



PROTECTIVE ROLE OF *CRATAEGUS MONOGYNA* ON SPERM QUALITY AND TESTIS OXIDATIVE STRESS AGAINST COPPER-INDUCED TOXICITY

CRATAEGUS MONOGYNA 'NIN, BAKIR KAYNAKLI TOKSİSİTEYE KARŞI SPERM KALİTESİ VE TESTİS OKSİDATİF STRESİ ÜZERİNDEKİ KORUYUCU ROLÜ

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ABSTRACT

Objective: The purpose of this study was to investigate the possible beneficial use of common hawthorn *Crataegus monogyna* aqueous extract at 1.5g/Kg bw/day against chronic copper sulfate intoxication (100mg/Kg bw) in Wistar rats.

Material and Method: Animals were divided into 6 groups; the untreated control (C), 2 positive controls treated respectively with hawthorn fruits (F) and leaves (L), 1 group treated with copper (Cu) and 2 combined treatment groups treated with Cu and hawthorn-fruits (CuF) and Cu and hawthorn-leaves (CuL). After 30 days of oral administration, testis weight and plasma testosterone levels were evaluated, in addition to the epididymal sperm concentration, motility, vitality, velocity (VCL, VSL and VAP), the amplitude of lateral head displacement (ALH), and the beat cross frequency (BCF). Testicular glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPx) were also evaluated.

Result and Discussion: Cu exposure reduced testosterone, sperm concentration, live sperm, VCL, VSL, VAP, ALH, BCF, GSH, and GPx levels compared to control groups. Dead sperm and MDA levels were increased in rats of Cu group compared to the untreated control. When compared to the Cu group, levels of testosterone, sperm concentration, sperm motility, live sperm, VCL, VSL, VAP, ALH, BCF, GSH, and GPx were much higher in the CuF and CuL groups, along with a significantly lower MDA concentration. In conclusion, hawthorn, when co-administered as an aqueous extract with Cu, protected most biological markers against copper toxicit, while positive control (s) boosted sperm concentration and velocity (VCL and VAP).

Keywords: *C. monogyna*, CASA, Copper, sperm, testosterone

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ÖZ

Amaç: Bu çalışmanın amacı, Wistar sıçanlarında, kronik bakır sülfat zehirlenmesine (100mg / Kg bw) karşı yaygın alıç *Crataegus monogyna* sulu ekstraktının (1.5g / Kg bw/ gün) olası faydalı kullanımını araştırmaktır.

Gereç ve Yöntem: Hayvanlar, tedavi edilmemiş kontrol (C), sırasıyla alıç meyveleri (F) ve yaprakları (L) ile muamele edilmiş 2 pozitif kontrol, bakır (Cu) ile muamele edilmiş grup ve Cu ve alıç meyveleri (CuF) ve Cu ve alıç yaprakları (CuL) ile muamele edilmiş 2 kombine tedavi grubu olmak üzere 6 gruba ayrılmıştır. 30 günlük oral uygulamadan sonra, epididimal sperm konsantrasyonu, motilite, canlılık, hız (VCL, VSL ve VAP), dış yana baş deplasmanının amplitüdü (ALH) ve sperm kuyruğu vuruş sıklığının (BCF) yanısıra testis ağırlığı ve plazma testosteron seviyeleri değerlendirilmiştir. Ayrıca, testis glutatyonu (GSH), malondialdehid (MDA) ve glutatyonperoksidaz (GPx) da değerlendirilmiştir.

Sonuç ve Tartışma: Cu maruziyeti, kontrol gruplarına kıyasla testosteron, sperm konsantrasyonu, canlı sperm, VCL, VSL, VAP, ALH, BCF, GSH ve GPx seviyelerini azaltmıştır. Cu grubunun sıçanlarında ölü sperm ve MDA seviyeleri, tedavi edilmeyen kontrole kıyasla artmıştır. Cu grubuyla karşılaştırıldığında, testosteron seviyeleri, sperm konsantrasyonu, sperm motilitesi, canlı sperm, VCL, VSL, VAP, ALH, BCF, GSH ve GPx seviyeleri CuF ve CuL gruplarında çok daha yüksek ve MDA konsantrasyonları ise anlamlı olarak düşüktür. Sonuç olarak, alıç, Cu ile sulu bir özüt olarak uygulandığında, bakır toksisitesine karşı çoğu biyolojik belirteci korumuş ve sperm konsantrasyonunu ve hızını (VCL ve VAP) artırmıştır.

