Applications of Sumach extract in reduction of male reproductive parameters damages following morphine administration through down-regulated apoptotic genes, antioxidants regulation, and inflammatory markers suppression

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SUMMARY

Morphine (MO), a psychoactive member of opium family, causes free radicals' accumulation in cells. *Sumach* (SU) is a medicinal plant with antioxidative activities. This study was designed to assess the probable ameliorative effects and alteration of apoptotic genes expression after SU extract administration on testopathy caused by chronic MO contamination. Sixty-four male rats were divided into 8 groups: 1, normal; 2, MO; 3, 200 mg/kg SU; 4, 400 mg/kg SU; 5, 800 mg/kg SU; 6, MO + 200 mg/kg SU; 7, MO + 400 mg/kg SU; and 8. MO + 800 mg/kg SU. All intraperitoneal injections of MO (10 mg/kg) were applied on the first day of the experiment, and SU extract was also treated orally (through a nasogastric tube) on the other 2-28 days. Apoptotic genes expression (*P53*, *Bcl2* and *caspase-3*) and inflammatory cytokines were measured by Real-Time PCR and ELISA techniques, respectively. Also, total antioxidant capacity (TAC) and male reproductive parameters were detected quantitatively. In MO group, significant detrimental changes of testes (including all investigated parameters) were detected in comparison with the normal group ($P < 0.01$), but genes expression of P53 and caspase-3 and inflammatory cytokine showed a significant incremental trend. In *SU* (200, 400, and 800 mg/kg) and *SU* (200, 400, and 800 mg/kg) + MO treated groups, all values were accelerated significantly in comparison with MO group ($P<0.01$), but genes expression of P53 and caspase-3, along with inflammatory cytokine indices, were down-regulated. Totally, therapeutic effects of SU extract were approved biochemically and histologically to scavenge MO impacts.

Key words: *Sumach* – Male reproductive – Morphine – Apoptosis – Inflammation – Antioxidant

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INTRODUCTION

Typically, MO is used as an opiate for acute pain medication by direct effects on the nervous system (Jalili et al., 2016). Ward et al. (2020), scientifically proved that opioids could accelerate free radical production in the body to an unhealthy state, which leads to widespread cell death. Mainly, MO is detected as an opioid with damaging consequences on the male sexual system. These adverse effects included hypogonadism, histopathological transitions, and severe sexual hormonal imbalances (Karami et al., 2019). MO disrupts regular spermatogenesis and pituitary-hypothalamic testicular axis (Roshankhah et al., 2017). Apart from demolition effects on either sexual system, apoptosis was also observed on non-sexual cells, including neurons and hepatocytes (Jabari et al., 2019). Chronic consumption of MO can lead to aggregation of free radicals. Regarding MO administration, produced ROS neglect the three-dimensional DNA molecule and membrane organelles (Famitafreshi et al., 2020). In these cases, the intervention of the immune function to stand detrimental effects is often in a slow reaction. Also, there is no absolute therapeutic protection against such irreversible disorders. In this situation, utilization of plant-based antioxidants with lesser side-effects becomes a safe and healthy procedure (Jalili et al., 2019a).

SU is an herb with antioxidant features belonging to *Anacardiaceae* family. This plant generally grows in the mountainous regions of Iran (Gharaei et al., 2013). In traditional medicine, the SU is hired to cure insect bites, hemorrhoids, and colic pain (Shabbir et al., 2012). Anwer et al. (2013), confirmed that the SU is high in tannins and flavonoids as critical sources of antioxidant. Multiple literatures have indicated the hypoglycemic, antitumor, and antioxidant activities of Flavonoids (González et al., 2011). Based on the theoretical hypotheses, hydroalcoholic products of SU improves enzymatic efficacy of cellular antioxidants, including catalase and superoxide dismutase (Ahangarpour al., 2014).

According to the physiology of reproductive system components, any minor changes in structure or role of this organ could cause considerable disruptive results, such as lost fertility or full infertility conditions (Schilit et al., 2020). As was suggested in the previous study, infertility can be seen in more than 50% directly related to men's origins (Ho et al., 2020). Free radicals invade unsaturated fatty acids in cell membranes and trigger intracellular alkylation of protein molecules. They could also provoke necrosis via various mechanisms, including membranous lipid peroxidation and cytoplasmic partial removal of necessary enzymes (Tremellen et al., 2008). Reactive oxygen species (ROS) could arrest cell cycle leading to programmed cell death. Outcomes of this alteration can be more severe in poor-proliferating cells such as spermatozoa (Jalili et al., 2014; Ellis et al., 2016). Although MO administration is extremely common in medications and its adverse reactions on male sexual operation is authorized, also due to the antioxidant quality of SU to eliminate free radicals, this experiment was intended to investigate the potential therapeutic properties of SU on male rat reproductive dysfunction during MO administration.

MATERIALS AND METHODS

SU seed extraction method

Fresh SU plants were obtained from the local grocery (Javanrood town, Kermanshah, Iran). A botanist (Department of Pharmacognosy, Faculty of Pharmacy, Hamadan Medical Sciences University, Hamadan, Iran, voucher specimen NO. PCT-2037) identified the accuracy of the plant. An automatic grinder was also used to grind dried seeds. Then hydroalcoholic compound was constructed as follows: 300 gr of SU powder was dissolved in 1300 mL of concentrated mixture of water-ethanol. The solution was allowed to stay for 2 days at room temperature and purified by the use of filter paper; then the centrifugation was accompanied (4000 rpm, 15 min). The extract was preserved for potential administration at 37^{O}C after withdrawal of supernatant (Ahangarpour et al., 2014).

