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Investigating the Influence of Aqueous Khat (*Catha edulis*) Leaves Extract and Saffron Extract (*Crocus sativus*) on Prostate Tissue in Male Albino Rats.

Ferial Ahmed El-Messady[1], Sherif Adel Elagamy[2], Ahmed Mahmoud Abdeen[3], Doaa Abdelhameed Ali [4], Eman Hossny Kandil[1]*

[1]Zoology Department-Faculty of Science- Menoufia University-Shebin EI-Kom 32551- Menoufia- Egypt. ferialahmed64@yahoo.com, eman.hosney@science.menoufia.edu.eg

[2] Biology Department-College of Science- Jazan University-Jazan P.O. Box 2079, Jazan 45142-Saudi Arabia. elagamy.sherif2020@gmail.com

[3]Zoology Department-Faculty of Science-Mansoura University-Mansoura- 35516- Egypt. AMabdeenR@gmail.com

[4]Biology Department, College of Science, Taibah University- Al-Madinah Al-Munawarrah P.O.BOX 344, Zip Code: 41477 Saudi Arabia. dabdelsalam@taibahu.edu.sa

***Corresponding author**: Eman Hossny Kandil

ORCID:

Ferial A. El-Messady: https://orcid.org/0000-0003-2890-9374

Eman H. Kandil: https://orcid.org/0009-0002-7671-5574

Doaa A. Ali: https://orcid.org/0000-0002-6133-9599

Sherif A. Elagamy: https://orcid.org/0000-0002-1806-3953

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Abstract

Background: Chewing Khat (*Catha edulis*) is widespread in many parts of the world for its stimulating and euphoric effect, although Numerous studies have recently demonstrated the harmful effects of chewing khat.

Aim: examine the impact of saffron in minimizing khat-induced prostate toxicity in male rats.

Materials and methods: Four equal groups of 40 male albino rats were used. Control, Saffron: Saffron extract was given to rats orally at a dose of 100 mg/kg body weight. Khat: Khat extract was orally administered to rats at a dose of 200 mg/kg body weight. And finally, Khat + Saffron: Rats were given the same previous doses of khat and saffron. **Result:** Rats treated with Khat showed marked changes in the prostate which included degeneration of the glandular lining epithelium with an exfoliation within the lumen, marked edema with dilation in blood vessels, hemorrhage in the interstitial tissue, abnormal flattened epithelial layers of the prostatic acini and Substantial hyperplasia were founded in the prostatic acini's lumens. The histochemical analysis clarified that there was a significant decrease in proteinic and polysaccharide contents. Also, a decrease in the vimentin, E-cadherin proteins, and BCL2 expression while increasing BAX expression. Superoxide dismutase and catalase activity showed a marked decrease while malondialdehyde levels were. elevated. However, rats given both saffron and khat showed less harm than the khat group in prostatic histology. **In Conclusion,** saffron demonstrated its strong antioxidant feature besides its effectiveness in mitigating and ameliorating the histological changes induced by khat in the prostate of male rats.

Keywords: Khat, saffron, prostate, Bax-BCL2, Vimentine, E-Cadherin.

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Introduction:

Native to the Arabian Peninsula and the Horn of Africa, khat (*Catha edulis*) is a blooming evergreen shrub plant with young leaves **(1)**, however, it is now widespread in many regions worldwide **(2).** The leaves are traditionally chewed for their stimulant and euphoric properties **(3),** which result from the presence of cathine and cathinone, two alkaloids chemically similar to amphetamine, besides norephedrine **(4)**. Khat is usually chewed for several hours at a time **(5)**. The leaves are first chewed into a paste, then placed on the cheek and absorbed **(6).** Khat's constituents are easily absorbed in the stomach, and cathinone is quickly changed into cathine and norephedrine. Monoamine oxidase inhibition may play a role in their actions, primarily mediated via the reduction of monoamine release and reuptake **(7)**.

The effects of khat typically include increased alertness, energy, euphoria, and body temperature **(8)**. Also, elevated blood pressure and heart rate **(5)**. Khat is also thought to be a risk factor for schizophrenia and other mental health disorders, including addiction, insomnia, anxiety, paranoia, psychosis **(9)**. Moreover, oral health problems, digestive problems like stomatitis and esophagitis **(10),** renal failure **(11)**. Animal studies revealed that high concentrations of *Catha edulis* had the opposite effects on male reproductive health and were linked to lower levels of luteinizing hormone (LH); also, in human trials, users experienced significantly decreased libido or erectile dysfunction, as well as significantly decreased semen volume, sperm count, and motility. This suggests that khat consumption may have detrimental effects on male reproductive and sexual health. **(12).**

In addition to its adverse health effects, khat can also hurt society **(13).** Chronic khat chewing stimulates the creation of ROS, which raises the risk of oxidative toxicity, whereby can lead to DNA damage and a drop in superoxide dismutase (SOD) levels and catalase (CAT) activities, while the malondialdehyde (MDA) levels were increased in male Wistar rats or even in Chinese hamster cell lines; in addition of lowering the survival and proliferation of several cell types in in-vitro experiments and raising cell apoptosis **(14, 15).**

Because they are believed to be safer than allopathic therapies, the use of herbal medicines for the prevention, treatment, and prophylaxis of many illnesses is growing globally **(16).** One such significant example is the perennial stemless plant *Crocus sativus L.* a member of the Iridaceae family. This plant, extensively grown in Iran (80% of the world's production) and also cultivated in India, Afghanistan, Greece, Morocco, Spain, and Italy, holds promise as a medicinal herb **(17).**

