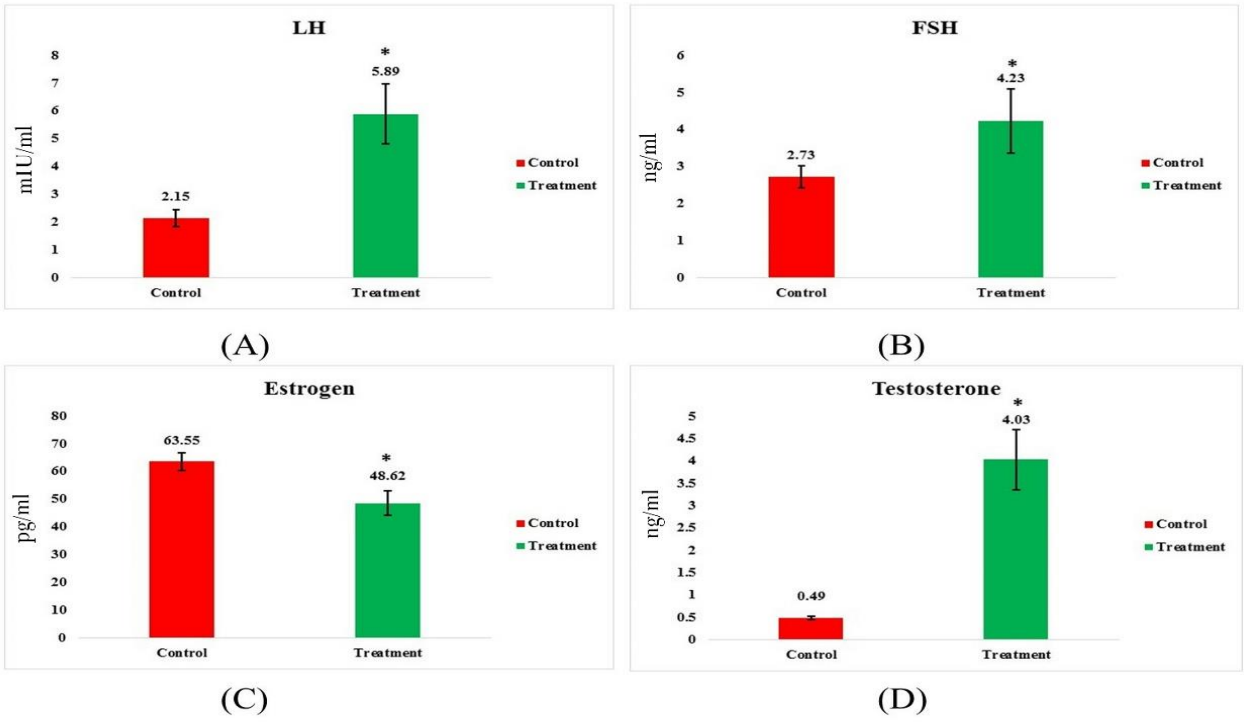

Figure (4): Effect of khat (*Catha edulis* Forsk.) aqueous leaves extract on some sexual hormones for the treated group compared to the control.

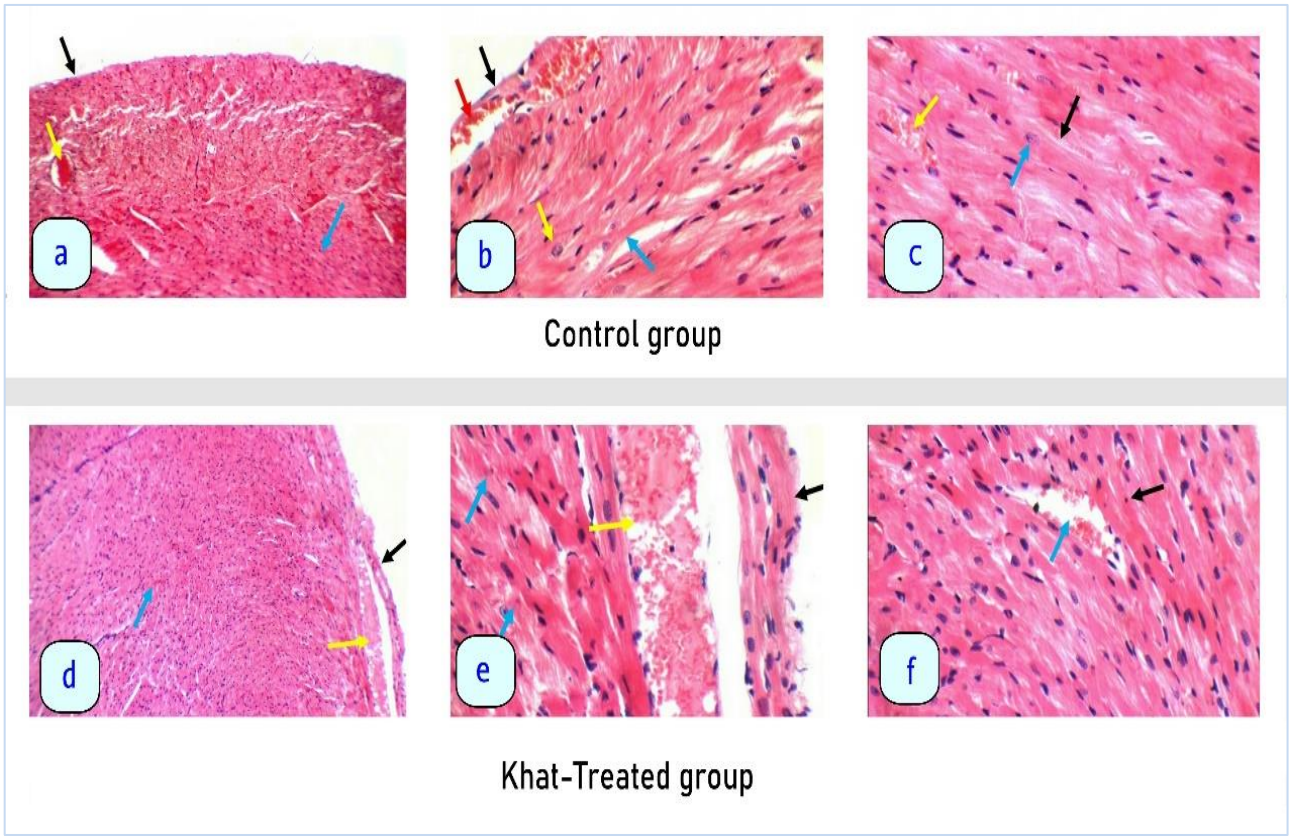

Figure (5): Photomicrographs describe sections in the hearts of two different rat groups., (a) low power photomicrographs (HX&E, 200X) control rat group illustrating, average blood vessels (yellow arrow), a cardiac wall appears intact with a visible pericardium (black arrow), and viable cardiac muscle fibers (blue arrow), (b) high power photomicrographs (HX&E, 400X) showing average blood vessels are visible (red arrow), intact pericardium (black arrow), cardiac muscle fibers with distinct cell borders and central oval/elongated nuclei (blue arrow), (c) high power photomicrograph (HX&E, 400X) showing average intervening blood capillaries (yellow arrow), central oval/elongated nuclei (blue arrow) and viable cardiac muscle fibers with distinct cell borders (black arrow), (d) low power photomicrograph (HX&E, 200X) treated rat group showing mildly congested blood vessels (yellow arrow), scattered apoptotic cardiac muscle fibers (blue arrow), the cardiac wall shows an intact thick pericardium (black arrow), (e) high power photomicrograph (HX&E, 400X) showing mildly congested blood vessels (yellow arrow), intact thick pericardium is visible (black arrow), scattered cardiac muscle fibers with pyknotic nuclei (blue arrow), (f) high power photomicrograph (HX&E, 400X) showing mildly congested intervening blood capillaries (blue arrow), scattered cardiac muscle fibers with pyknotic nuclei (black arrow).