Phytochemical analysis

Semi-quantitative phytochemical analysis was used based on standard protocols (Treas and Evans, 1989). Ethanol (80%) was percolated

with 5 gr of SU powder. Vaporization was then established. TLC on silica gel Merck 60F245 (thickness 0.2 mm) was recruited to identify in 80% of ethanol extract; Liebermann-Burchard as a reagent and hexane/ethyl acetate; 1:1 as a mobile layer for terpenes and sterols (color range was produced after 10 min at 100° C sprayed plate heating) were also provided. Classical acid/base separation strategies were also established and examined in chloroform/methanol/ammonia solution by TLC as a solvent method for alkaloids. Then, patches were observed by applying Dragendorff 's reagent spry method. TLC was formed in 4:1:5 (top layer) n-butanol/acetic acid/water for flavonoid recognition, and 1% aluminum chloride solution in methanol (under UV, 366 nm) for spot representation. Tannin and saponin recognition was applied by adding 1% gelatin solution and froth analysis separately. In methanol, anthraquinones and phlobatannins were also reported by 10% potassium hydroxide solution (Roshankhah et al., 2020).

Antioxidant capacity assessment by 2,2, dipheny l,1, picrylhydrazyl (DPPH)

DPPH-free radical assay was used to assess antioxidant levels. In reaction among DPPH and an antioxidant agent which provide hydrogen, the DPPH is modified into a reduced state. Color adjustment (deep violet to pale yellow) is recorded using a spectrophotometer. DPPH (200 µl) was added to each dilution. During a half-hour in dark incubation, its emission was documented (517 nm). As a positive control, butylated hydroxytoluene (BHT, a typical antioxidant) with equal concentration was hired. The following method was used for the scavenging of free radicals; AA%= [A0-A1 / A0]100 (A0; DPPH absorption, A1; butylated hydroxytoluene and SU absorption) (Hosseinipour et al., 2019).

Animals

The University's animal house was considered as a center of animal training. Sixty-four male Wistar rats (200-230 gm) were collected. All typical environment requirements were equipped, including 12:12 hour light/dark period and 22 ± 2°C. This experimental approach was authorized by the ethical committee of the Kermanshah University of Medical Sciences and implemented in line with the National Institution of Health Guide for Laboratory Animals (IR.KUMS.REC.1397.500).

Design and settings

Sixty-four male Wistar rats were randomly divided into 8 groups (8 rats in each group). The first group (normal) handled with normal saline (resemble experimental groups in quantity (orally); the second group (MO) was handled as an established procedure (injection of 10 mg/kg on first day, followed by 20 mg/kg/day on 2-28 days); the third to fifth groups (SU) were handled with doses of 200, 400 and 800 mg/kg daily for 28 consecutive days; the sixth to eighth groups (MO+SU) were handled with MO followed by daily specific doses of 200, 400 and 800 mg/kg of SU (days 1–28). Oral (nasogastric tube) and injection procedures were handled for SU and MO administration, respectively. MO was treated from 9:45 AM to 10 AM (Ahangarpour et al., 2014; Jalili et al., 2016).

Sperm sample preparation

At the end of the procedure, the animals were anesthetized by intraperitoneal injection of ketamine HCl (100 mg/kg) and Xylazine (70 mg/kg). Blood samples were aspirated by subxiphoid pathway. Samples were placed for 20 min at atmospheric pressure and temperature. They were centrifuged for 10 min at 300 g. For biochemical study, purified blood serum was placed at -70 °C refrigerator. DMEMF12/FBS5% culture medium was used for scrotum dissection. Right testes were used for biochemical assays, and the left were fixed for histopathological evaluations in 10% formalin. A warmed petri dish (37 °C) comprising 10 ml Hank's balanced salt solution was also used for dissection of caudal portion of both samples. Dissolved sperms were used in order to assess sperm parameters (400x) (Roshahnkhah et al., 2017).

Histopathological methods

The left sample was immersed in standard saline and fixed in formaldehyde (10%) for 48 h (right specimen was used for biochemical studies). Five-micrometer cuts of the left testis

were processed using a microtome and stained with hematoxylin and eosin. Histological and GLH morphometric evaluations were treated under light microscopy and recorded by a Motic camera.

Sperm viability

Eosin penetrated into cells to discriminate between destroyed sperm cells and living ones. 20µl of condensed semen was diluted with the same quantity of eosin staining. 2-5 min later, half of the mixture was moved to a neobar slide culture to determine live (no pink) and dead (cytoplasm-pink) samples (40x). 100 sperm cells were included (from each sample) in 10 imaging fields. Eventually, sperm ratio was reported (Roshankhah et al., 2017).

Progressive sperm motility assessment

Only progressive movement was regarded as healthy and appropriate sperm motility according to the WHO recommendation (2010). 100 µl sperm storage was imposed on a slide field (Jalili et al., 2014).

Sperm count

400 µL suspension (sperm) was dissolved in formaldehyde fixative (Sigma; USA) to evaluate sperm count. 15 µL of sample was mounted on a hemocytometer. After 20 minutes, the steady cells were monitored and measured per 250 minor hemocytometer quadrangles (40x magnification). Semen concentration (per mm^3) equated to the sperm count (Jalili et al., 2016).

Morphology of sperm cells

Phenotype of sperms was selected based on sperm in right cauda epididymis. For morphology assessments, an aliquot of each specimen was used. Later, for a closer evaluation, a light microscope (400x) was employed through Eosin/nigrosine staining. Overall, 500 spermatozoa were counted on each slide (5000 in each group) (Roshankhah et al., 2017).

GLH morphology evaluation

Fixed testicular samples were handled for these histological assessments through standard tissue processing, including dehydration, clearing and embedding. Hematoxylin and eosin stained the 5-µm slices. At least 30 frameworks were prepared. GLH evaluation was applied using a Motic camera (Jalili et al., 2016).