Saffron considered the red stigmas obtained from *Crocus sativus L.* flowers. There are more than 150 volatile and non-volatile chemicals in stigma. However, the primary ones that have bioactivities of importance for human health include flavonoids like crocin, crocetin, safranal, quercetin kaempferol, and picrocrocin **(18).**

Mohd Hatta *et al.*, (2023) **(19)** Demonstrated the ability of the carotenoids in *Crocus sativus* stigma to capture peroxyl radicals and quench singlet oxygen making them efficient free-radical scavengers. In addition to being a natural colorant, crocin is also a potent antioxidant because its molecular structure contains conjugated double bonds that allow it to absorb electrons from reactive species and neutralize free radicals **(20)**. The process of oxidative stress can happen either intra- or extracellularly **(21).** Intracellular oxidative stress can result in cell necrosis and cell disorganization, with potentially disastrous consequences if a cell is unable to procreate. Meanwhile, extracellular oxidative stress has deleterious effects **(22)**.

The primary constituent of saffron's volatile oils, safranal, has a variety of pharmacological actions, such as anti-inflammatory, antihypertensive, anti-

oxidant, anti-ischemic, anti-asthmatic, anti-anxiety, anticonvulsant, analgesic, antinociceptive and cytotoxic properties. Safranal has been discovered to possess lung, gastrointestinal, neuroprotective, nephroprotective, and cardioprotective properties **(23).** Additionally, crocin showed good suppression against the pathogenic bacterium S. epidermidis **(24),** beside saffron's anti-inflammatory and anti-depression properties **(25)**. **Wang *et al*., (2022) (26)** explained the antiproliferative effect of saffron and especially crocin on human cancer cell lines.

The significance of the current study lies in its evaluation of the effects of khat extract on prostatic tissues. This evaluation, conducted from the perspectives of histopathology, histochemistry, and immunohistochemistry, aims to provide crucial insights. Furthermore, the study assesses the potential of saffron as an antioxidant agent, particularly its ability to protect the prostate tissues from any damage.

Material and methods:

Materials: (all the chemicals used are of highly analytical grade**)**

Khat:

Khat was obtained from Substance Abuse Research Centre (SARC) - Jazan University- Saudi Arabia.

Saffron extract (SE):

Saffron was purchased from Spain. IBERPRIME IMPORT & EXPORT SL - Lot No. 130416.

Methods:

Khat extract (KE) preparation:

The khat extract was prepared with meticulous care. The green plant leaves (350 grams) were repeatedly rinsed with distilled water, then dried, and finally crushed to a fine powder (dry weight became 56 grams). The ground dried khat powder was soaked (4 h) and then stirred in 500 ml of distilled water for two hours. Then, filtration was dried by using a rotary evaporator as modulated according to **(27)** the final weight of the extract was calculated, and it was 24 grams. To prepare a dose of 200 mg/ kg body weight of khat, 3 g of khat extract was dissolved in 60 ml distilled water and prepared freshly before being orally administrated to the animals **(28).**

Saffron extract (SE) preparation:

One gram of *Crocus sativus L.* powder was immersed in twenty ml of warmed distilled water, stirred for two hours, and then filtrated. A dose of 100 mg/kg body weight of saffron was orally administered to animals according to **(29).** The aqueous solution of the extract was prepared freshly before administration.

Animals and experimental design:

The study was conducted with the utmost respect for ethical standards. Albino rats aged ten weeks were reared and maintained on a 12h light/12h dark cycle under definite conditions of temperature (20±4 OC) and Rh (55 ± 10%). Rats had unlimited access to food and water. The study was authorized by Menoufia University's Animal Care and Bioethics Committee, Egypt (Approval No. MNSH 175).

The experimental animals were grouped into four groups, each with ten rats: Group 1 (Control group): Rats received distilled water only. Group 2 (Saffron group): orally administered 100 mg/kg body weight of saffron extract every other day. Group 3 (Khat group): orally administered 200 mg/kg body weight of khat extract every other day. Group 4 (Khat + Saffron group): Rats were given khat extract and saffron extract with the same doses in the second and third groups every other day for three months. At the end of the 3 months, animals were fasted for 12 hours before being dissected the following morning.

Histological preparation

Immediately after dissection, the prostate was removed, washed in saline, and put in formalin solution (10% neutral buffered) for 24 hours, and sections of 5-micron were prepared and then stained with hematoxylin and eosin **(30).**

Histochemical investigation

The total polysaccharide was determined by using the PAS reaction, and the total protein was determined by the mercury bromophenol blue method as described in **(Mahran *et al.*, 2017) (31).**

Immunohistochemical investigation

The sections were subjected to incubation with an anti-rabbit monoclonal antibody to assess the expression of BCL2 (B cell lymphoma 2) (Abcam, Shanghai Trading Co. Ltd. China, catalog code: ab194583; 1:100), Bcl-2-associated X protein (Bax) (LabVision, Thermo Fisher Scientific; catalog code: MA5–14003; 1:100), vimentin (Abcam, Shanghai Trading Co. Ltd. China, catalog code: EPR3776; 1:100) and Epithelial Cadherin (E- Cadherin) (LabVision, Thermo Fisher Scientific; catalog code: PA5– 8508; 1:100) **(32).**

Biochemical estimations

The other parts of the prostate were separated, cleaned as rapidly as possible, weighed, then homogenized in ice-cold water and frozen at -20°C for subsequent analysis of catalase (CAT) **(33)**, superoxide dismutase (SOD) **(34)** and malondialdehyde (MDA) **(35)**.