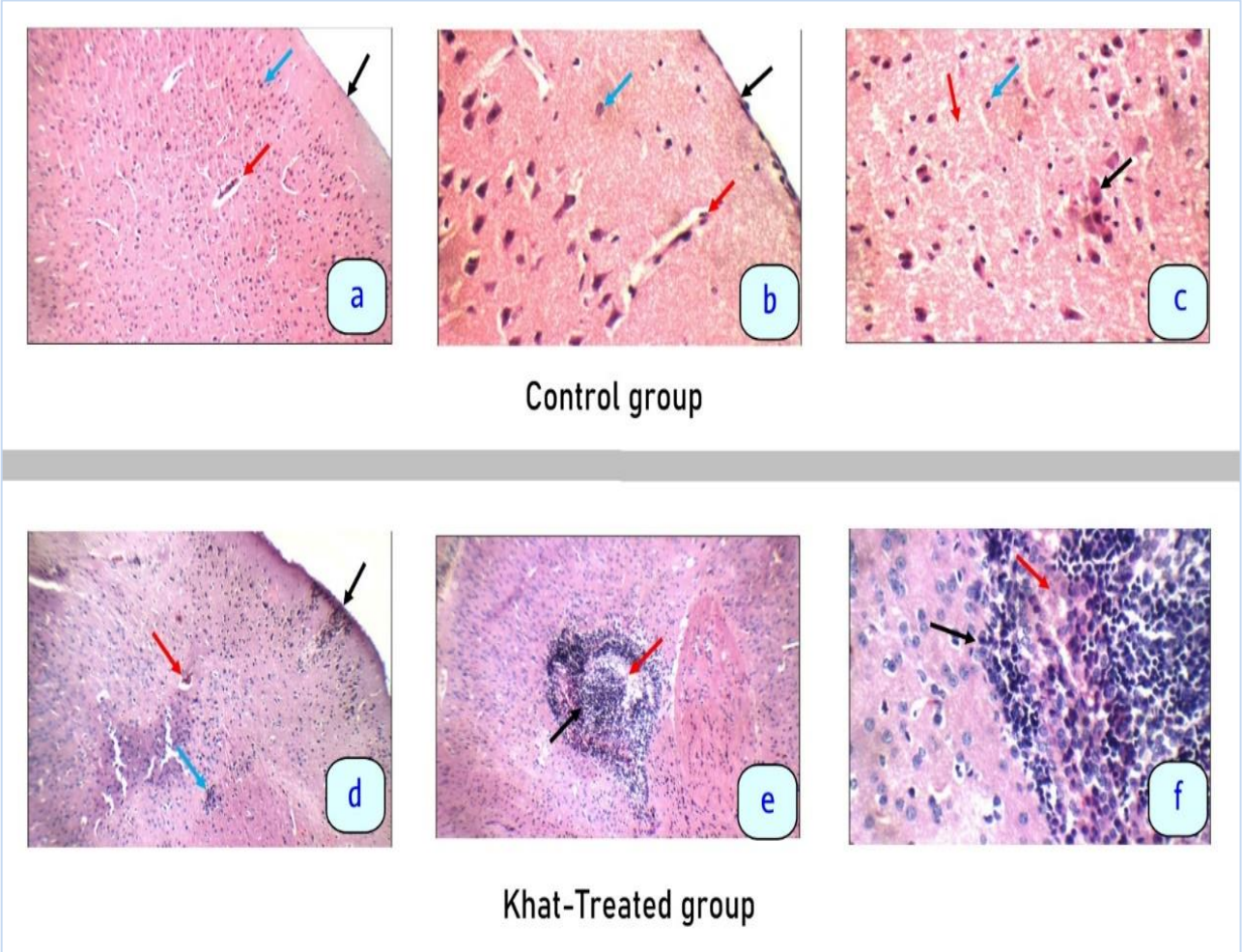

Figure (6): Photomicrographs describe sections in the brain of two different rat groups. (a) low power photomicrographs (HX&E, 200X) control rat group showing average blood vessels (red arrow), cerebral cortex with average neurons (blue arrow and average meninges (black arrow), (b) high power photomicrograph (HX&E, 400X) showing average blood vessels (red arrow), cerebral cortex displays average neurons (blue arrow), average meninges are visible (black arrow), (c) high power photomicrograph (HX&E, 400X) showing a fibrillary background (red arrow), average glial cells (blue arrow), white matter reveals average neurons (black arrow), (d) low power photomicrographs (HX&E, 200X) treated rat group showing, mildly congested blood vessels (red arrow), cerebral cortex shows astrogliosis (blue arrow), the brain exhibits thick meninges with a mild inflammatory infiltrate (black arrow), Another view at 200X magnification (e) low power photomicrographs (HX&E, 200X) showing deep cortex displays marked inflammatory infiltrate (black arrow) with the formation of an abscess (red arrow), (f) high power photomicrograph (HX&E, 400X) showing deep cortex reveals marked astrogliosis (black arrow) with abscess formation (red arrow).