Strategy of plasma ferric reduction capacity (FRAP)

In the FRAP approach, the possible capacity of blood plasma to regain ferric ions is stately as a serum TAC index. This procedure required Fe^{III} ions. Blue stain was developed when the acid-pH Fe^{III}-TPTZ was converted into Fe^{III}. Absorption occurred at 500 nm wavelength. TAC levels were also plotted via the default iron sulfate concentration curve (Jalili et al., 2019b).

Sexual hormones examination

Serum and frozen specimens were used for hormonal assessment. ELISA (Abcam 107555, USA) method examined serum testosterone levels (Jalili et al., 2016).

Real-time PCR

Real-time PCR was used to examine the gene expression of caspase-3, Bcl2 and p53 [Table 1]. The testis specimen was suspended in liquid

Table 1. Real-time PCR primers.

Primer ID	Primer sequences
GAPDH	F: 50- AAGCTCATTTCCTGGTATG-30 R: 50- CTGCCACAAGAACTAGAGA-30
p53	F:50-AGAGACCGCCGTACAGAAGA-30 R:50-GCATGGGCATCCTTTAACTC-30
caspase-3	F: 50-ATGGCGAAATGGAGATGAATA-30 R: 50-ACTGCCCATGATGGTTCTGTG-30
Bax	50-TGG GATGCCTTTGTGGAACT-30 R:50-GCATGGGCATCCTTTAACTC-30

nitrogen and stored at -80 °C in a freezer. RNA was extracted with a RNeasy kit (Qiagen), and DNA samples were prepared with a DNase kit (Qiagen). cDNA was developed using cDNA Synthesis Kit (Fermentas). Gene expression rate was calculated using the Maxima SYBR Green (Fermentas) approach (Esfandiari et al., 2014).

RESULTS

Phytochemical screening of SU

Pharmacologically, the product was formulated in 9-Octadecenoic acid (Z), methyl ester, Dodecanoic acid, Saponins, and tannins. Natural herbal products were also measured, including flavonoids, Phlobatannins, Anthraquinones, and Phlobatannins (Table 2).

Table 2. Phytochemical screening of hydroalcholic extracts of SU.

Phytochemical tests	Extract
Saponins	++
Flavonoids	++
Alkaloids	+
Tannins	++
Anthraquinones	+
Phlobatannins	+
9-Octadecenoic acid (Z), methyl ester	++
Dodecanoic acid	++

+ Mild presence, ++ Strong presence, SU: Sumach

Inflammatory cytokines assessments

Interleukin 1 beta (IL-1β) (Abcam Cambridge, UK) and tumor necrosis factor-alpha (TNFα) (Abcam, Cambridge, UK) of testis samples were analyzed using ELISA approach. RIPA (Abcam, Cambridge, UK) lysed full testis proteins and centrifuged at 15,000 g for 30 min. 1:20 supernatants/dilutions ratio was implanted into covered microplates with antibodies to provoke enzyme-substrate interaction. Standard solutions for drawing standard curves were also hired. ELISA kits explored the protein levels in supernatant divisions. Absorbance percentage was measured at 450 nm (Jalili et al., 2019b).

Statistical evaluation

Kruskal–Wallis was used to assess data normality and variance homogeneity at significance of 0.05. Both data analyzes were conducted using SPSS Statistics ver. 16 (SPSS Inc., Chicago, IL, USA). Variance analysis (ANOVA) was used. Mean ± standard error of mean and $P<0.05$ were considered to be statistically significant.

Sperm viability, motility, count, and morphology

MO, due to the inhibitory aspects, limited viability, progressive motility, and count of sperms, and even substantially improved typical morphology to pathological form compared to normal category ($P < 0.01$). Compared to standard group, SU displayed no significant alterations in SU (200, 400, and 800 mg/kg) groups ($P > 0.05$). Furthermore, these parameters were significantly enhanced in both SU (200, 400 and 800 mg/kg) and MO + SU (200, 400 and 800 mg/kg) groups compared to MO group (Table 3).

GLH

MO diminished GLH value in the MO group more than the normal group ($P < 0.01$). SU had no significant effects on SU group compared to the control group ($P > 0.05$). In SU (200, 400 and 800 mg/kg) and MO+SU (200, 400 and 800 mg/kg) groups, the GLH value was slightly higher than in the MO group ($P < 0.01$) (Fig. 1). Figure 2 demonstrated the GLH histopathological characteristics.

Table 3. Effect of SU and MO on sperm parameters (n=8).

Groups	Mean of sperm count (106)	Sperm progressive motility (%)	Sperm viability (%)	Normal sperm morphology (%)
Normal	93.36±4.64	33.36±3.10	91.36±6.8	82.64±4.62
MO	41.08±3.59*	5.39±0.33*	35.65±3.35*	31.83±2.11*
SU 200mg/kg	92.64±6.78†	31.34±3.61†	92.71±5.18†	81.04±3.09†
SU 400 mg/kg	94.82±6.77†	35.28±4.71†	90.03±5.94†	78.41±4.64†
SU 800 mg/kg	93.66±3.91†	33.12±4.34†	89.66±6.20†	83.64±6.71†
MO+SU 200 mg/kg	64.71±3.34¶	15.36±2.82¶	62.71±3.14¶	59.71±3.32†
MO+SU 400 mg/kg	59.55±4.85¶	12.81±3.43¶	60.38±4.72¶	59.91±4.02†
MO+SU 800 mg/kg	58.01±3.40¶	12.69±2.02¶	48.71±3.22¶	48.09±4.19†

Data presentation as mean ± SEM. * P < 0.01 compared to the normal group. † P < 0.01 compared to MO group. ¶ P < 0.01 compared to the MO group. MO: Morphine, SU: *Sumach*.