Statistical analysis:

Advanced statistical analysis was performed using SPSS version 20. The data were presented as mean ± standard deviation (SD). Data were obtained as average concentration ± standard deviation. Data normality was determined using the Kolmogorov-Smirnoff test ($p < 0.05$), followed by homogeneity assessment using the Levene test ($p < 0.05$). Data were analyzed using one-way ANOVA ($p < 0.05$) and the Tukey test to determine the significance between cohorts.

Image Analysis:

The immunohistochemical expressions of Bcl2, Bax, vimentin, and E-Cadherin positive cells (brown stained) in prostate sections were analyzed by a semi-quantitative scoring system (Fiji-Image J software, Java based application for analyzing images). The percentage of colored stained area (area fraction) per field area was determined by measuring five randomly photographed high-power fields (X400 magnifications).

Results:

Biochemical results

The control and saffron groups exhibited normal MDA levels and normal CAT and SOD activity in prostate homogenate. While, the khat extract group showed a decrease in CAT enzyme and SOD content, along with an increase in lipid peroxidation product (MDA) concentration. On the other hand, the combined group of saffron and khat extract demonstrated a decrease in khat effects; with increased CAT and SOD activity and decreased MDA levels in the prostate's homogenate (**Table 1**).

Table (1): change in MDA level, CAT, and SOD activity in the experimental groups.

Animal groups	MDA (nmol/g)	CAT (U/mg)	SOD (µg/g)
Control group	20.86 ±1.5	4.56 ±0.3	993.04 ±3.5
Saffron group	23.29 ±1.4	2.89 ±0.3	1033.82 ±2.8
Khat group	43.80±1.9	2.27 ±0.7*	687.8 ±3.9
Khat+ saffron	40.09±1.9	3.27 ±0.1**	781.88±3.6

Data were expressed as mean ± standard deviation (*n*=10). MDA: malondialdehyde, CAT: catalase, SOD: superoxide dismutase

(*) Significant compared to the control group at P<0.05.

(**) Significant compared to the khat group at P<0.05.

Histological observations:

The prostate gland, an important part of the male reproductive system, comprises many small compound tubule-alveolar acini of varying sizes encased in a thick outer capsule. The secretory tubules, responsible for producing prostatic fluid, differ in size and are irregularly shaped, featuring a lining of folded simple columnar or cuboidal cells. The prostatic acini have narrow lumens that contain prostatic concretions (corpora amylacea). Between the secretory tubules is a fibromuscular stroma of fibro-elastic tissue, smooth muscle fibers, and blood vessels. The typical structure of the Prostate to control untreated rats is shown in (**Fig1.a, b**).

The contrast between the control group and the khat-treated group was stark, with marked histological alterations in the prostate gland observed after 3 months of khat treatment. The prostatic acini were observed to consist of aberrant flattened epithelial layers, with significant hyperplasia extending into the lumens of the acini. Besides the degeneration of glandular lining epithelium with exfoliation within the lumen, marked oedema in the interstitial tissue was noticed and congested blood vessels with hemorrhage appeared (**Fig1.c, d**).

However, when rats were subjected to the dual treatment (khat and saffron extract together) every other day for 3 months, the prostatic gland showed a significant improvement in histopathological alterations when compared with animals treated with khat only. The glandular lining epithelium of the prostatic acini returned to its distinctive shape and was healthier than that in the khat-treated group in most of the tested tissue sections, except rarely mild degeneration of small parts of epithelial tissues without oedema or congested blood vessels (**Fig1.e**).