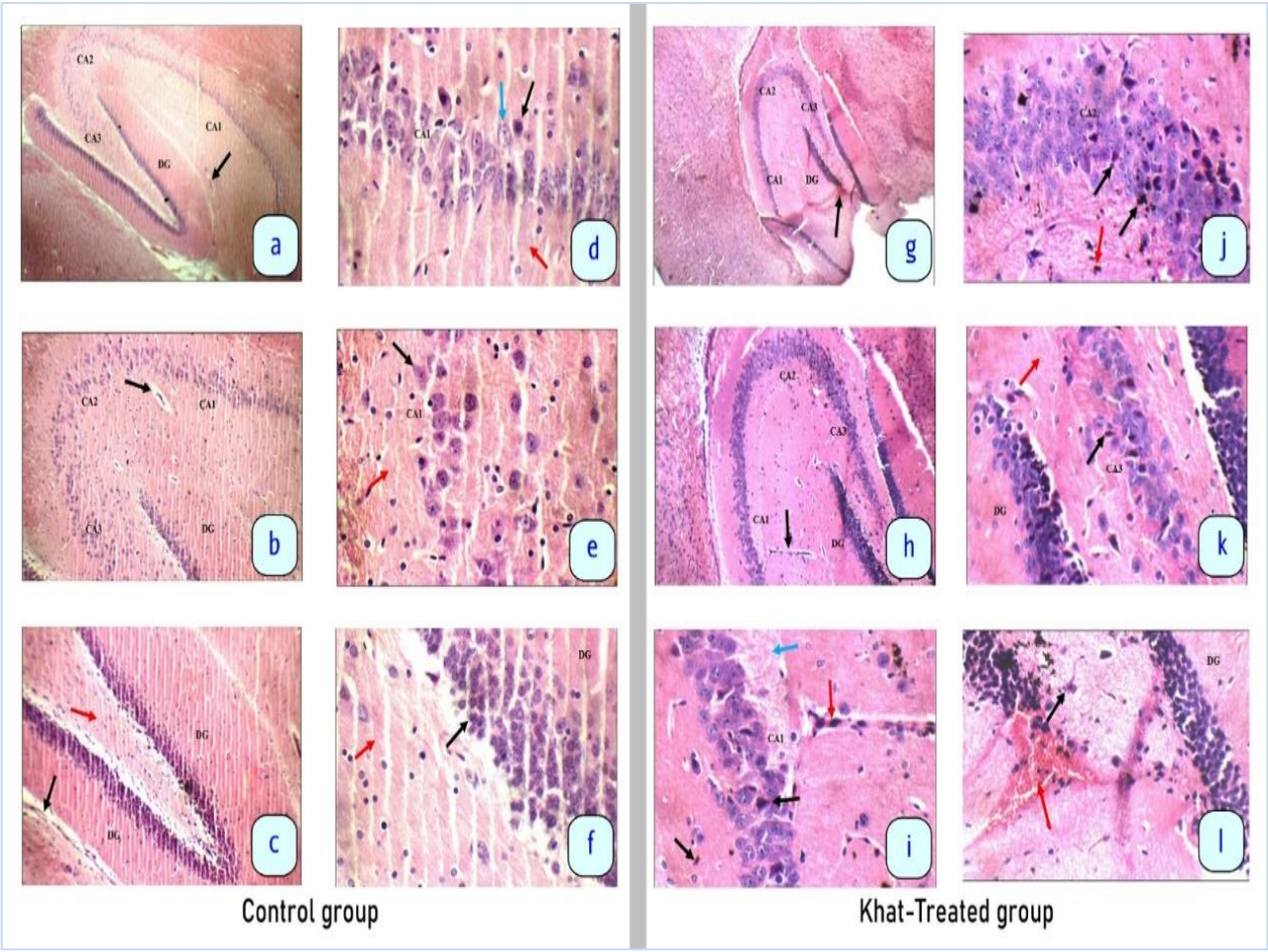

Figure (7): Photomicrographs describe sections in the hippocampus of two different rat groups. (a) low power photomicrographs (HX&E, 100X) control rat group showing, Cornu Amonis regions (CA1, CA2, CA3), the CA1describes average blood vessels (black arrow), average dentate gyrus (DG), (b) low power photomicrographs (HX&E, 200X) showing the average Cornu Amonis regions (CA1, CA2, CA3), average blood vessels (black arrow) in CA1, average dentate gyrus (DG), (c) low power photomicrographs (HX&E, 200X) displays average blood vessels (black arrow), average inter-neuron area (red arrow), average dentate gyrus (DG), (d) high power photomicrograph (HX&E, 400X) displays Cornu Amonis region (CA1) with average pyramidal neurons (black arrow), average inter-neuron area (red arrow), average granule cells (blue arrow), Additionally, (f) another view in the dentate gyrus (DG) shows average inter-neuron area (red arrow), average pyramidal neurons (black arrow), (g) low power photomicrographs (HX&E, 100X) treated rat group showing hippocampus with average Cornu Amonis regions (CA1, CA2, CA3), average dentate gyrus (DG) with mildly dilated congested blood vessels (black arrow), (h) low power photomicrographs (HX&E, 100X) showing average Cornu Amonis regions (CA1, CA2, CA3), the average dentate gyrus (DG) with average blood vessels (black arrow), (i) high power photomicrograph (HX&E, 400X) showing Cornu Amonis region (CA1) with scattered degenerated pyramidal neurons (black arrow), mild edema (blue arrow), and average blood vessels (red arrow). Furthermore, (j) another view high power photomicrograph (HX&E, 400X) in the Cornu Amonis region (CA2) displaying markedly degenerated pyramidal neurons (black arrow) and markedly degenerated neurons in the inter-neuron area (red arrow), (k) high power photomicrograph (HX&E, 400X) in the Cornu Amonis region (CA3) showing scattered degenerated pyramidal neurons (black arrow), an average inter-neuron area (red arrow), and the average dentate gyrus (DG). Lastly, (l) high power photomicrograph (HX&E, 400X) in the dentate gyrus (DG) reveals scattered degenerated pyramidal neurons in the inter-neuron area (black arrow) and mildly dilated congested blood vessels (red arrow).