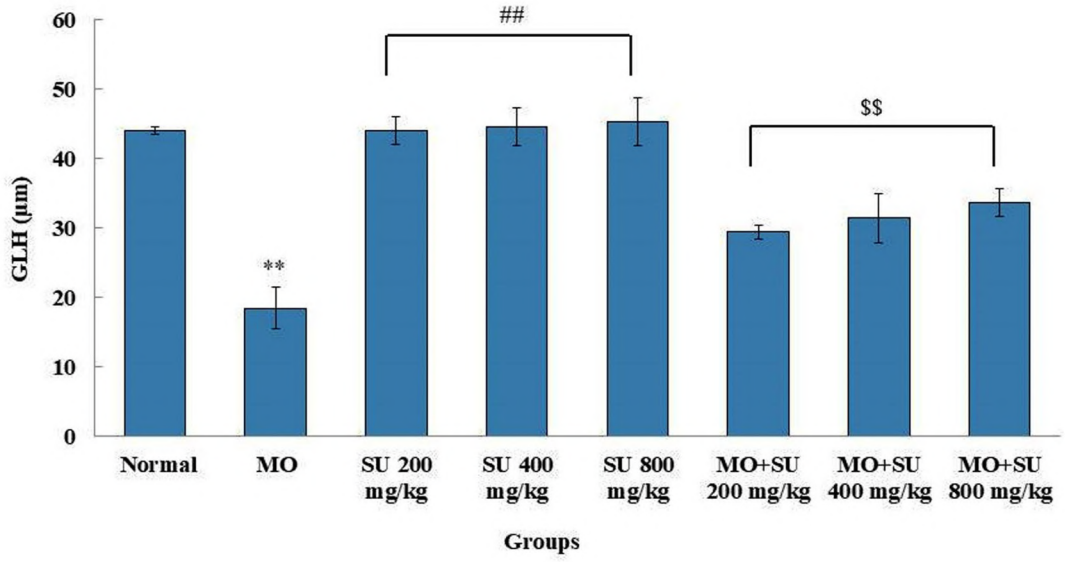

Fig. 1.- Comparison of GLH in all groups. **Significant reduction in MO group than normal group (P < 0.01). ## Significant differences in SU (200, 400 and 800 mg/kg) groups than MO group (P < 0.01). $$ Significant alteration in SU (200, 400 and 800 mg/kg) + MO groups than MO group (P < 0.01). MO: Morphine, SU: Sumach, GLH: Germinal layer height.

TAC

MO reduced TAC serum concentrations in the MO group in comparison with the normal group (P < 0.01). Also, SU elevated TAC levels in both SU ((200, 400 and 800 mg/kg) and MO + SU (200, 400 and 800 mg/kg) groups in comparison with the MO group (P < 0.01) (Fig. 3).

Testosterone levels

MO substantially decreased testosterone levels in the MO group in comparison with the normal group (P < 0.01). There were no significant biochemical differences in the SU group if compared to the normal group (P > 0.05). Similarly, testosterone in both SU (200, 400 and 800 mg/kg) and MO + SU (200, 400 and 800 mg/kg) groups displayed slightly higher amounts than MO (P < 0.01) (Fig. 4).

Gene expression rates

Up-regulated modifications of apoptotic p53 and caspase-3 genes and down-regulated differences of Bcl2 gene in MO-treated animals were identified as significant (P < 0.01). Similarly, a significant down-regulation of p53 and caspase-3 genes and up-regulated Bcl2 apoptotic gene were characterized in all SU doses and MO+SU (200, 400 and 800 mg/kg) groups than MO group (Fig. 5).