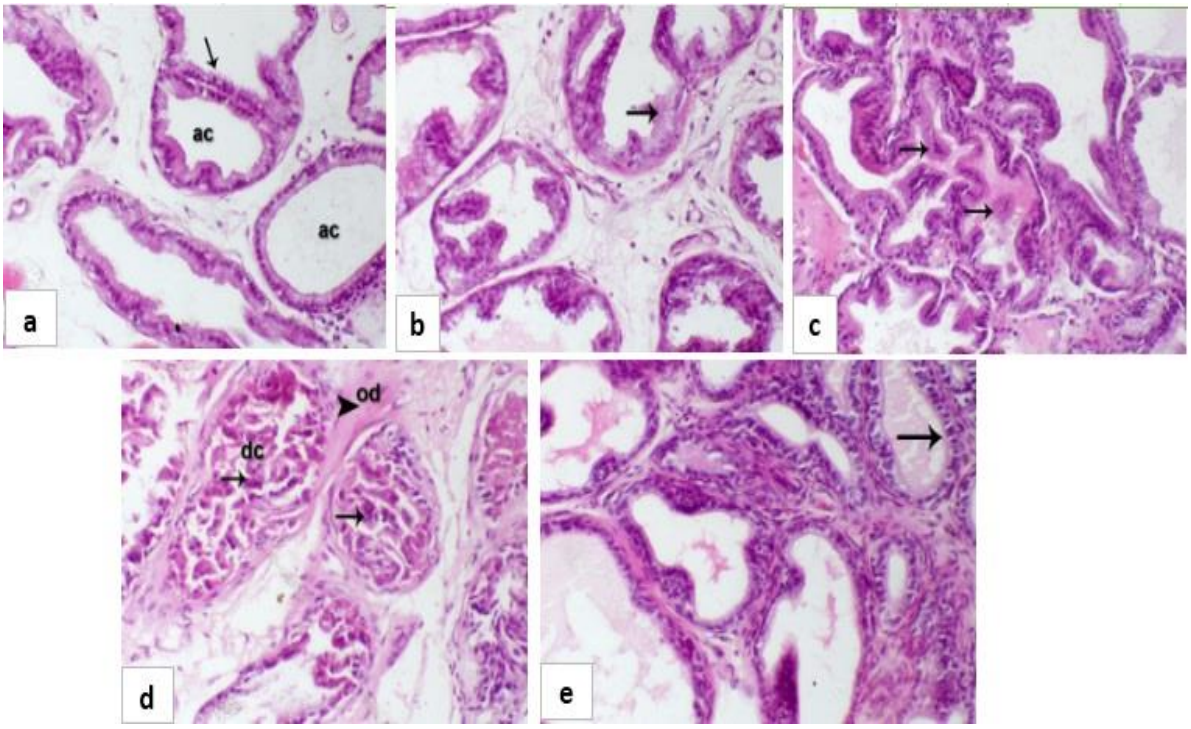

Fig. (1): Photomicrograph in: (a): Prostate of control rat showing normal glandular acinar structures (ac) lined with normal basal and acinar cells (arrow), **(b):** Prostate of rat treated with saffron showing normal glandular acinar structures lined with normal epithelium forming minutes papillae (arrow), **(c, d):** Prostate of rat treated with khat showing degeneration of glandular lining epithelium with exfoliation within the lumen (arrows), severe degree of degenerative changes (dc) within the glandular lining epithelium (arrows) and marked oedema (od) in the interstitial tissue (arrowhead) and **(e):** Prostate of rat treated with khat & Saffron showing mild degeneration of the glandular epithelium (arrow). **(Cross sections 6µm stained with H&E., ×200).**

Histochemical observations:

Polysaccharides

The distribution of polysaccharides in the prostate tissue from the control and saffron groups was meticulously examined and showed a magenta color which indicated a PAS-positive reaction. The reaction appeared strong in the lining epithelium of the acini (**Fig.2 a, b**).

On the other hand, examination of the prostatic tissue from rats in the khat group showed a reduction in the number of polysaccharides particularly in the epithelia of prostatic acini (**Fig.2c**).

Examination of PAS reaction in prostate sections of rats treated with khat and saffron revealed a gradual increase in polysaccharide content compared to the khat group (**Fig.2d**).

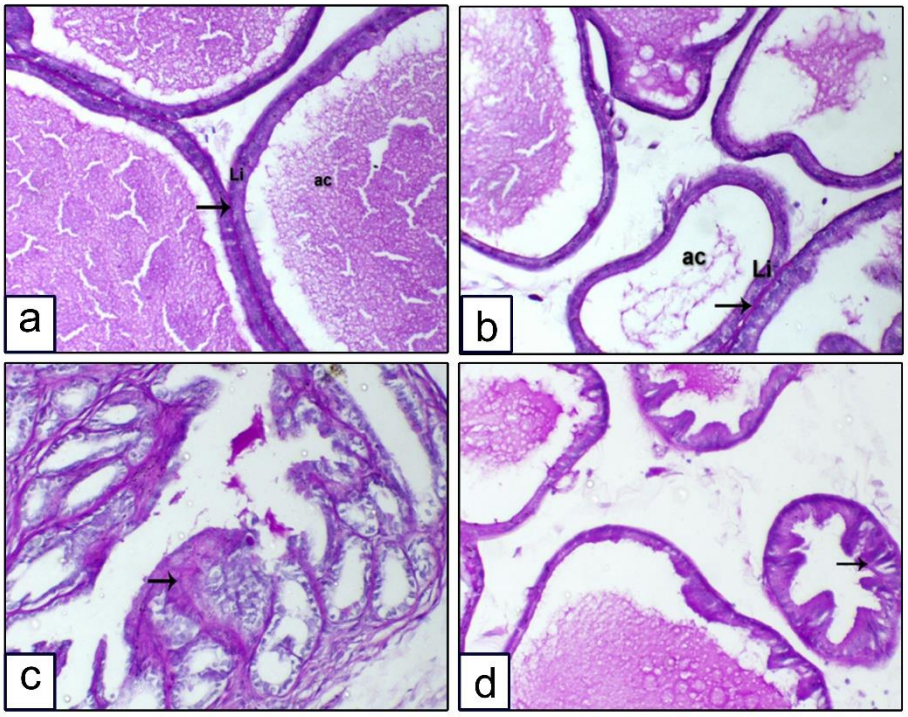

Fig (2): Photomicrograph in: (a): Prostate of a control rat showing strong polysaccharides staining (magenta) (arrow) in the lining epithelium (Li) of the acini (ac), **(b):** Prostate of saffron treated rat showing dense polysaccharides content (arrow) in the lining epithelium (Li) of the acini (ac), **(c):** Prostate of khat treated rat showing moderate polysaccharides staining with low polysaccharides content and **(d):** Prostate of khat & saffron treated rat showing moderate polysaccharides content. **(Cross sections 6µm stained with PAS stain ×200).**

Total proteins

Examination of the prostate of the control and saffron rats group showed high protein content with intensive bromophenol blue staining within the lining epithelium of the acini **(Fig.3a, b)**.