Anahtar Kelimeler: Bakır, *C. monogyna*, CASA, sperm, testosteron

INTRODUCTION

Copper is a trace element and important component of numerous metalloenzymes that are involved in energy and antioxidant metabolisms. However, some of copper's chemical forms, such as copper sulfate are very toxic [1]. Copper binds to binding proteins in the bloodstream and, distributes to all tissues especially the brain and the liver, which can secrete the excess of the metal into bile. Hypercupremia may cause several oxidation reactions, inflammations, and tissues damage by inducing free radical generations [2]. Copper can enter the body orally through food, by inhalation into the lungs or through the skin by direct contact. Copper is used in agriculture as a fungicide, herbicide, and insecticide [3]. Also, it is used as an electrical conductor in several industries, it has many chemical applications, and is known as a coinage metal. In nature, copper exposure may be caused by clouds of dust, volcanoes and forest fires. Copper dyshomeostasis has been linked to a variety of disorders. For example, ATP7A and ATP7B are both involved in copper metabolism; mutations of the former lead to Menkes' disease, but that of the latter causes Wilson's disease [4].

Previous studies showed that human spermatogenesis has decreased by up to 60% after 40 years [5]. In addition, many couples today are infertile [6]; this male sexual disorder may be related to pollutants [7]. The discharge of heavy metals into the environment has led to a harmful deterioration of the ecosystems [8]. Copper can cause several problems both in excess and deficiency. Copper imbalance in both men and women might affect reproduction, and women are said to be copper-dominant and men are zinc-dominant [1]. Excess copper harms male reproduction; metal

contamination provokes certain pathophysiological alterations in humans and animals, affecting sperm quality and causing infertility [9]. In its ionic form, copper is toxic to a variety of cells, including human spermatozoa [10]. According to Wong *et al.* [11], a positive correlation between blood Cu concentration and sperm motility dysfunction was found. However, cytosolic Cu is mainly bound to metallothioneins that may reduce its toxicity to some extent. The hydroxyl free radicals induced by the Fenton reaction of copper are very destructive to tissues [12] of the testis and epididymis, and also reduce antioxidant biomarkers such as catalase, superoxide dismutase, glutathione, and glutathione peroxidase. This oxidation can change sperm quality by modifying spermatozoa shape and movement. In addition, copper's effect on the pituitary receptor can provoke hormonal imbalance. Copper exposure causes several symptoms such as erectile dysfunction, anxiety, and testicular pain [1]. Furthermore, high copper levels can lead to a decrease in sperm concentration, motility, and vitality [13].

Today, numerous plants are used as remedies in the treatment of many ailments [14]. Hawthorn *Crataegus monogyna* is a very common shrub plant in the Mediterranean basin that is used by the local population in North Africa in certain traditional therapeutic applications including for hypertension, heart disorders, diabetes, anxiety, cancer, and some abdominal symptoms. The *Crataegus* spp have been used in medicinal treatments and as food [15]. Many studies conducted in Europe, Asia, and the USA showed that hawthorn has a high content of phenolic compounds that show antioxidant activity by scavenging superoxide anions, hydroxyl radicals, hydrogen peroxides, and by reducing lipid peroxidation [16].

This study aims to investigate the possible protective role of common hawthorn *Crataegus monogyna* aqueous extract against the induced toxicity of copper sulfate in Wistar rats by measuring certain reproductive and oxidative stress markers.

MATERIAL AND METHOD

Plant preparation

Fruits and leaves of the common hawthorn *Crataegus monogyna* were harvested every 3 days from the region of Annaba in the northeast of Algeria in November. Each of the 2 aqueous extracts of fruits (F) and leaves (L) was prepared daily by crushing 1.5g/kg bw (fruits and leaves) in an appropriate volume of distilled water and letting the mixtures steep overnight (12 hours) at room temperature in order to obtain 10 ml of filtered solutions of each of fruits and leaves in the morning. The aqueous extracts were administrated to rats *per os* daily for a period of 30 consecutive days.

Preparation of copper solution

Copper sulfate powder (Cu) was freshly dissolved in distilled water; this solution was administered to animals by gavage at a dose of 100mg/kg bw/day for 30 days.