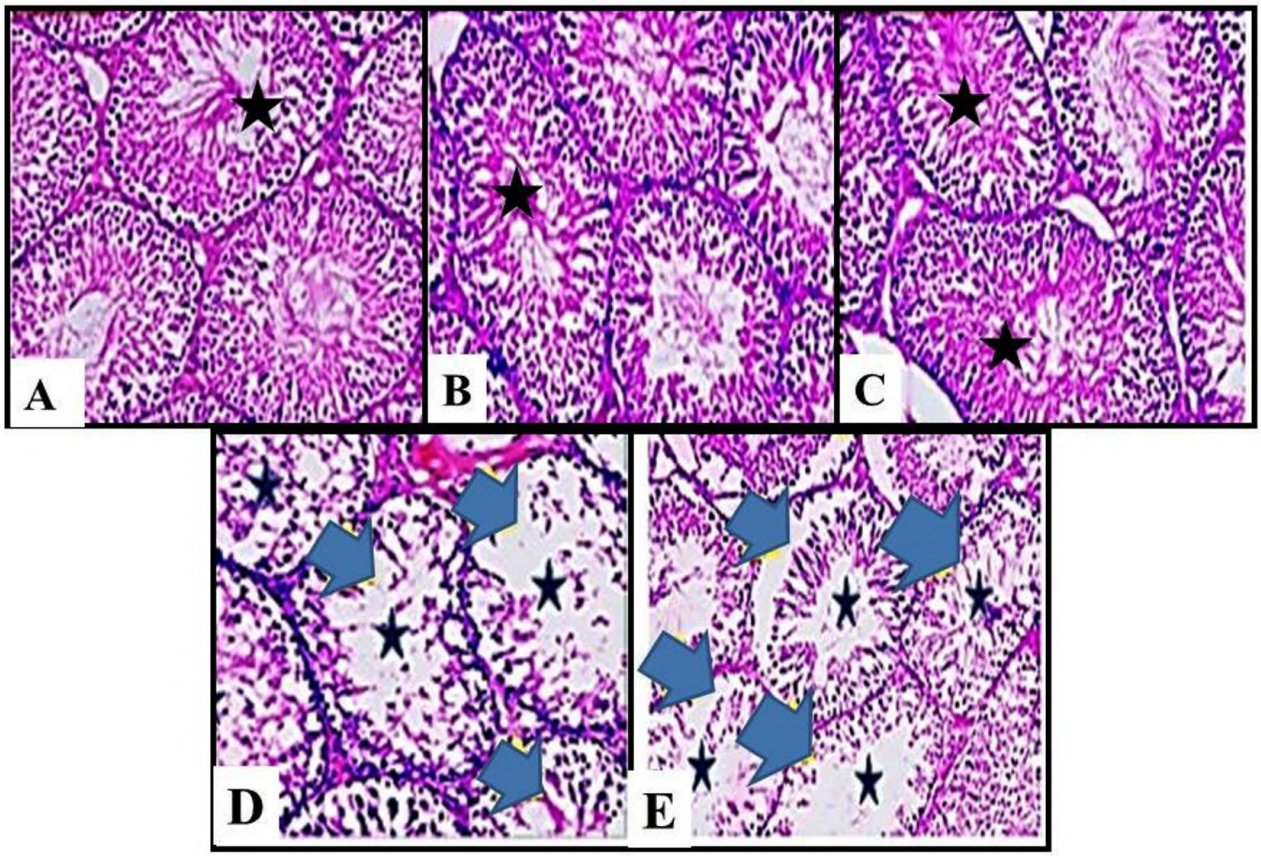

Fig. 2.- Effects of MO and SU on GLH value (×40). Healthy GLH structure was detected in groups of **A** (normal), **B** (SU, 800 mg/kg) and **C** (SU + MO, 800 mg/kg). A decreased level in GLH and sperm cells within the seminiferous tubule was observed in MO groups (**D** and **E**). Blue arrows indicated GLH value (decreased in GLH and irregularities in morphology of seminiferous tubule margin); and stars indicate sperm cells. MO: Morphine, SU: *Sumach*, GLH: Germinal layer height.

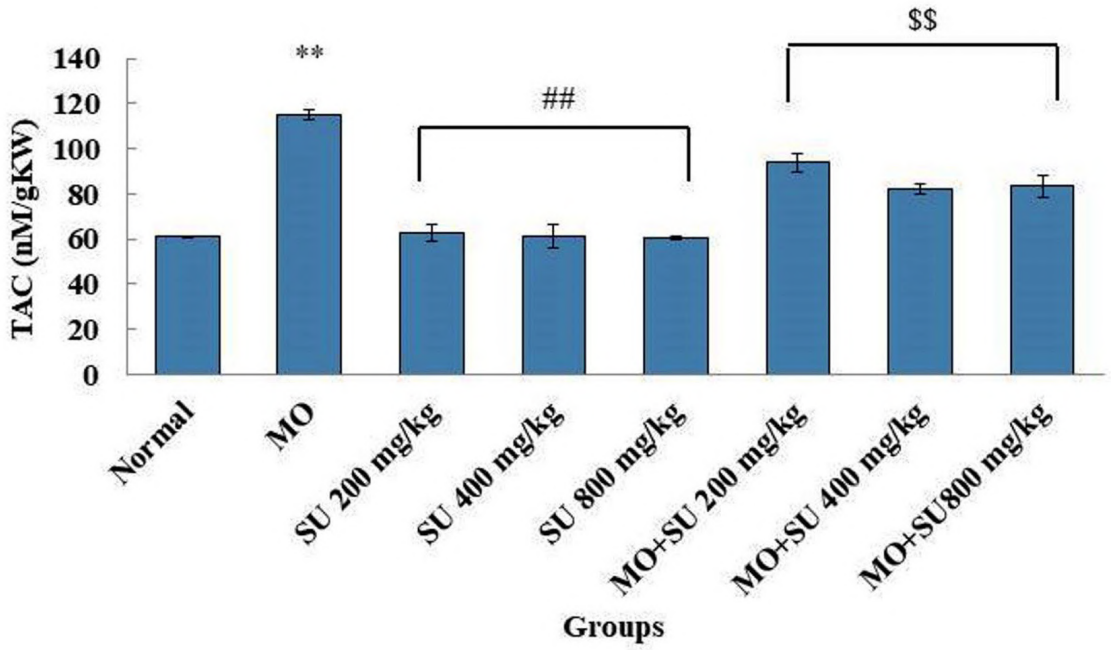

Fig. 3.- Comparison of TAC among treatment groups. **Significant reduction in MO group compared to normal group ($P < 0.01$). ##Significant increase in SU (200, 400 and 800 mg/kg) groups compared to MO group ($P < 0.01$). $$ Significant increase in SU (200, 400 and 800 mg/kg) + MO groups compared to MO group ($P < 0.01$). MO: Morphine, SU: Sumach.