This bluish staining level of bromophenol blue decreased in the lining epithelium of the acini of the prostate gland of rats treated with khat extract as a result of decreasing protein content compared with the control group. **(Figs.3c).**

On the other hand, treated animals with khat and saffron together every other day for 3 months showed a gradual increase in the protein content that appeared in the lining epithelium of the prostate acini compared with the khat group **(Figs.3d).**

Immunohistochemical observations:

BCL2 expression

A sections of the prostate of control and saffron-treated rats were examined after 3 months, the glandular epithelial cells showed high cytoplasmic BCL2 expression as a brown color (**Fig.4a, b**). The area percentage of BCL2 expression did not show any significant change between the control and saffron groups (**Table 2**).

While in the khat-treated group, a few glandular acini epithelial cells showed BCL2 expression in their cytoplasm (**Fig.4c**). The area percentage in the khat group increased substantially compared to the control group (**Table 2**), suggesting a potential link between khat treatment and Bcl2 expression. In the khat and saffron group, the BCL2 expression increased in the cytoplasm of the epithelial cells when compared to khat alone (**Fig.4d**).

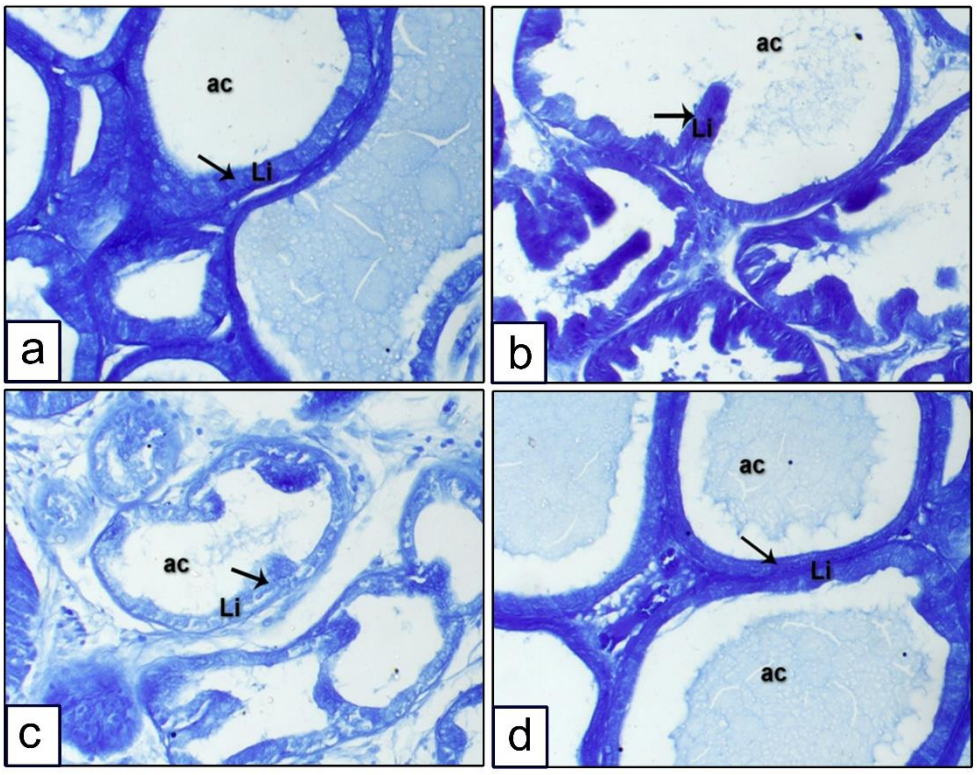

Fig (3): Photomicrograph in: (a, b) Prostate of normal & Saffron rat groups showing marked highly stainability by normal blue color within the lining epithelium of the acini (arrow), **(c),** Prostate of rat treated with khat showing decrease in the proteinic content by decreasing of Bb stainability within the lining epithelium of the acini (arrow) and **(d),** Prostate of rat treated with khat & Saffron showing marked increase of staining of the epithelial lining of acini (arrow**). (Cross sections 6µm were stained with bromophenol blue, ×200.)**

Table (2): The area percentage (Mean±SD) of Bcl2 and Bax expressions in the different groups.

Animal groups	The area percentage of Bcl2, Bax, Vimentin and E-cadherin (Mean±SD)			
	Bcl2	Bax	Vimentin	E-cadherin
Control	10.701±0.3	0.051±0.01	7.859±0.28	5.371±0.23
Saffron	11.336±1.6	0.071±0.01	9.175±0.24	3.633±0.21
Khat	1.166*±0.1	7.097*±0.2	0.373*±0.06	0.112±0.01
Khat+saffron	8.205**±0.2	0.985**±0.1	2.74**±0.33	2.2±0.43

n= 10 animals per group
(*) Significant compared to the control group at P<0.05.
(**) Significant compared to the khat group at P<0.05.