Experimental design

Wistar rats were purchased from the Pasteur Institute (Algiers), each weighing 196 ± 8 g. Thirty-six males were divided into 6 equal groups: the control (C), the copper group (Cu) which received 100 mg/kg bw/day, the fruits group (F) which received an aqueous extract of 1.5 g fruits/kg bw/day, the leaves group (L) which received an aqueous extract of 1.5g leaves/kg bw/day, and 2 other groups that were treated with a combination of copper and fruits (CuF) or copper and leaves (CuL). Rats received tap water and standard diet ad libitum. After 30 days of continuous treatment, animals were sacrificed by decapitation; the blood was collected in heparinized tubes and then centrifuged at 3000 rpm for 10min. The plasma obtained was stored at -20 °C along with the testes, which had already been weighed, till further analysis. Animals' treatments were authorized by the Ethical Committee of Animal Sciences at the University of Badji Mokhtar-Annaba, before starting the experimental work.

Measurement of testosterone

The Ultrasensitive TESTOSTERONE ELISA test (DRG instrument GnbH) is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact testosterone molecule. Mouse monoclonal anti-testosterone antibody was used for solid phase (microtiter wells) immobilization, and goat anti-testosterone antibody was used in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies. After 2 hours of incubation at room temperature with shaking, the solid phase and enzyme were washed with distilled water to remove unbound labeled antibodies. A solution of tetramethylbenzidine (TMB) was added and incubated for 20 minutes, resulting in the development of a blue color. The color development was stopped with the addition of 1N HCl, and the resulting yellow color was measured using a spectrophotometer at 450 nm. The concentration of testosterone was directly proportional to the color intensity of the sample.

Semen analysis

Semen analysis was realized using the Computer-Assisted Sperm Analysis Method (CASA) using Sperm Class Analysis (SCA®, Microptic, Barcelona, Spain). The epididymal semen was

obtained immediately after sacrifice, and then a drop of semen (about 1 μ l) was diluted with a physiological solution of NaCl 0.09%, and 5 μ L of the mixture was placed in an empty chamber slide (GoldCyto model). The slide was then placed on a Nikon Eclipse (Nikon E200-LED) microscope at the phase objective (x4). The sperm markers of concentration, motility, vitality linearity (VCL, VSL), velocity (VAP), the amplitude of lateral head displacement (ALH) and the beat cross frequency (BCF) were automatically calculated.

Hypo-osmotic swelling (HOS) test

The HOST test was used to evaluate the integrity of sperm by exposing a drop of sperm, derived from the epididymis cauda, to the hypo-osmotic solution composed of fructose and sodium citrate [17], after which 100 spermatozoa were observed and the number of live sperms (as shown by an inflation of the tail) were counted.

Measurement of oxidative stress parameters

Frozen stored samples of testis were thawed and 100mg of each sample was taken and transferred to test tubes for the determination of glutathione (GSH) using the method of Cory and Weckbecker [18]. The testicular total proteins were quantified according to the colorimetric method of Bradford [19] by using the Coomassie Brilliant Blue G-250. Malondialdehyde (MDA) was estimated by using the method of Ohkawa *et al.* [20]. The measurement of glutathione peroxidase (GPx) was realized by the method of Flohe and Günzler [21].

The dosage of glutathione (GSH) was carried out according to the method of Wekbeker and Cory (1988). The principle of this assay is based on the measurement of the optical absorbance of the acid 2-nitro-5-mercapturic. The latter results from the reduction of 5,5'-dithio-bis-2- acid nitrobenzoïque (Ellman's reagent, DTNB) by groups (-SH) of glutathione. For this deproteinization of the homogenate is essential in order to keep only specific thiol groups of glutathione.

The tissues proteins were quantified according to the colorimetric method of Bradford (1967) who uses Gloss Blue Coomassie G250 (BBC) as a reagent and the serum albumin of breef (BSA) as standard. The BBC reacts with the amino groups (-NH₂) for protein to form a complex of blue color. The emergence of this color reflects the degree of ionization of the acid and intensity established the concentration of protein which is measured spectrophotometrically at 595nm.

MDA is a product of lipid peroxidation reactions that forms during the attack of polyunsaturated lipids by reactive oxygen species generated by certain contaminants. In our study, testicular MDA levels were assessed using the method of Ohkawa *et al* (1979). The dosage is based on the formation in an acidic and hot environment (100 ° C) between MDA and thiobarbituric acid

(TBA) of a colored pigment absorbing at 530 nm, extractable by organic solvents like butanol.

The enzymatic activity of glutathione peroxidase (GPx) was measured by the method of Flohe and Gunzler (1984). This method is based on the reduction of hydrogen peroxide (H₂O₂) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of the GPx.