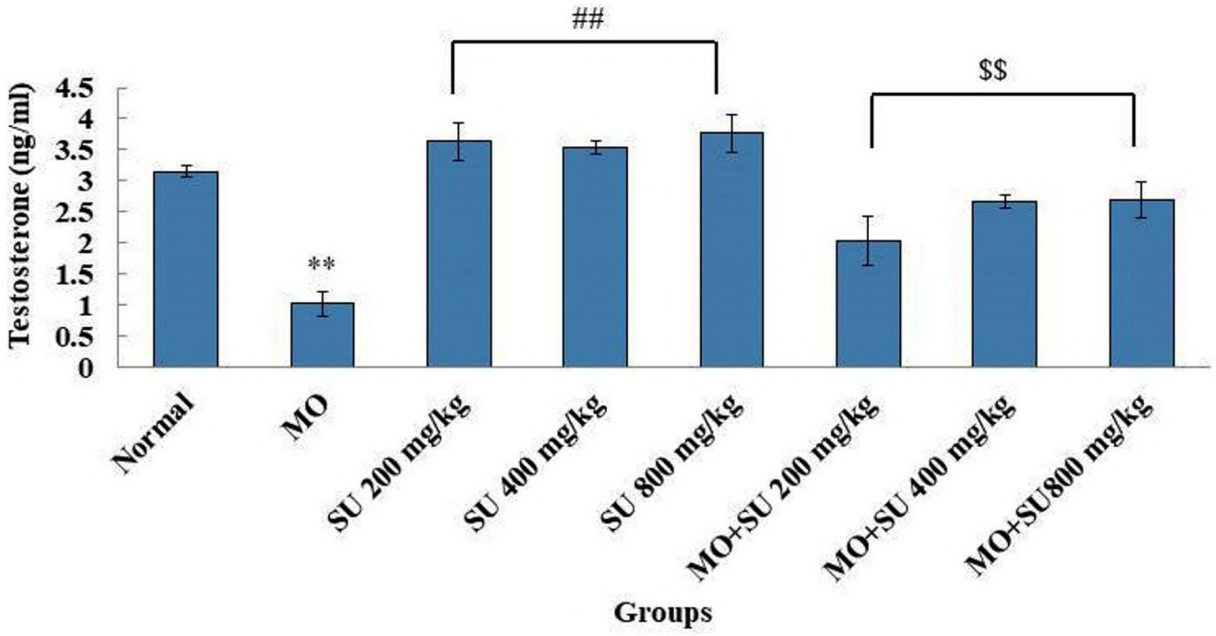

Fig. 4.- Comparison of testosterone hormone levels among treatment groups. **Significant decrease in MO group compared to normal group ($P < 0.01$). ## Significant increase in SU (200, 400 and 800 mg/kg) groups compared to MO group ($P < 0.01$). $$ Significant increase in SU (200, 400 and 800 mg/kg) + MO groups compared to MO group ($P < 0.01$). MO: Morphine, SU: Sumach.

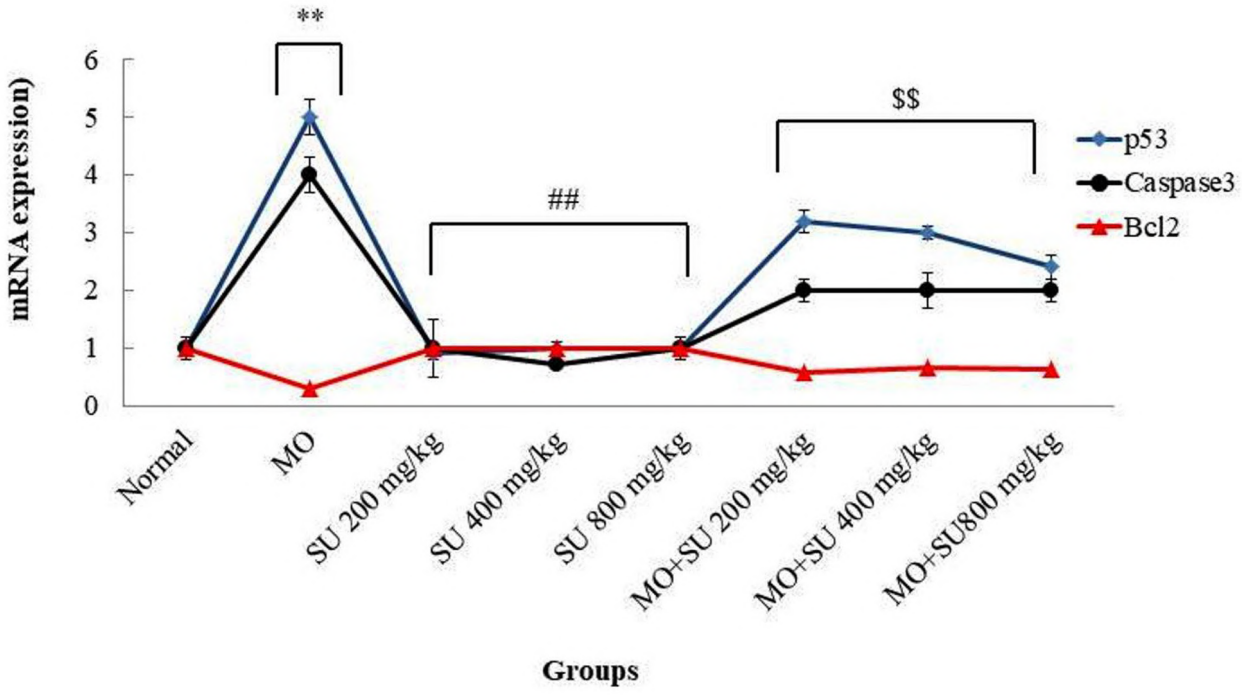

Fig. 5.- Effects of SU on Caspase 3, p53 and Bcl2 genes expression in normal, MO, and SU (200, 400 and 800 mg/kg) groups. ** Statistically significant ($P < 0.01$) between MO and normal groups. ## Statistically significant ($P < 0.01$) between SU and MO groups. $$ Significant increase in SU (200, 400 and 800 mg/kg) + MO groups compared to MO group ($P < 0.01$). MO: Morphine, SU: *Sumach.*

Inflammatory cytokines

In the morphine group, the inflammatory cytokines were elevated significantly if compared to the normal group ($P < 0.01$). No significant variations were observed in inflammatory cytokines in all SU groups, which remained similar to the normal group ($P > 0.05$). Subsequently, multiple doses of SU (200, 400 and 800 mg/kg) in SU and MO+SU (200, 400 and 800 mg/kg) groups exhibited a significant reduction in inflammatory cytokines compared to the MO group ($P < 0.01$) (Table 4).