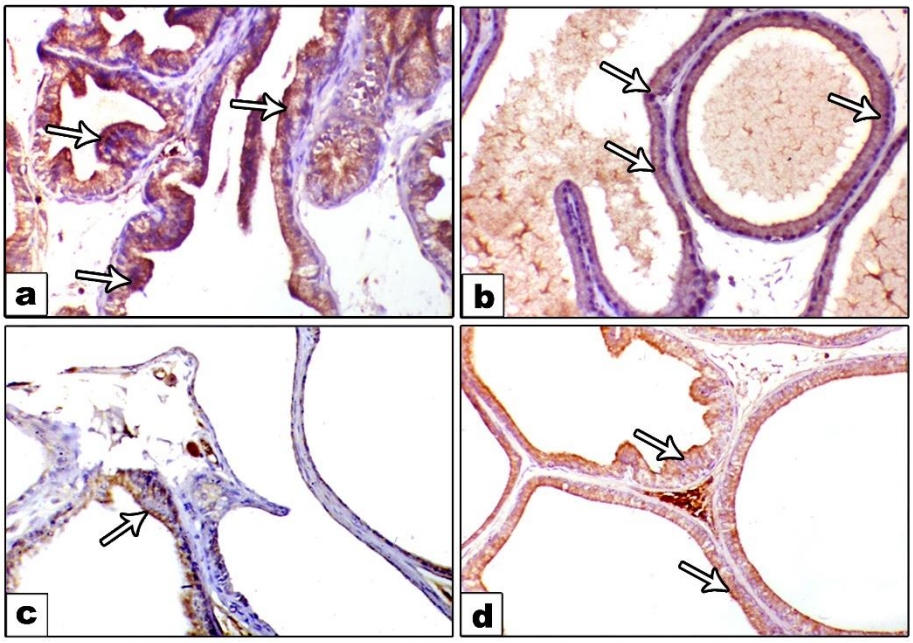

Fig. (4): Photomicrograph in: (a & b): prostate of control & saffron rat groups showing marked brownish cytoplasmic BCL2 immunostaining within the glandular lining epithelium (arrow), **(c):** Prostate gland of khat treated animal showing mild/high decrease of the cytoplasmic expression of BCL2 antibody within the epithelial lining of the acini (arrow) and **(d):** Prostate gland of khat and saffron treated animal showing an increase of the cytoplasmic expression of BCL2 antibody within the epithelial lining of the acini (arrow) **(Cross sections 6µm stained with BCL2 IHC, ×200).**

BAX expression

In control and saffron-treated rats, the nuclei of the prostatic epithelial cells showed negative Bax expression. Between these two groups, the area percentage did not show any significant difference **(Table 2 and Fig. 5a, b)**. In the khat group, a strong positive expression of Bax (as a brown color) appeared in a large number of the nuclei of epithelial cells. Furthermore, rats given khat showed a highly remarkable increase in the percentage of area of the Bax expression **(Table 2 and Fig. 5c).** However, when rats were treated with both khat and saffron, the number of nuclei of prostatic epithelial tissues showed a decrease in the Bax expression **(Fig. 5d).**

E-Cadherin expression

The prostate sections of the control and saffron-treated rats were examined, and most of the glandular epithelial cells showed high cadherin expression as a brown color in between cells (**Fig.6a, b**). The area percentage of cadherin expression did not show any significant change between the control and saffron groups (**Table 2**).

Conversely, a stark contrast was observed in the khat-treated group. the glandular epithelia acini cells displayed low cadherin expression in their cytoplasm and near cell walls, indicated by a less intense brown color (**Fig.6c**). Notably, the area percentage in the khat group showed a substantial increase compared to the control group, as detailed in (**Table 2**). In the khat and saffron group, the cadherin expression exhibited a more intense brown color in the cytoplasm and near cell walls of the epithelial cells, a notable difference from khat alone (**Fig.6d**).

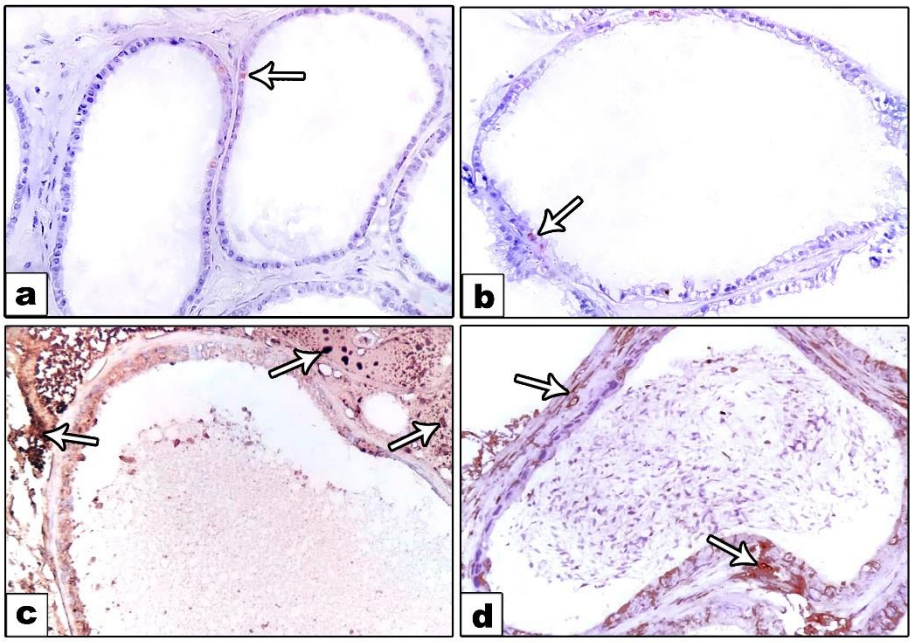

Fig. (5): (a-b) Photomicrograph in the rat prostate of control and saffron group showing negative Bax expression in the nuclei of glandular lining epithelium of prostatic acini cells; (c): Khat group showing condensed Bax expression in the of lining epithelial cells; (d): Khat and saffron group showing moderate expression of Bax expression in nuclear of the epithelial cells (BAX immunostain).

Vimentin expression:

When the prostate sections of the control and saffron-treated rats were examined, almost glandular epithelial cells showed high vimentin expression as a brown color in the cytoplasm (**Fig.7a, b**). There was no significant change between the control and saffron groups in the area percentage of vimentin expression (**Table 2**).