Statistical analysis

Statistics was realized using (MINITAB 18 Software ANOVA Tukey). Results are expressed as mean \pm standard deviation. The significant test was considered at $p < 0.05$.

RESULT AND DISCUSSION

Testicular weights and testosterone

Results presented in Table 1 showed that differences between the absolute testicular weights of all groups were statistically non-significant when compared to the control. Testosterone concentration (Table 1) was significantly lower in Cu group compared to the untreated control. When compared to the Cu group, testosterone was significantly higher in the CuF and CuL groups. Testosterone levels of the positive control F and L groups were significantly lower compared to the control.

Table 1. Mean testicular absolute weights (g), and testosterone level (ng/ml) in wistar rats treated with copper sulphate and *C. monogyna* leaves and fruits extracts for one month. Results are expressed as mean \pm SD.

Groups	Testis (g)	Testosterone (ng/ml)
Control	1.67 \pm 0.31 ^a	5.80 \pm 0.007 ^a
Cu	1.67 \pm 0.015 ^a	1.32 \pm 0.150 ^f
F	1.67 \pm 0.01 ^a	5.55 \pm 0.121 ^b
L	1.69 \pm 0.02 ^a	5.23 \pm 0.012 ^c
CuF	1.67 \pm 0.012 ^a	3.10 \pm 0.008 ^e
CuL	1.67 \pm 0.01 ^a	4.10 \pm 0.003 ^d

Means that do not share the same letter are significantly different at $p < 0.05$.

Sperm concentration

Results of semen analysis indicated a significant lower sperm concentration in the Cu group compared to the control, Sperm concentration showed no significant difference between the F group and the control but was significantly higher in the L group than in the control, and significantly higher in the CuF and CuL groups compared to the Cu group (Figure 1).

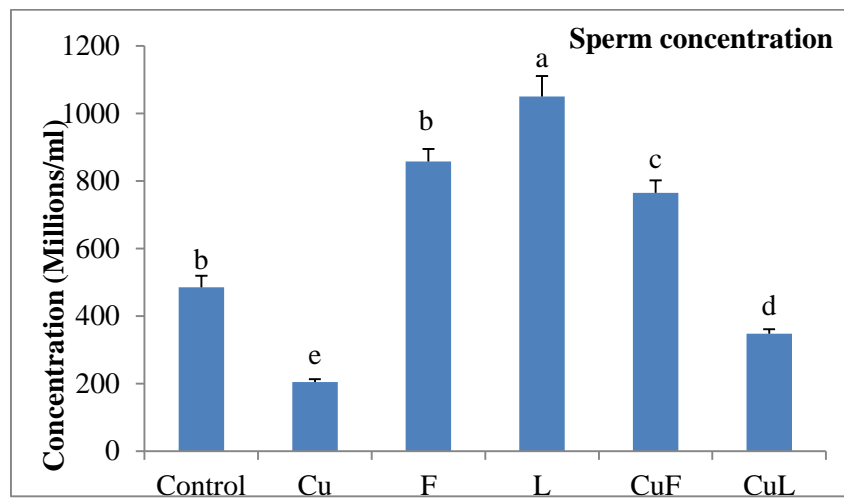


Figure 1. Evaluation of sperm concentration (Millions/ml) of Wistar rats exposed to copper sulphate and *C. monogyna* leaves and fruits for one month. Results are expressed as mean \pm SD. Means that do not share the same letter are significantly different at $p < 0.05$.

Sperm motility

Sperm motility was significantly lower in both the Cu group and the F group compared to the untreated control. Motility was also significantly lower in the CuF and CuL groups compared to the control but higher than the Cu group (Figure 2).