According to the results of this study, there was no significant relationship between various increasing doses of SU and its effects on sperm parameters compared to the MO group. Besides, all doses of SU increased the quality of sperm parameters compared to the MO group. It is noteworthy to state that in the present study, the MO group was compared with the control group, and also the SU+MO groups was compared with MO group. All doses of SU in this group improved the quantity of sperm parameters. This phenomenon suggested that the therapeutic effects of SU are not related to dose. Previous studies on acceleration of sperm parameters using other antioxidant plants and morphine showed similar results (Jalili et al., 2016; Salahshoor et al., 2018).

Table 4. Effects of MO and SU on testicular levels of TNFα and IL-1β (n=8 for each group).

Groups	TNFα (pg/ml)	IL-1β (pg/ml)
Normal	75.88±4.2	102.44±9.0
MO	162.34±9.1*	222.34±11.4*
SU 200 mg/kg	70.71±5.3†	99.82±6.4†
SU 400 mg/kg	68.08±4.8†	103.71±9.3†
SU 800 mg/kg	72.64±4.9†	101.34±8.0†
MO+SU 200 mg/kg	110.82±8.3¶	141.83±7.5¶
MO+SU 400 mg/kg	109.64±9.2¶	133.73±10.7¶
MO+SU 800 mg/kg	99.78±3.4¶	130.83±8.3¶

Data presentation as mean ± SEM. * $P < 0.01$ compared to the normal group. † $P < 0.01$ compared to MO group. ¶ $P < 0.01$ compared to the MO group. MO: Morphine, SU: *Sumach*.

Hydro-alcoholic extract of SU with antioxidant efficacy

SU extract demonstrated higher antioxidant capacity in comparison to the standard value of butylates hydroxytoluene (Fig. 6).

DISCUSSION

MO administration creates free radicals which damage male reproductive system leading to minimized sperm parameters index. This harmful transition refers to oxidative characteristics of MO. As suggested previously, the harmful effects of MO could be decreased if it is administered with an antioxidant substance. According to extensive application of MO in medical interventions, the objective of this project was to prevent detrimental symptoms of MO on male reproductive system through an herbal antioxidant.

SU has varied benefits from conventional to modern medical sciences. This herb was first known for its abundance in the Western regions of Iran and usage in conventional medicine. Yet, the physiological functions of SU to relieve oxidative effects of MO is now uncertain. Based on the findings of this analysis, SU repelled MO's oxidative impact on sperm parameters. As we know, the male reproductive system is amongst the most important organs affected by free radicals of MO implementation. Furthermore, the SU seems to have inhibition effects on oxidative stress induced by other opioids. Based on significant statistical findings, medical MO intervention could decrease detrimental effects on total serum

antioxidant potential and fertility indices. MO also increased the levels of inflammatory cytokines and apoptotic genes expression (P53 and Caspase 3) in spermatogenic cell lines. Moreover, hydro-alcoholic extract of SU increased TAC levels and reproduction parameters in MO+SU (200, 400 and 800 mg/kg) groups by its antioxidant capabilities. In medical researches, MO is recognized as an effective promoter of oxidative stress production, especially in testes. Oxidative stress in reproductive organs induces mitochondrial ROS deposition, lipid peroxidation, and enzyme activity limitation (Jalili et al., 2019a). Tuerxun et al. (2019), in an experimental research, show that MO can stimulate hepatocarcinogenesis process in male rats.

TAC serum levels are considered a guideline for antioxidant levels. In this report, this value was decreased significantly. Whole genetic structures are vulnerable to ROS attacks, including nuclear DNA, mitochondrial DNA, and different cytoplasmic RNA classes. In ROS attack, the vital cellular and mitochondrial activities can also be interrupted, particularly in germ cells (Barroso et al., 2000). Instability of spermatogenesis and cellular and molecular processes could contribute to subfertility or infertility conditions (Houston et al., 2018). As such, all types of spermatogenic lines become vulnerable to free radicals' attacks (Leisegang et al., 2017). Parallel to our analysis, in an analytical study performed by Khan et al. (2013), it was concluded that MO could theoretically trigger the abnormal ROS level in cells. They reported that deformity, motility, and numbers of sperm cells are affected. Testosterone serum levels were also declined. In the primary ROS attack, the membrane was affected by lipid peroxidation. Also, ROS attacked to protective enzymes in sperm cytoplasm is reported. A large amount of cytoplasm was discarded in spermatogenesis, contributing to a weakened immune system. During ROS attack, Ca^{2+} channels in smooth endoplasmic retinaculum (SER) is also interrupted. This mechanism reduced sperm motility (Roshankhah et al., 2020). Non-motile sperms often report a decreased glutathione amount in their cytoplasm (Gomez et al., 1998).

Medical evidence has shown that every type of fatty acid oxidation induced by ROS attack can lead to reduced progressive sperm motility (Jalili et al., 2016). As described in the experimental research by Jalili et al. (2014), nicotine-induced oxidative stress can decrease sperm count, fertility index, testosterone level, and sperm motility. SU (200, 400 and 800 mg/kg) and MO+SU (200, 400 and 800 mg/kg) groups displayed a dramatic improvement in sperm parameters if compared to MO group, suggesting antioxidant effects of SU (Kosar et al.,