On the other hand, the khat-treated group shows low vimentin expression in the cytoplasm of the glandular epithelia acini cells by decreasing the brown color (**Fig.7c**). Also, when compared to the control group, the area percentage in the khat group increased substantially (**Table 2**). In the khat and saffron group together, the vimentin expression showed an increased brown color in the cytoplasm of the epithelial cells of the prostatic acini when compared to khat alone (**Fig.7d**).

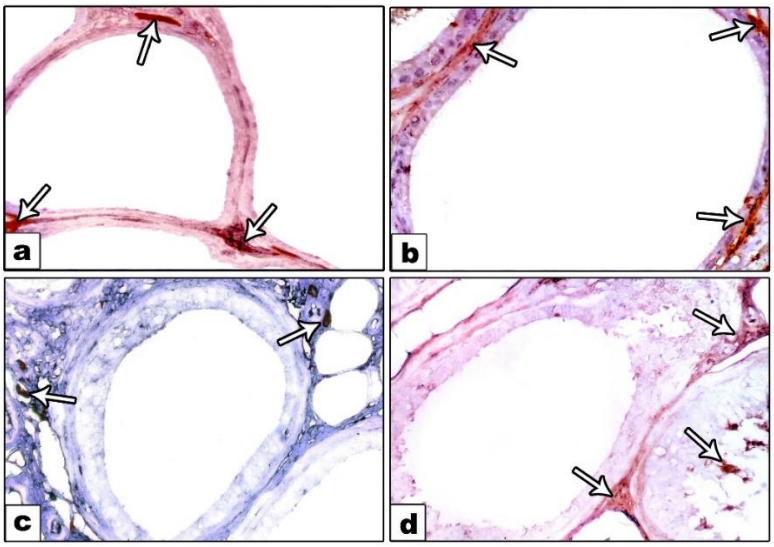

Fig. (6): (a-d) E-Cadherin immunoreaction (a, b): control and saffron groups showing strong expression of e-Cadherin with brown color; (c): khat group showing a marked reduction in E-cadherin immunoreaction with less brown color; (d): khat and saffron group showing a moderate expression of cadherin reaction.

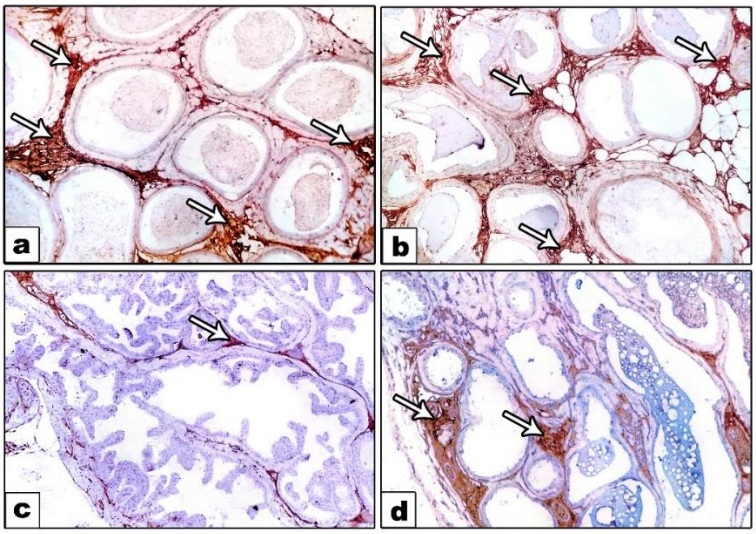

Fig. (7): A photomicrograph from Prostatic tissue (a-d) vimentin immunoreaction; (a, b) control and saffron groups: showing strong positive expression of vimentin as a brown color; (c) khat group showing a highly significant reduction in the vimentin expression as faint brown color; (d) khat and saffron group showing vimentin expression as a moderate brown color;

4. Discussion:

In the present histological investigation results rats treated with khat recorded abnormally flattened epithelial layers in the prostatic acini, along with many alterations in the prostate gland tissues when compared with the control group. These findings may be due to the high oxidative stress of khat and its active component cathinone which increases the free radicals and hampers the body's ability to clear these free radicals which in turn cause tissue damage **(36).** The imbalance in the biological system's ability to produce reactive oxygen species (ROS) and its capability to detoxify reactive intermediates and repair damage leads to oxidative stress. This, in turn, results in the production of free radicals and peroxides, which harm every cell component **(37)**. Our results align with those of **(38)**, who also observed that khat extract increased oxidative stress and elevated ROS production, potentially affecting sterility in female rats.

Dosing saffron and khat together reduced the harmful effect of khat by inhibiting the histopathological changes in prostate tissues. This may be attributed to the saffron scavenging activity against the free radicals, which shield cells from oxidative damage **(39).** Our results agree with **Sharma, *et al.*, (2023) (40)** who suggested using saffron as a powerful antioxidant to increase antioxidant capacity and prevention from the destruction of tissue cells caused by the high oxidation pressure which is induced by the incomplete combustion of oxygen during the mitochondrial cycle.