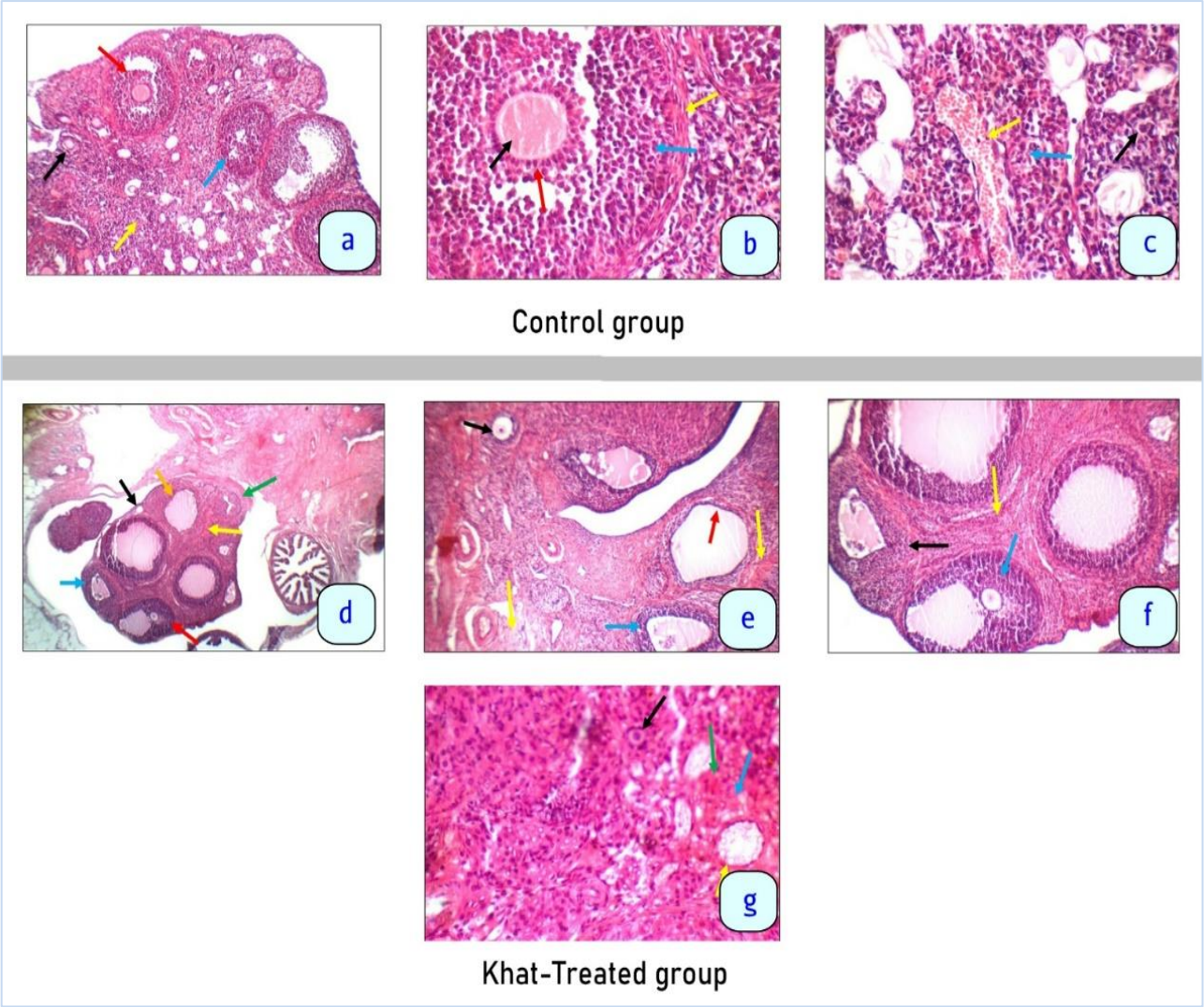

Figure (8): Photomicrographs describe sections in the ovary of two different rat groups. (a) low power photomicrographs (HX&E, 200X) control rat group showing an average stroma with average blood vessels (yellow arrow), primary follicles (black arrow), secondary follicles (blue arrow), and antral follicles (red arrow), (b) high power photomicrograph (HX&E, 400X) showing an antral follicle with a cumulus oophorus containing an average oocyte (black arrow), moderate corona radiata (red arrow), moderate granulosa cells (blue arrow), mild theca cells (yellow arrow), (c) high power photomicrograph (HX&E, 400X) displays primordial follicles (black arrow) in an average stroma (blue arrow) with average blood vessels (yellow arrow), (d) low power photomicrographs (HX&E, 100X) treated rat group showing the ovary with primary follicles (black arrow), secondary follicles (blue arrow), antral follicles (red arrow), scattered follicles with atrophied lining (orange arrow), and multiple corpora lutea (green arrow) in stroma showing markedly dilated congested blood vessels (yellow arrow), (e) low power photomicrographs (HX&E, 200X) treated rat group showing primary follicles (black arrow) and secondary follicles (blue arrow) along with scattered follicles with atrophied lining (red arrow), in markedly edematous stroma (yellow arrow). Additionally, (f) a view at 200X magnification displays secondary follicles (black arrow) and antral follicles (blue arrow) in markedly cellular stroma (yellow arrow), (g) high power photomicrograph (HX&E, 400X) showing primordial follicles (black arrow) in markedly edematous stroma (blue arrow) with scattered apoptotic stromal cells (green arrow) and mildly dilated blood vessels (yellow arrow).

4. Discussion

This study intends to explore the impact of (*Catha edulis*) extract on female albino rats. The data reveals a considerable decline in the activity of the SOD enzyme. This decrease shows that the enzyme plays a critical role in countering the excessive generation of free radicals and boosting the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen (O_2), thereby exerting an antioxidant effect [4]. Two previous studies have drawn attention to the contrasting levels of SOD activity in the follicular fluid of individuals with polycystic ovarian syndrome. Specifically, these studies found that SOD activity was significantly diminished in the follicular fluid of infertile patients compared to those with confirmed fertility [5, 6]. Wang et el. [7], found that women who have mutations or impairments in genes responsible for antioxidants or genes involved in antioxidant production may experience adverse impacts on their reproductive health. Furthermore, prior research conducted on mice with deleted antioxidant genes has demonstrated that adult female mice, when subjected to a reduction in the number of pre-ovulatory follicles and corpora lutea, experienced a subsequent decline in fertility. These findings show that the dysfunction of late follicular development or ovulation contributes to the infertility reported in these mice [9]. The study revealed a notable increase in Malondialdehyde (MDA) levels in the treated rats, indicating a significant difference when compared to the control group. MDA is a byproduct created during the peroxidation process of polyunsaturated fatty acids within cells. The observed surge in MDA levels suggests a potential disruption in the delicate balance between reactive oxygen species production and the body's antioxidant defense system, possibly due to an overproduction of free radicals. Moreover, in an organism, free radicals trigger the lipid peroxidation process. The level of malondialdehyde is widely utilized in cancer patients as a valuable indicator of oxidative stress and antioxidant status and reflects the degree of cell injury [11]. MDA levels in the ovaries increase in epithelial ovarian cancer [12]. Also, malondialdehyde (MDA) increases dramatically in PCOS patients compared with the standard [16]. The histological study of the ovary demonstrated that the administration of Khat (*Catha edulis* Forsek.) extract had adverse effects. The detected modifications included the presence of primary, secondary, and antral follicles, as well as dispersed cystically dilated follicles with atrophied linings. Additionally, numerous corpora lutea were identified in the highly edematous stroma, coupled with dispersed apoptotic stromal cells and greatly dilated congested blood vessels.

In the context of antioxidants, scavenging antioxidants such as glutathione play a significant role. They operate as second-line defensive antioxidants by scavenging or neutralizing free radicals, thereby preventing the onset and propagation of oxidative chain reactions. Antioxidants play a crucial role in preserving the delicate equilibrium between free radicals and the body's antioxidant defense mechanisms, thereby contributing to overall health and well-being [4,20]. GSH is vital to the intracellular defense of homeostasis and redox equilibrium [17]. GSH depletion below an actual threshold is considered a sign of oxidative stress (OS), which underpins the pathophysiology of several age-related degenerative illnesses, including inflammatory and tumor processes [17]. The findings of the present investigation indicated a considerable decline in the levels of glutathione (GSH) in the treatment group when compared to the control group. GSH is a key antioxidant molecule involved in the fight against oxidative damage. The decrease in GSH levels suggests a potential disruption in the antioxidant capacity of the treated group, which may contribute to increased oxidative damage or an imbalance in the redox status.

Glutathione levels fluctuate dramatically throughout meiosis and the early embryonic development of oocytes. A high fertilization level is

required to form male pronuclei and embryonic development to the blastocyst stage [9]. The present results are congruent with the findings of Devine et al. [9], who reported that oxidative stress had significant adverse effects on female fertility and gamete health in a previous study. Investigations have shown that multiple reproductive processes are substantially regulated by reactive oxygen species (ROS) and antioxidant enzyme systems [18]. The reproductive cycle involves several major processes: ovarian follicular development, luteal steroidogenesis, endometrial preparation and shedding, embryonic growth, and implantation. The balance between ROS and antioxidants is critical for preserving reproductive health and successful reproduction. On the other hand, extended treatment of Khat extract in mice yielded distinct effects. Specifically, it led to total and partial infertility in males, post-implantation loss in females, and reduced conception rates. This suggests that the extended use of Khat extract may interfere with reproductive processes in mice, leading to fertility difficulties and lower pregnancy success [19]. The considerable increase in GSH levels in the brain and spleen of khat-addicted mice suggests a heightened oxidative burden in these organs. This spike in GSH is assumed to be a reaction to the oxidative damage caused by khat, as the body strives to offset the challenge given by the chemical [21]. This study observed that the administration of *Catha edulis* (Khat) to rats caused the CAT's activity to decline. Molecules. This shows that the extract created free radicals or directly hindered the synthesis of antioxidant enzymes. These findings confirm the research conducted by Al-Hashem et el. [22], where it was established that the administration of *Catha edulis* extract or its alkaloid component produced alterations in the activity of enzymes involved in metabolizing and scavenging free radicals. This investigation revealed the adverse effects of khat extract on the functioning of the ovary, heart, and brain in white albino rats. Furthermore, it was discovered that this toxicity is associated with oxidative stress in the tissues of the ovary, heart, and brain, as indicated by a significant increase in lipid peroxidation biomarkers (MDA) and a significant decrease in the levels of antioxidant components such as GSH, CAT, and SOD.

In this experiment, the group treated with khat extract displayed more significant levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone. Conversely, a different study conducted in Pakistan and Iran revealed a drop in estrogen levels. In the treatment group, greater estrogen levels were observed than in the control group, showing that Khat impacts estrogen regulation, leading to enhanced expression. The persistent increase in estradiol levels may potentially contribute to the occurrence of abortion. Additionally, a comparable elevation in estrogen levels was detected among male individuals who chew Khat, as described in a study conducted by Al-Ghamdi [23]. While several studies have studied the effects of Khat on reproductive hormones, these investigations have primarily focused on male rats or humans. Previous studies evaluating the influence of khat extract on FSH, LH, Estrogen, and testosterone levels in female rats were relatively scarce. In their research, Toennes et al. [24] evaluated the immediate impact of Khat chewing on the levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in the plasma of male participants.