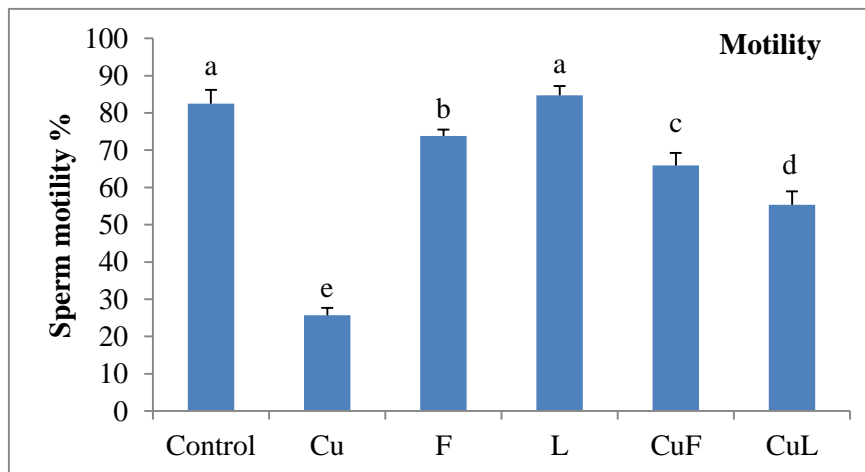


Figure 2. Evaluation of sperm motility percentage (%) of Wistar rats treated with copper sulphate and *C. monogyna* leaves and fruits for one month. Results are expressed as mean \pm SD. Means that do not share the same letter are significantly different at $p < 0.05$.

Dead and live sperm

Percentage of dead sperm in the Cu group was significantly higher compared to the control, while F, L, CuF, and CuL group showed levels close to that of the control (Figure 3). On the other hand, percentage of dead sperm was remarkably lower in the combined treatments of CuF and CuL groups compared to the Cu exposed group. Live sperm of the Cu group was slightly decreased when compared to the control, with a weak reduction in the CuF and CuL groups, whereas F, CuF and CuL have kept in close percentage as that of the control (Figure 3).

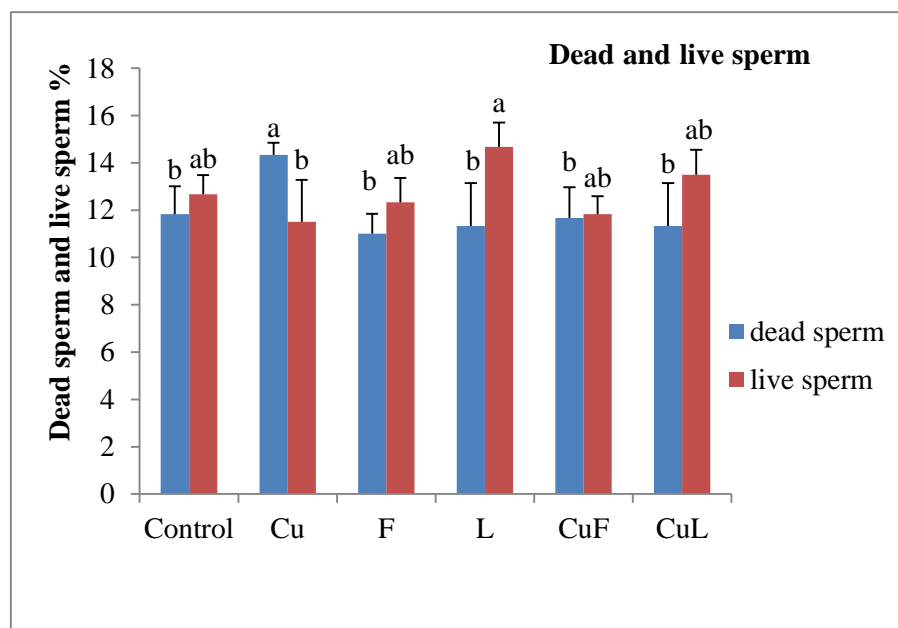


Figure 3. Evaluation of sperm vitality (dead sperm and live sperm percentage %) of Wistar rats exposed to copper sulphate and *C. monogyna* leaves and fruits for one month. Results are expressed as mean \pm SD. Means that do not share the same letter are significantly different at $p < 0.05$.

Sperm velocity

The VCL, VSL, and VAP of sperm from the Cu group were significantly lower compared to the control. VCL, VSL, and VAP of sperm in the CuF and CuL groups were significantly higher than the Cu group, and not statistically different from the control group (Figure 4). The VCL and the VAP of the L positive control were significantly higher than that of the control group.