Histochemical studies on rats treated with khat revealed a remarkable reduction in the cytoplasmic polysaccharides and proteinic materials in the prostatic tissue**.** These results, which may be due to the high oxidative stress caused by cathinone and khat components, underscore the potential health risks of khat consumption. **Tarboush, *et al.*, (2019) (41)** discuss that oxidative stress and free radical reactions are involved in the etiology of various diseases, where free radicals can react with lipids, proteins, and DNA or RNA. Also, **Amin *et al.*, (2020) (42)** explained that cathinone which is considered the primary active ingredient in khat; increases serum cortisol levels, stimulates catecholamine release, activates adrenergic receptors, and contributes to the inhibition of insulin release; which lowers the amount of polysaccharides in the cells**.**

These findings, supported by **Wabe, (2011**) **(43)** who reported that khat extract caused a decrease in the glycogen content in rabbits due to the stimulating effect of khat on adrenocortical function. Likewise, **Muema *et al.*, (2016) (44)** reported that khat consumption leads to decreasing serum albumin concentration, he explained this deficiency in the albumin protein due to the effect of khat components, which led to liver and kidney damage.

In the present study, polysaccharides and protein content are greatly recovered in the prostate tissue after rats received saffron and khat together. The improvement in protein and carbohydrate contents by saffron may be attributed to the scavenging ability of saffron components to free radicals. **Sen *et al.*, (2010) (45)** discuss that the body obtains energy by the oxidation of carbohydrates, fats, and proteins through both aerobic and anaerobic processes, which leads production of free radicals, which explains this overconsuming of carbohydrates and proteins and considers evidence of the sever oxidation happened by khat components, he also cleared the reason of recovering in protein and carbohydrates by scavenging the free radicals and decreasing the oxidative stress. This result by **Khadfy *et al.*, 2023 (46)** confirmed the ability of saffron and its components to devour free radicals and protect against oxidation.

In the present study, the treatment with khat resulted in a decrease in the cytoplasmic expression of BCL2, E-cadherin, and vimentin, while increasing BAX expression in the prostate cells. These changes in protein expression are significant as they are associated with the regulation of apoptosis, a highly

regulated process to eliminate unwanted or defective cells (47). The decrease in BCL2, E-cadherin, and vimentin, and the increase in BAX expression, could be attributed to toxicity at the cellular level. In contrast, it is well known that the BCL2 protein family regulates the release of apoptosis-inducing factors, where the ratio of BCL2 to Bax determines the direction of cell death or maintenance of cell survival (48-50). Also, khat caused apoptotic cell death by activating caspases -1, -3, and -8. (7).

The present findings are in line with previous research by **El-Setouhy and Hassan, (2022)** (14) who reported that khat inhibited the viability and proliferation of various cell types in vitro. The cells exposed to khat exhibited ultrastructural changes that resemble apoptosis features, suggesting a potential mechanism for the observed effects. This inhibition was likely mediated via the mitochondrial pathway of apoptosis and associated with an alteration in the mitochondria phenotype and increased Bax and decreased Bcl-2, E-cadherin, and vimentin protein expression. Similarly, cell death in khat-exposed cells was associated with increased ROS production, decreased mitochondrial function, and activation of mitochondria-mediated cell death pathways.

Rats treated with saffron and khat together exhibited a significant increase in the expression of BCL2, Vimentin, and E-cadherin, while the expression of BAX in their prostate was reduced. This finding underscores the potential impact of saffron and khat on gene expression. The presence of safranal in saffron, with its unique mechanism, including anti-oxidant, anti-apoptotic, and regulatory effects on the expression of genes and proteins implicated in different signaling pathways linked to oxidative stress, apoptosis, and proliferation, may have contributed to this result (23). Furthermore, the present outcome might be explained by the anticancer-related crocin action mechanisms, including cell cycle arrest at G0/G1 or G2/M phases, induction of caspase-dependent apoptosis, and signaling pathway-linked tumor metabolism regulation (26). This result by **(Albalawi *et al.*, 2023)** (51) reported that crocin significantly reduced expression levels of BAX in conjunction with increased levels of expression of BCL2.

Concerning the biochemical results, an increase in the MDA concentration and a decrease in SOD, and CAT enzyme activity were detected in khat-treated rats. These results are consistent with the current histological and immunohistochemical observations. The biochemical alterations induced by khat could be attributed to the oxidative damage of the khat extract, in which the administration of khat can induce tissue toxicity in rats through the induction of oxidative stress by either depleting antioxidative mechanisms or by enhancing prooxidant ingredients of tissues, according to new research suggest (52). These results are also in parallel with (**El-Setouhy and Hassan, 2022**) (14), who reported that chronic khat chewing induces ROS production and potentially causes oxidative toxicity and decreased SOD level.

In the current study, we demonstrate the effect of Saffron as an antioxidant by observing the reduction of MDA concentration and the increase in the activity of SOD and CAT in rats treated with both khat and saffron. These results can be attributed to Saffron, specially to crocin and safranal which have a strong effectiveness in regulating the state of oxidative stress and work to restore the balance of oxidants and antioxidants, leading to a significant decrease in the levels of oxidants in the blood and a significant increase in antioxidants (53).

Kocaman *et al.*, (2021) (54) in line with the current results, explained that 100 mg/kg/day of crocin, the responsible agent in saffron, reduced MDA levels and maintained the rate of SOD and CAT activity.

Conclusion

From the present study, we conclude that khat adversely affected the prostate of male rats via enhancement of oxidative stress. also, Saffron approved when used in conjunction with khat

extract, significantly protects the health of the prostate in male rats. This was evidenced by the improvement in histological, immunohistochemical, and biochemical results compared to the khat group. This could be linked to the ability of saffron to restore the balance of oxidants and antioxidants by scavenging the free radicals produced due to khat components.

Conflict of interest statement:

None of the authors has a conflict of interest.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The study has been authorized by the Animal Care and Bioethics Committee, Egypt (Approval No. MNSH 175).

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