Similarly, a study by Al-Motarreb et al. [25] evaluated the effects of Khat on male reproductive hormones, reporting that khat use may boost testosterone levels. However, it may also lead to decreased sperm count and motility. The current findings are in keeping with the results obtained in the present investigation since they reveal a significant increase in testosterone levels among the treated group compared to the control group. Estrogen levels may continue to rise and cause many health concerns during ovulation and pregnancy [26]. The research by Mwenda et al. revealed that the ingestion of Khat could contribute to hormonal abnormalities [3]. Their study indicated a drop in

serum estrogen levels and found modifications in the estrogen/progesterone ratio in rodents. Reduced estradiol may promote post-implantation embryonic resorptions in the uteri and degeneration of early embryos [27, 28].

The present data also reveal that Khat boosted TNF-α secretion, which concurs with Ismaeel et el. [29] argument that the substantial elevation in the level of pro-inflammatory cytokine TNF-α could be related to Khat's propensity to induce apoptosis via caspase activation by TNF-α. Kennedy et al. [30], found that co-treatment of Coenzyme-Q10 (Co-Q10) with Khat decreased the khat-induced rise in TNF-α. Co-treatment of Coenzyme-Q10 (Co-Q10) with Khat considerably reduced the khat-induced elevation in TNF-α [31, 32]. Earlier research has provided evidence that the proliferation of monocytes and neutrophils can promote the production of pro-inflammatory cytokines, including IL-6, IL-8, IFN-γ, and TNF-α. This cascade of events contributes to the development of illnesses related to inflammation [33,34]. Khat induced a considerable rise in TNF-α expression, which could explain its participation in increasing inflammation and illnesses. The histological examination revealed an ovary with a diverse follicular landscape, including primary, secondary, and antral follicles, alongside scattered cystic dilations and atrophied linings. Within the edematous stroma, several corpora lutea were observed, accompanied by apoptotic stromal cells and dilated, congested blood vessels. These data demonstrated that Khat consumption damages ovaries and fertility. Such results coincide with Lee et al. [35], showing that high levels of TNF-α in follicular fluids are connected to low-quality oocytes and embryos. The abnormal changes of immunoreactive chemicals in the pelvic environment can produce female infertility [36-38]. Furthermore, TGF-β1 is indeed a crucial factor in promoting embryo implantation at the uterine surface. Its role extends to modulating immunological tolerance, thereby creating an environment conducive to successful embryo embedding [39]. The results of the present study indicated a considerable increase in TGF-β1 in the Khat-treated group. This conclusion is congruent with the results of Yan et al. [40], who discovered that the levels of TGF-β1 and IL-8 rose in infertile patients; these data suggested that IL-8 and TGF-1 might be utilized to identify tubal factor infertility (TFI). Murdoch et al. have uncovered intriguing insights into the potential impact of Khat on systemic and oral health [41]. Their findings suggest that Khat may significantly influence the phenotype and function of immune cells, thereby altering their behavior and potentially affecting overall health. Based on the current findings, the histological investigation of the brains of Khat-treated rats showed considerable anomalies compared to the control group. The thick meninges with modest inflammatory infiltration seen in the Khat-treated rats suggest inflammation of the protective membranes protecting the brain. Thickening of the meninges has been connected with chronic inflammation and may indicate long-term exposure to Khat.

Indeed, detecting significant astrogliosis followed by degenerated neurons in the cerebral cortex of khat-treated rats implies probable injury to the nerve cells inside this specific brain region. Astrogliosis increases the number and size of astrocytes, a kind of glial cell, and is typically related to brain damage and neurodegenerative illnesses [42]. The loss of neurons seen in the cerebral cortex gives evidence of a probable connection between khat consumption and neurotoxicity. This finding aligns with the research by Alle et al., which demonstrates that concurrent exposure to Khat and ethanol can differentially influence the sizes of specific neurons in the motor cortex and hippocampus, thereby reinforcing the understanding of Khat's harmful effects on brain cell integrity [43]. The deep cortex with marked inflammatory infiltration with abscess formation found in khat-treated rats showed the existence of an infection or inflammation. Abscesses signal an initial inflammatory response and may indicate a disease caused by the bacteria typically found in the oral

cavity of khat consumers. The presence of slightly congested blood vessels and an eosinophilic plaque-like area in the brains of khat-treated rats suggests potential disruptions in blood flow and possible neuronal damage. These data show that khat use may have deleterious effects on cerebral vascular health and neuronal integrity. Degenerative neurons, neurons with nuclear vacuoles, and average glial cells observed in the white matter of khat-treated rats suggest potential adverse effects of khat on neural health in this brain region. The observation of scattered degenerative neurons, neurons with nuclear vacuoles, and average glial cells in the white matter of khat-treated rats suggests potential adverse effects of khat on the neural health of this specific brain region. This observation further underscores the potential neurotoxicity linked with khat consumption. Nuclear vacuoles in some neurons signal cellular injury, which may contribute to cognitive impairment and other neurological symptoms. The present results correspond with Al-Motarreb et al. [44], who showed that the Khat extract may have neurotoxic effects on the brain, notably the dopaminergic and serotonergic systems. Chronic ingestion of Khat can lead to neurodegenerative changes, oxidative stress, and inflammation, thereby affecting cognitive function and raising the likelihood of developing psychiatric illnesses.

The hippocampus is vulnerable to the effects of various medications and poisons. It plays a critical role in learning and memory, and injury to this area can lead to cognitive impairment. The study's findings imply that Khat extract may have neurotoxic effects on the hippocampus, which could contribute to cognitive impairment in persons who use Khat over a lengthy period. Specifically, the CA1 region displayed dispersed degraded pyramidal neurons—specialized neurons that make up the hippocampus—as well as moderate edema. The CA2 region revealed severely damaged pyramidal neurons, indicating that this section of the hippocampus may be particularly prone to the effects of Khat extract. Additionally, scattered degraded pyramidal neurons were observed in the CA3 region, indicating that the damage is not limited to a particular location of the hippocampal region. This widespread damage could lead to significant cognitive impairment, affecting memory and learning processes.

In addition, the dentate gyrus, a part of the hippocampus that is responsible for the formation of new memories, revealed scattered degenerated pyramidal neurons in the inter-neuron area and modestly dilated congested blood vessels. This finding suggests that Khat extract may also alter the blood vessels in the hippocampus, which could contribute to the reported damage to the neurons. The damage in the dentate gyrus could lead to significant impairment in the formation of new memories, further exacerbating the cognitive effects of Khat extract.

This conclusion accords with the results of Berihu et al. [45], who suggested that Khat extract may have neurotoxic effects on the hippocampus and may lead to cognitive impairment in persons who use Khat over a more extended period. According to Al-Motarreb et al. (2002) [44, 46], Khat use has also been associated with cognitive deficiencies, including attention, memory, and executive function. They indicated that these effects may be linked to the stimulating and psychoactive qualities of the active chemicals in Khat, such as cathinone and cathine, which can alter the release and absorption of neurotransmitters in the brain. According to the study by Kassim et al. (2006) [47], which studied the prevalence and correlates related to Khat use among UK-resident Yemeni Khat chewers, Khat usage was connected to cognitive deficiencies such as attention deficits. According to Wabe et al. [8], using Khat has been related to adverse effects on cognitive function. They hypothesized that these effects could be caused by the stimulating and psychoactive effects of the active compounds in Khat, such as cathine and cathinone, which can alter the brain's neurotransmitter release and reuptake. They also talked about the possible neurotoxic effects of Khat

on the hippocampus, including damage to the blood vessels and pyramidal neurons, which could lead to cognitive impairment.