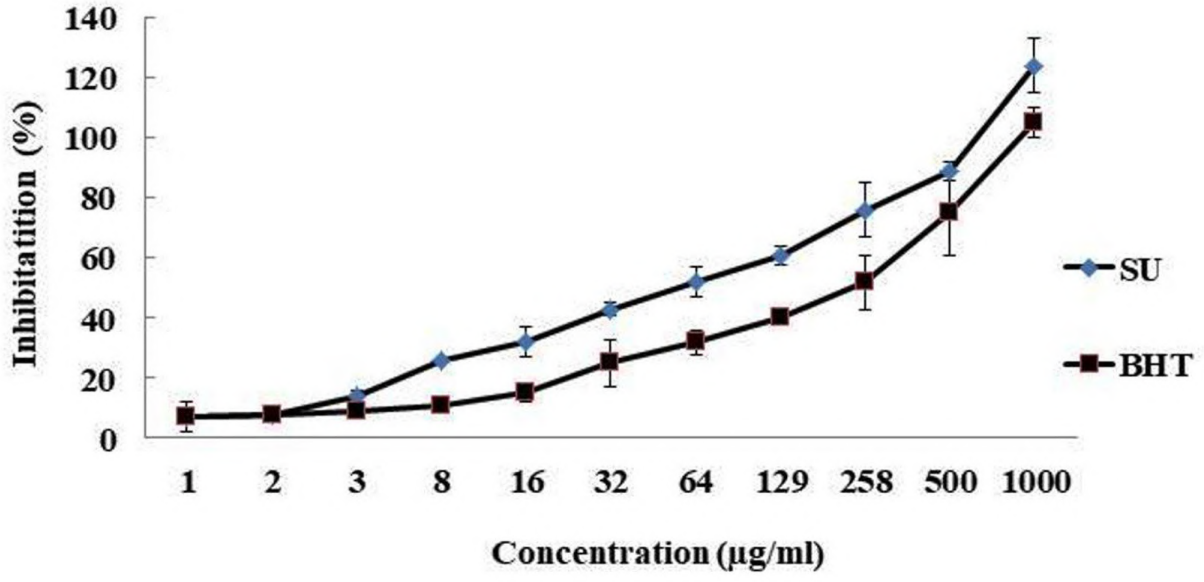

Fig. 6.- Antioxidant activities of different dilutions of hydro-alcoholic extract of SU in comparison with Butylate hydroxytoluene. SU: *Sumach*.

2008). Biochemical composition of semen fluid suggested the existence of a strong antioxidant mechanism which compensates for this system's deficiency in sperm with low cytoplasm content (Jalili et al., 2016). In relation to the findings of this research, Ahangarpour et al. (2014), also found that sperm parameters can be elevated in diabetic mice. Salimi et al. (2015), have reported that SU has antioxidant properties on diabetic animals induced by Streptozotocin. SU could disable receptor-alpha co-activator mechanism. This phenomenon triggers adenosine monophosphate protein kinase, and regulate mitochondrial activity (Kahale et al., 2015). TAC levels were higher in SU (200, 400 and 800 mg/kg) and SU (200, 400 and 800 mg/kg) + MO groups. In this research, following SU prescription, TAC levels were elevated showing anti-lipid peroxidation and antioxidant effects of SU. Testosterone and GLH levels were also decreased in MO group. Moreover, following implementation of SU (in SU (200, 400 and 800 mg/kg) and MO+SU (200, 400 and 800 mg/kg) groups), the GLH index appears to increase level, presumably based on the presence of Saponins and Flavonoids (Nozza et al., 2020). MO infiltration into intracellular cell-matrix can lead to damage of genetic material, induction of lipid peroxidation, and enzyme deformation (such as superoxide and hydrogen peroxide) (Jalil et ali., 2014). These biological changes, along with alteration of morphological feature,s could cause seminiferous tubules and apoptosis atrophy in specialized cells from germ cells, Sertoli cells, to Leydig cells (Roshankhah et al., 2020). Elevated blood flow is often recorded due to the SU administration (Ahangarpour et al., 2014). Cytochrome-c channels release cellular tumor antigen p53 to regulate the activities of key pro-apoptotic factors, including Bax, caspases, and endonucleases (Li et al., 2020). MO up-regulates these variables. Based on their intrinsic structure, the cells can escape from oxidants, but this aspect is significantly diminished by the presence of MO (Maher et al., 2020). In spermatogenesis, the apoptotic Fas/FasL cascade is widely used. However, in impaired testes, the caspase-3 and cytochrome-c expression could elevate the apoptotic rate (Wang et al., 2012). As Ibrahim et al. (2019), revealed the same results, the MO up-regulated caspase-3 genes and apoptosis frequencies.

MO increased expression of inflammatory factors. Experimental animal studies showed a strong link towards NO levels and inflammatory factors in lipopolysaccharide-induced lung damage (Somasundaram et al., 2020). In allergic rhinitis conditions, the concentrations of pro-inflammatory cytokine were diminished after SU prescription (Gharaei et al., 2020). There is a typical significant correlation among NO, TLRs, and inflammatory cytokines. However, critical role of cell lines in intercellular relationship between certain TLR and pro-inflammatory cytokine cannot be dismissed during inflammation. Equally, we expected a decent positive association between NO and TNFα expression in MO-induced testicular inflammation, and IL-1β, while SU reduced these dysfunctions and irregularities. These outcomes were exacerbated in the report by Jalili and colleagues. They established a relationship between TNFα and IL-6 in Acacetin-induced preventive cascade via antioxidants control in subsequent ischemia-reperfusion hepatitis (Roshankhah et al., 2020). The current study is valuable to describe either the IL-1β interaction or this dependency in MO administration and SU inflammation rescue.

CONCLUSION

In this research, SU amplified male infertility or subfertility triggered by MO contact. SU elevated TAC serum level, inflammatory cytokine and testosterone, changed impaired sperm parameters to physiological type, and arrested p53 and Caspase 3 gene expression. Thus, SU can improve the fertility rate or regain male infertility status dependent on natural antioxidants. Besides, further studies on animal models are required to achieve in-depth definitive proof of the molecular relationship between SU and MO, contributing to male reproductive degradation.

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