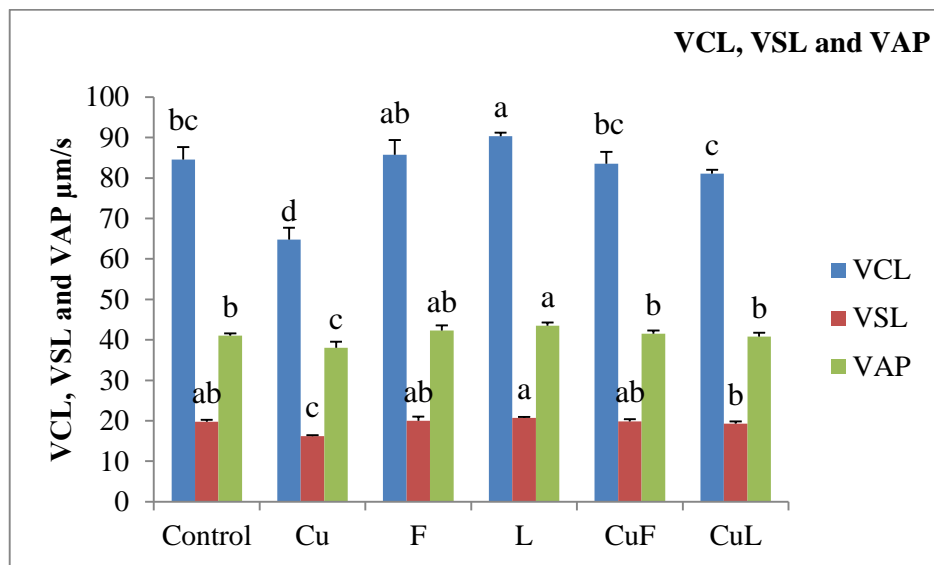


Figure 4. Evaluation of sperm velocity (VCL, VSL and VAP) of Wistar rats exposed to copper sulphate and *C. monogyna* leaves and fruits for one month. Results are expressed as mean±SD. Means that do not share the same letter are significantly different at $p < 0.05$.

Amplitude of lateral head displacement

The sperm ALH was significantly lower in the Cu group compared to the control (Figure 5). Sperm ALH in the other groups were not significantly different from that of the control.

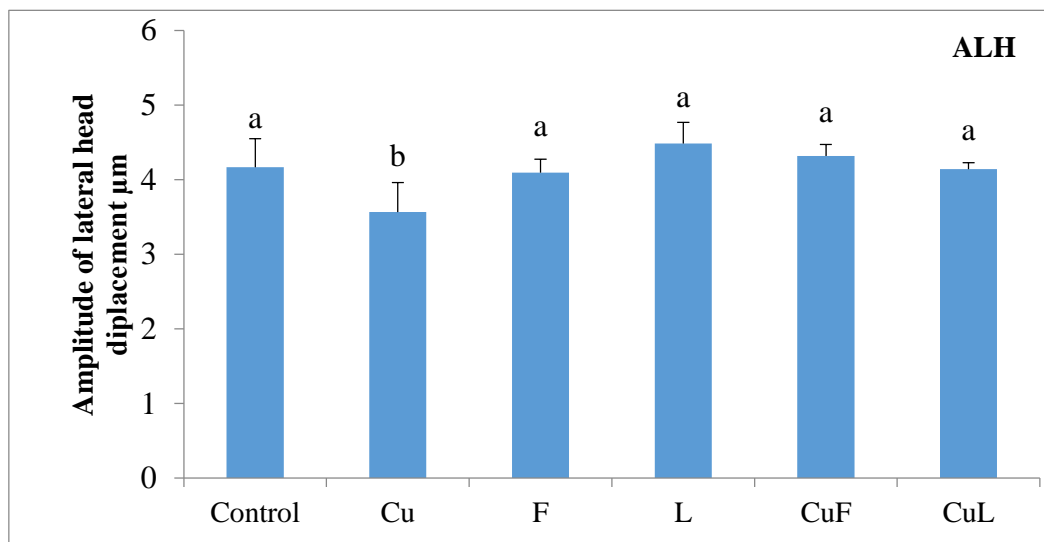


Figure 5. Evaluation of sperm amplitude lateral head displacement (ALH) of Wistar rats exposed to copper sulphate and *C. monogyna* leaves and fruits for one month. Results are expressed as mean±SD. Means that do not share the same letter are significantly different at $p < 0.05$.

Beat cross frequency

The BCF was lower in the Cu group compared to the control, while F, L, CuF and CuL groups did not differ significantly from the control (Figure 6).