The considerable increase in TNF-α levels seen in the Khat-treated group, as indicated in our experiment, coincides with the findings of Hennessy et al. [48]. This consistency shows a potential link between high TNF-α levels and hippocampal injury, which can contribute to cognitive impairment. TNF-α's significance in neuro-inflammation and its participation in several neurological disorders further supports the hypothesis that greater TNF-α levels may contribute to the development and progression of these conditions. These potential implications of our research underscore its potential impact on the field of neuroscience and immunology, inspiring further exploration and investigation.

TGF-β1 is a cytokine that modulates inflammatory responses and is implicated in developing several disorders, including cancer and cardiovascular disease. The present data demonstrated increased TGF-β1 levels in the Khat-treated group, indicating an inflammatory response that may contribute to tissue damage and dysfunction. According to Derynck et al. [49], TGF-β1 is a multifunctional cytokine that regulates immune responses and inflammation. Dysregulation of TGF-β1 signaling can lead to persistent inflammation, tissue damage, and malfunction, which may contribute to the development of many diseases. The increase in TGF-β1 levels in the Khat-treated group is consistent with the role of TGF-β1 in controlling inflammation and immunological responses. On the other hand, IL-4 is an anti-inflammatory cytokine that is involved in the control of immune responses. The drop in IL-4 levels in the Khat-treated group, as opposed to the control group, may indicate a shift towards a more pro-inflammatory state, which could contribute to tissue damage and dysfunction. Islam et al. [50] studied the potential cardiotoxicity of Khat in rats. They discovered that Khat consumption led to a significant reduction in the levels of IL-4 compared to the control group. The decrease in IL-4 levels may signal a shift towards a more pro-inflammatory state, which could contribute to tissue damage and dysfunction. Furthermore, a prior study by Murdoch et al. [41] found that Khat use was substantially linked with lower IL-4 levels in the study subjects. IL-4 is an anti-inflammatory cytokine that plays a vital function in immune control and is involved in allergy and autoimmune illnesses. The fall in IL-4 levels may signal a shift towards a more pro-inflammatory state, which could contribute to tissue damage and dysfunction. Inflammation is necessary for ensuring successful pregnancies, but if it becomes aberrant and persistent, and anti-inflammatory cytokine-producing cells fail to resolve it, numerous pregnancy problems may ensue. The study demonstrated a substantial drop in IL4 in the Khat-treated group compared to the control group. This finding supports the statement made by Chatterjee et el. [51], which suggests that IL-4 and IL-10 are essential for a successful pregnancy, and a deficiency in these cytokines can contribute to infertility, spontaneous abortion, preterm birth (PTB), fetal growth restriction (FGR), and hypertensive disorders of pregnancy.

Khat leaves (*Catha edulis*) contain various pharmacologically active alkaloids, including cathulidins and Cathinone, which elicit sympathomimetic effects [52]. These actions cause the release of noradrenaline from peripheral sympathetic neurons and serotonin and dopamine in the central nervous system, comparable to the effects of amphetamine and cocaine. Cathinone, in particular, elevates blood pressure and heart rate proportionate to blood levels, peaking at 1.5-3.5 hours after chewing [53]. According to Yanagita [51], Cathinone, a main component of khat, has a critical function in enhancing noradrenergic transmission, which is responsible for the sympathomimetic condition typically noticed following khat ingestion. This syndrome is characterized by elevated sympathetic nervous system activity, leading to numerous physiological outcomes. Cathinone is also connected with an

improved inotropic effect, which refers to strengthening the heart's contractile force, further contributing to the complicated physiological responses induced by khat. The potential implications of this research are vast and intriguing, opening up new avenues for further study and understanding.

The present investigation, conducted over a period of [time], involved the treatment of rats with *Catha edulis* (Khat) to study its effects on cardiovascular and neurological health. The study demonstrated that Khat significantly elevated serum troponin, creatine kinase (CK), CPK, and aspartate transaminase (AST) levels in rats compared to the control group. These findings are in line with Alkadi et al. [55], which indicated that acute administration of Khat extract could generate a chronotropic impact and an increase in the amplitude of ventricular activity. The study also showed increased CK-MB and AST in the serum linked with myocardial infarction. The Khat-induced myocardial infarction may be linked to an increase in myocardial oxygen demand, followed by platelet aggregation due to the activation of catecholamine and coronary vasospasm [44, 52, 55]. The microscopic analysis of histological sections of the cardiac tissues of rats treated with *Catha edulis* (Khat) exhibited thick pericardium, significantly apoptotic cardiac muscle fibers, others with pyknotic nuclei, and moderately congested blood vessels with mildly congested blood capillaries. This discovery coincides with the findings of Saha and Dollery [52], who stated that Long-term khat usage had been related to significant cardiovascular adverse effects. Several research studies have explored the effects of Khat on the heart, and their findings suggest that khat use may injure the heart muscle. For example, histological studies of rats treated with khat extract have demonstrated thickening of the pericardium, apoptotic cardiac muscle fibers, pyknotic nuclei, and mildly congested blood arteries with mildly congested blood capillaries [2].

Additionally, investigations have revealed modifications in cardiac contractility following khat consumption, including an increase in the inotropic effect and an increase in the amplitude of ventricular activity [55]. Furthermore, long-term khat intake has been related to an increased risk of cardiovascular illnesses, such as hypertension, atherosclerosis, and myocardial infarction [56]. These effects may be associated with the sympathomimetic qualities of khat alkaloids, which excite the sympathetic nervous system, elevate blood pressure and heart rate, and promote oxidative stress and vasoconstriction.

In conclusion, the existing data suggests that khat use may adversely damage the heart and brain. Khat use has been related to different harmful consequences on both the heart and brain. Structural changes and modifications in heart contractility have been reported, potentially raising the risk of cardiovascular illnesses. Additionally, Khat is related to brain inflammation, nerve cell damage, decreased blood flow, and the chance of infection. The use of Khat may also upset the balance of cytokines, resulting in a rise in pro-inflammatory markers and a decrease in anti-inflammatory ones, contributing to tissue damage and dysfunction.

Furthermore, our study reveals that khat extract may have neurotoxic effects on the hippocampus, potentially leading to cognitive impairment. However, further research is needed to fully understand the mechanics and health implications of these effects. These findings underscore the importance of continuous investigation into the potential dangers of khat usage and the need for further inquiry to better our understanding of these risks.

Acknowledgements:

The facilities provided by the Faculty of Science at Jazan University, Saudi Arabia, are greatly acknowledged and appreciated.

Funding information: None

Conflict of interest:

The author declares that no conflict of interest.

Ethical statement:

All the experiments agreed with the regulations of the institutional animal ethics committee of Jazan University, Jazan, Saudi Arabia kingdom.

5. REFERENCES

[1] A. El-Menyar, A. Mekkodathil, H. Al-Thani, and A. Al-Motarreb, "Khat use: history and heart failure," *Oman Medical Journal*, vol. 30, no. 2, pp. 77, 2015.

[2] M. Abebe, S. Kindie, and K. Adane, "Adverse health effects of khat: a review," *Fam Med Med Sci Res*, vol. 4, no. 1, pp. 2-5, 2015.

[3] J. M. Mwenda, R. A. Owuor, C. M. Kyama, E. O. Wango, M. M'Arimi, and D. K. Langat, "Khat (*Catha edulis*) up-regulates testosterone and decreases prolactin and cortisol levels in the baboon," *Journal of Ethnopharmacology*, vol. 103, no. 3, pp. 379-384, 2006.

[4] O. Ighodaro, and O. Akinloye, "First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid," *Alexandria Journal of Medicine*, vol. 54, no. 4, pp. 287-293, 2018.

[5] A. K. Seleem, A. A. El Refaeey, D. Shaalan, Y. Sherbiny, and A. Badawy, "Superoxide dismutase in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection," *Journal of assisted reproduction and genetics*, vol. 31, pp. 499-504, 2014.

[6] L. Sabatini, T. Al Shawaf, C. Wilson, A. Lower, and J. Grudzinskas, "Follicular fluid superoxide dismutase (SOD) activity in women with polycystic ovarian syndrome (PCOS)," *Fertility and Sterility*, vol. 74, no. 3, pp. S253-S254, 2000.

[7] S. Wang, G. He, M. Chen, T. Zuo, W. Xu, and X. Liu, "The role of antioxidant enzymes in the ovaries," *Oxidative medicine and cellular longevity*, vol. 2017, 2017.

[8] N. T. Wabe, "Chemistry, pharmacology, and toxicology of khat (*Catha edulis* forsk): a review," *Addiction & health*, vol. 3, no. 3-4, pp. 137, 2011.

[9] P. J. Devine, S. D. Perreault, and U. Luderer, "Roles of reactive oxygen species and antioxidants in ovarian toxicity," *Biology of reproduction*, vol. 86, no. 2, pp. 27, 1-10, 2012.

[10] U. F. Shaik Mohamed Sayed, S. Moshawih, H. P. Goh, N. Kifli, G. Gupta, S. K. Singh, D. K. Chellappan, K. Dua, A. Hermansyah, and H. L. Ser, "Natural products as novel anti-obesity agents: Insights into mechanisms of action and potential for therapeutic management," *Frontiers in pharmacology*, vol. 14, pp. 1182937, 2023.

[11] S. Gaweł, M. Wardas, E. Niedworok, and P. Wardas, "Malondialdehyde (MDA) as a lipid peroxidation marker," *Wiadomosci lekarskie (Warsaw, Poland: 1960)*, vol. 57, no. 9-10, pp. 453-455, 2004.

[12] A. Caglayan, D. C. Katlan, Z. Selcuk Tuncer, K. Yuce, H. B. Sayal, M. Coskun Salman, and B. Kocer-Gumusel, "Impaired antioxidant enzyme functions with increased lipid peroxidation in epithelial ovarian cancer," *IUBMB Life*, vol. 69, no. 10, pp. 802-813, Oct, 2017.

[13] A. S. Abou-Elhamd, G. Kalamegam, F. Ahmed, M. Assidi, A. F. Alrefaei, P. N. Pushparaj, and M. Abu-Elmagd, "Unraveling the *Catha edulis* Extract Effects on the Cellular and Molecular Signaling in SKOV3 Cells," *Front Pharmacol*, vol. 12, pp. 666885, 2021.

[14] M. El-Setouhy, and A. A. Hassan, "Cytotoxicity and Genotoxicity of Khat (*Catha edulis* Forsk)," *Handbook of Substance Misuse and Addictions: From Biology to Public Health*, pp. 1-11: Springer, 2022.

[15] G. Al-Qadhi, M. M. Ali Mohammed, M. Al-Ak'hali, and E. A. Al-Moraissi, "Khat (*Catha edulis* Forsk) induced apoptosis and cytotoxicity in cultured cells: A scoping

review," *Heliyon*, vol. 7, no. 12, pp. e08466, Dec, 2021.

[16] M. Mohammadi, "Oxidative stress and polycystic ovary syndrome: a brief review," *International journal of preventive medicine*, vol. 10, 2019.

[17] D. Acevedo-León, L. Monzó-Beltrán, S. Á. Gómez-Abril, N. Estañ-Capell, N. Camarasa-Lillo, M. L. Pérez-Ebri, J. Escandón-Álvarez, E. Alonso-Iglesias, M. L. Santaolaria-Ayora, and A. Carbonell-Moncho, "The effectiveness of glutathione redox status as a possible tumor marker in colorectal cancer," *International Journal of Molecular Sciences*, vol. 22, no. 12, pp. 6183, 2021.

[18] A. Ramadan, R. Hemida, A. Nowara, L. A. Eissa, and A. M. El-Gayar, "Role of oxidative stress in epithelial ovarian cancer in Egyptian patients," *Journal of Experimental Therapeutics & Oncology*, vol. 12, no. 1, 2017.

[19] M. Tariq, I. Al-Meshal, N. Parmar, A. Ageel, and S. Qureshi, "Evaluation of genotoxic potential of khat (*Catha edulis*) in Swiss albino mice," *Mutagenesis*, vol. 1, no. 5, pp. 381-382, 1986.

[20] Falih, S., Al-Ali, A. Determination of ex vivo chemical and in vivo biological antioxidant activities of clary sage essential oil. *Journal of Bioscience and Applied Research*, 2024; 10(3): 290-301. doi: 10.21608/jbaar.2024.259473.1030

[21] C.-l. Fu, Y. Liu, J. Leng, J. Zhang, Y.-f. He, C. Chen, Z. Wang, and W. Li, "Platycodin D protects acetaminophen-induced hepatotoxicity by inhibiting hepatocyte MAPK pathway and apoptosis in C57BL/6J mice," *Biomedicine & Pharmacotherapy*, vol. 107, pp. 867-877, 2018.

[22] F. H. Al-Hashem, I. Bin-Jaliah, M. A. Dallak, L. O. Nwoye, M. Al-Khateeb, H. F. Sakr, R. A. Eid, K. S. Al-Gelban, H. S. Al-Amri, and M. A. Adly, "Khat (*Catha edulis*) extract increases oxidative stress parameters and impairs renal and hepatic functions in rats," *Bahrain Medical Bulletin*, vol. 33, no. 1, pp. 1-9, 2011.

[23] S. S. Al-Ghamdi, "The reproductive and thyroid hormonal profile of khat (*Catha edulis*) chewers," *Pharmacology & Pharmacy*, vol. 3, no. 04, pp. 447-452, 2012.

[24] S. W. Toennes, S. Harder, M. Schramm, C. Niess, and G. F. Kauert, "Pharmacokinetics of cathinone, cathine and norephedrine after the chewing of khat leaves," *British journal of clinical pharmacology*, vol. 56, no. 1, pp. 125-130, 2003.

[25] A. Al-Motarreb, M. Al-Habori, and K. J. Broadley, "Khat chewing, cardiovascular diseases and other internal medical problems: the current situation and directions for future research," *J Ethnopharmacol*, vol. 132, no. 3, pp. 540-8, Dec 1, 2010.

[26] J. Mwenda, M. Arimi, M. Kyama, and D. Langat, "Effects of khat (*Catha edulis*) consumption on reproductive functions: a review," *East African medical journal*, vol. 80, no. 6, pp. 318-323, 2003.

[27] T. Misgana, D. Tesfaye, D. Alemu, B. Gebremichael, D. Tamiru, M. Tariku, A. B. Weldesenbet, and M. Dheresa, "Khat use and associated factors during pregnancy in eastern Ethiopia: A community-based cross-sectional study," *Frontiers in Global Women's Health*, vol. 3, pp. 941300, 2022.

[28] M. A. Alzahrani, M. A. Alsahli, F. F. Alarifi, B. O. Hakami, F. W. Alkeraithe, M. Alhuqbani, Z. Aldosari, O. Aldosari, A. E. Almhmd, S. Binsaleh, and R. Almannie, "A Narrative Review of the Toxic Effects on Male Reproductive and Sexual Health of Chewing the Psychostimulant, *Catha edulis* (Khat)," *Med Sci Monit*, vol. 29, pp. e939455, Apr 1, 2023.

[29] I. Bin-Jaliah, M. A. Dallak, F. H. Al-Hashem, L. O. Nwoye, H. F. Sakr, A.-M. Jamil, and M. Al-Khateeb, "Derangement of hemopoiesis

and hematological indices in Khat (*Catha edulis*)-treated rats," *African Journal of Biotechnology*, vol. 13, no. 2, 2014.