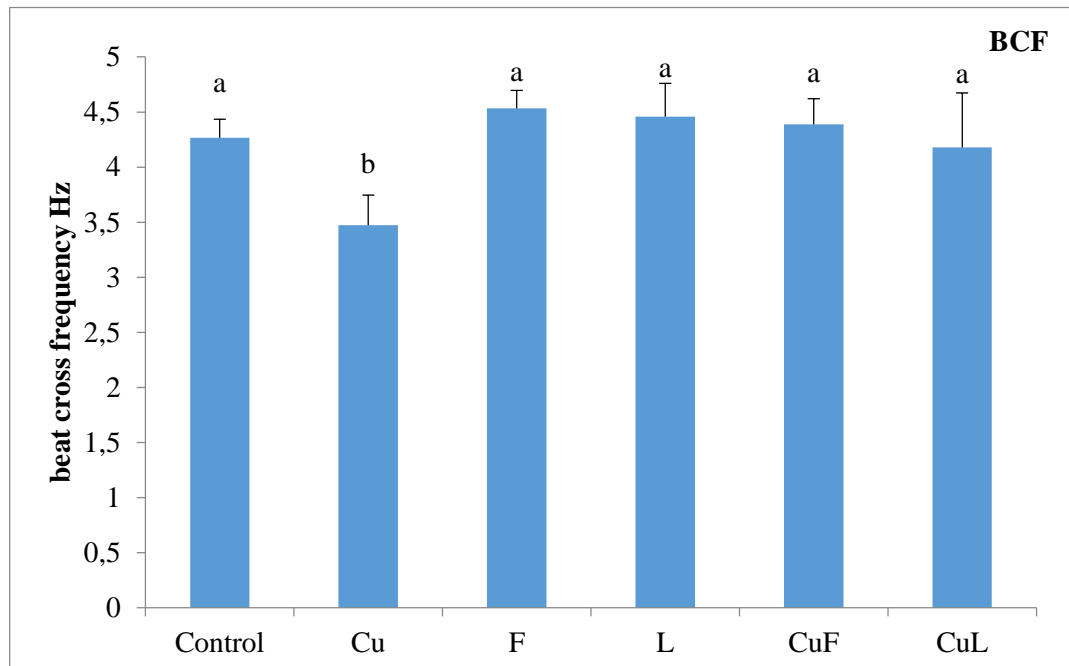


Figure 6. Evaluation of sperm beat cross frequency (BCF) of Wistar rats exposed to copper sulphate and *C. monogyna* leaves and fruits for one month. Results are expressed as mean \pm SD. Means that do not share the same letter are significantly different at $p < 0.05$.

Oxidative stress markers

The MDA level was significantly higher in Cu group compared to the control group, but it was significantly lower in the CuF and CuL groups compared to the Cu group (Table 2). The CuF group showed significantly higher levels than the control, while the CuL group was not significantly different from the control.

In contrast, the GSH and GPx levels were significantly lower in the Cu group compared to the control. These levels were significantly higher in the CuF and CuL groups compared to both the Cu group and the control (Table 2). There were no differences in the MDA, GSH and GPx of the positive controls F and L and that of the control.

Table 2. Mean testicular levels of MDA (nmol/g tissue), GSH (nmol/mg proteins), and GPx (μmol GSH/mg proteins) of wistar rats treated with copper sulphate and *C. monogyna* leaves and fruits for one month. Results are expressed as mean \pm SD.

	MDA (nmol/g tissue)	GSH (nmol/mg proteins)	GPx (μmol GSH/mg proteins)
	Testis	Testis	Testis
Control	0.015 \pm 0.001 ^c	0.206 \pm 0.005 ^{ab}	0.067 \pm 0.007 ^a
Cu	0.018 \pm 0.002 ^a	0.066 \pm 0.001 ^e	0.026 \pm 0.002 ^d
F	0.015 \pm 0.005 ^c	0.209 \pm 0.001 ^a	0.071 \pm 0.001 ^a
L	0.015 \pm 0.002 ^c	0.203 \pm 0.001 ^b	0.070 \pm 0.009 ^a
CuF	0.016 \pm 0.002 ^b	0.110 \pm 0.001 ^d	0.057 \pm 0.008 ^b
CuL	0.015 \pm 0.002 ^c	0.179 \pm 0.002 ^c	0.035 \pm 0.008 ^c

Means that do not share the same letter are significantly different at $p < 0.05$.

Results indicated a no difference in the absolute testicular weights of rats exposed to Cu for one month compared to the control group, which agrees with the finding of Chattopadhyay *et al.* [22]. Other researchers showed a slight decrease of rat testicular weight, in addition to a degenerative and necrotic effect of Cu on the seminiferous tubules and loss of spermatid from the center of the tubules [23]. In humans, Cu can produce adverse effects, including prostate enlargement, prostate infections, erectile dysfunction, depression, anxiety, testicular pain, and testicular cancer [1].