[30] C. Kennedy, P. Okanya, J. N. Nyariki, P. Amwayi, N. Jillani, and A. O. Isaac, "Coenzyme Q10 nullified khat-induced hepatotoxicity, nephrotoxicity and inflammation in a mouse model," *Heliyon*, vol. 6, no. 9, 2020.

[31] J. N. Nyariki, L. A. Ochola, N. E. Jillani, N. O. Nyamweya, P. E. Amwayi, D. S. Yole, L. Azonvide, and A. O. Isaac, "Oral administration of Coenzyme Q10 protects mice against oxidative stress and neuro-inflammation during experimental cerebral malaria," *Parasitology international*, vol. 71, pp. 106-120, 2019.

[32] J. Zhai, Y. Bo, Y. Lu, C. Liu, and L. Zhang, "Effects of coenzyme Q10 on markers of inflammation: a systematic review and meta-analysis," *PloS one*, vol. 12, no. 1, pp. e0170172, 2017.

[33] A. Das, M. Sinha, S. Datta, M. Abas, S. Chaffee, C. K. Sen, and S. Roy, "Monocyte and macrophage plasticity in tissue repair and regeneration," *Am J Pathol*, vol. 185, no. 10, pp. 2596-606, Oct, 2015.

[34] Yameny, A., Alabd, S., Mansor, M. MiRNA-122 association with TNF-α in some liver diseases of Egyptian patients. *Journal of Bioscience and Applied Research*, 2023; 9(4): 212-230. doi: 10.21608/jbaar.2023.329927

[35] K. S. Lee, B. S. Joo, Y. J. Na, M. S. Yoon, O. H. Choi, and W. W. Kim, "Relationships between concentrations of tumor necrosis factor-α and nitric oxide in follicular fluid and oocyte quality," *Journal of assisted reproduction and genetics*, vol. 17, pp. 222-228, 2000.

[36] J. Alijotas-Reig, E. Esteve-Valverde, R. Ferrer-Oliveras, E. Llurba, and J. M. Gris, "Tumor Necrosis Factor-Alpha and Pregnancy: Focus on Biologics. An Updated and Comprehensive Review," *Clin Rev Allergy Immunol*, vol. 53, no. 1, pp. 40-53, Aug, 2017.

[37] F. Atzeni, E. Grillo, I. F. Masala, P. Sarzi-Puttini, and G. T. Jones, "Do anti-TNF blockers increase the risk of lung involvement in patients with ankylosing spondylitis or psoriatic arthritis? A systematic review," *Israel Medical Association Journal*, vol. 18, no. 3-4, pp. 154-155, 2016.

[38] Yameny, A., Alabd, S., Mansor, M. Serum TNF-α levels as a biomarker in some liver diseases of Egyptian patients. *Journal of Medical and Life Science*, 2023; 5(1): 1-8. doi: 10.21608/jmals.2023.329303

[39] D. Jiang, L. Li, and B.-Y. Zeng, "Treatment of Chinese herbal medicine for female infertility," *International review of neurobiology*, pp. 233-247: Elsevier, 2017.

[40] J. Yan, C. Liu, H. Zhao, C. Wang, H. Yao, Q. Lu, J. Liu, and Y. Feng, "A cross-sectional study on the correlation between cytokines in a pelvic environment and tubal factor infertility," *BMC Pregnancy and Childbirth*, vol. 20, no. 1, pp. 1-6, 2020.

[41] C. Murdoch, H. A. Aziz, H.-Y. Fang, H. Jezan, R. Musaid, and M. Muthana, "Khat (*Catha edulis*) alters the phenotype and anti-microbial activity of peripheral blood mononuclear cells," *Journal of ethnopharmacology*, vol. 138, no. 3, pp. 780-787, 2011.

[42] S. M. Vita, A. M. Smith, T. J. Ray, and B. E. Grayson, "Blood–brain barrier damage following mild traumatic brain injury," *Handbook of Animal Models in Neurological Disorders*, pp. 133-144: Elsevier, 2023.

[43] P. E. Alele, D. Matovu, L. Imanirampa, A. M. Ajayi, and G. T. Kasule, "Projection neurons in the cortex and hippocampus: differential effects of chronic khat and ethanol exposure in adult male rats," *J Exp Pharmacol*, vol. 8, pp. 43-52, 2016.

[44] A. Al-Motarreb, K. Baker, and K. J. Broadley, "Khat: pharmacological and medical aspects and its social use in Yemen," *Phytother Res,* vol. 16, no. 5, pp. 403-13, Aug, 2002.

[45] B. A. Berihu, G. G. Asfeha, A. L. Welderufael, Y. G. Debeb, Y. B. Zelelow, and H. A. Beyene, "Toxic effect of khat (*Catha edulis*) on memory: Systematic review and meta-analysis," *J Neurosci Rural Pract,* vol. 8, no. 1, pp. 30-37, Jan-Mar, 2017.

[46] A. M. Oyugi, J. K. Kibet, and J. O. Adongo, "A review of the health implications of heavy metals and pesticide residues on khat users," *Bulletin of the National Research Centre,* vol. 45, pp. 1-22, 2021.

[47] S. Kassim, and R. Croucher, "Khat chewing amongst UK resident male Yemeni adults: an exploratory study," *International dental journal,* vol. 56, no. 2, pp. 97-101, 2006.

[48] E. Hennessy, S. GormLey, A. B. Lopez-Rodriguez, C. Murray, C. Murray, and C. Cunningham, "Systemic TNF-α produces acute cognitive dysfunction and exaggerated sickness behavior when superimposed upon progressive neurodegeneration," *Brain, behavior, and immunity,* vol. 59, pp. 233-244, 2017.

[49] R. Derynck, and Y. E. Zhang, "Smad-dependent and Smad-independent pathways in TGF-β family signalling," *Nature,* vol. 425, no. 6958, pp. 577-584, 2003.

[50] M. Islam, O. Al-Shabanah, M. Al-Harbi, and N. Al-Gharably, "Evaluation of teratogenic potential of khat (*Catha edulis* Forsk.) in rats," *Drug and chemical toxicology,* vol. 17, no. 1, pp. 51-68, 1994.

[51] P. Chatterjee, V. L. Chiasson, K. R. Bounds, and B. M. Mitchell, "Regulation of the Anti-Inflammatory Cytokines Interleukin-4 and Interleukin-10 during Pregnancy," *Front Immunol,* vol. 5, pp. 253, 2014.

[52] S. Saha, and C. Dollery, "Severe ischaemic cardiomyopathy associated with khat chewing," *Journal of the Royal Society of Medicine,* vol. 99, no. 6, pp. 316-318, 2006.

[53] Z. X. Chong, M. Alshagga, K. A. Saed, and S. Kassim, "Impact of khat (*Catha edulis*) chewing/use on heart rate and blood pressure: a critical review," *Malaysian Journal of Public Health Medicine,* vol. 17, no. 3, pp. 76-85, 2017.

[54] T. Yanagita, "Studies on cathinones: cardiovascular and behavioral effects in rats and self-administration experiment in rhesus monkeys," *NIDA Res Monogr,* vol. 27, pp. 326-7, 1979.

[55] H. O. Alkadi, M. A. Noman, A. K. Al-Thobhani, F. S. Al-Mekhlafi, and Y. A. Raja'a, "Clinical and experimental evaluation of the effect of Khat-induced myocardial infarction," *Saudi Med J,* vol. 23, no. 10, pp. 1195-8, Oct, 2002.

[56] A. Al-Motarreb, and J. Al-Suwaidi, "Khat Chewing and Cardiovascular Disease," *Heart Views,* vol. 23, no. 2, pp. 103-107, Apr-Jun, 2022.