In this study, Cu was associated with lower testosterone concentration in rats; this result is in line with previous research showing that overdose of copper may affect the pituitary receptors, causing a reduction in the concentrations of LH and FSH, the key hormones in controlling testosterone release [24]. As a copper an antagonist of zinc, high copper levels can reduce zinc concentration; notably, zinc plays an important role in the formation of androgens [25]. The accumulation of heavy metals in the testis may lead to the inhibition of steroidogenesis [26].

In this investigation, rats exposed to copper had poorer sperm motility, velocity (VCL, VSL, and VAP), lateral head displacement, beat cross frequency, and vitality. This suggest that accumulation of copper in the epididymis, prostate, and seminal vesicle may inhibit sperm motility, BCF, and ALH, possibly by affecting the spermatozoa energy source, and by modifying the sperm tail shape, which can explain the decrease of the velocity measures VCL, VSL, and VAP. Moreover, Cu ions may accumulate in sperm mitochondria due to attraction to sulfhydryl groups, causing less ATP production, which can reduce sperm motility [27,28]. A high copper level in the reproductive organs is probably responsible for lowering the pH of seminal plasma, which was reported to

decrease sperm motility and the percentage of live sperm [13]. Other authors have found that a pH between 6.2 and 5.2 decreased sperm concentration, movement, and velocity measures VCL, VSL and VAP [29] by deregulating the Na/K ATPase activity [30] that blocks the entrance of calcium, which is responsible for improving the amplitude of flagellar beat [13]. Moreover, as copper is an antagonist of zinc, the deficiency of the latter may lead to low sperm concentration. Compared to other cells, spermatozoa contain less cytoplasm. As the latter is where the antioxidants system is found, having less cytoplasm makes spermatozoa susceptible to oxidative stress through lipid peroxidation [31], which affects the acrosome reactivity, and increases DNA damage [32]. This may explain the increase in dead sperm observed in the copper treated-group.

In the Fenton reaction, the cupric ions Cu^{+2} is reduced to cuprous Cu^{+} ; the latter is able to catalyze the hydroxyl radical, which is highly reactive, and form lipid radicals from fatty acids [12]. In our study, the group treated with Cu showed higher MDA levels, suggesting that copper ions inhibited the antioxidant reactions [33]. Copper as a destructive metal to tissues can cause lipid peroxidation, which can increase the testicular MDA levels [34].

We expected that high levels of copper in different tissues could produce reactive oxygen species (ROS), DNA damage, and lipid peroxidation [35]. In the present study, the observed low levels of GSH and GPx in the Cu exposed rats were likely to be caused by ROS production [36], and by cellular usage of GSH and GPx in the metabolism and detoxification of copper [23]. In addition, GPx is involved in scavenging hydrogen peroxide and lipid peroxidase caused by copper ions [37].

Although the hawthorn has been reported to treat sexual weakness in North Africa [38,39], there are not many studies about the effect of this plant on male fertility. The positive control of L showed higher sperm concentration and VCL in rats after a one-month treatment. As a powerful antioxidant, the fruits of hawthorn (*Crataegus* spp) may protect Sertoli cells from oxidative stress and may improve sperm quality due to the presence of phenolic compounds, oils, and vitamins. Previous findings showed the existence of vitamin C in the hawthorn and established the role of vitamin C on sperm characteristics [40], and the improvement of sperm concentration in male rabbits [41]. Similarly, our results showed an improvement in sperm quality in the CuL and CuF groups over the Cu group. The vitamin C in *Crataegus* spp. and in Rosaceae plants in general [40] helps in neutralizing hydroxyl, superoxide, and hydrogen peroxide radicals to prevent sperm agglutination [42]. In addition, vitamin C can both inhibit copper intestinal absorption, and increase its excretion [43]. The ability of vitamin C to scavenge free radicals could explain the remarkable lower levels of MDA observed in the CuL and CuF groups.

Furthermore, the augmentation in sperm motility in the CuL group is in line with the finding of Hu and Xiong [44] who observed that sperm from patients with asthenospermia showed increased

motility when co-incubated with extracts from the genus hawthorn (*Crataegus* spp). Studies on the chemical composition of *C. monogyna* revealed the presence of vitamins, flavonoids, and oils [40,45,46] that can act as a source of energy to boost sperm movement, increasing VSL and VAP [46]. This is probably why, in this study, the velocity measures (VCL, VSL, and VAP), ALH, and BCR were higher in the groups administered with *C. monogyna* extract.

Researchers previously found an effective role of *C. monogyna* extracts as an antioxidant [47,48] through the scavenging of free radicals and the inhibition of LDL oxidation [49]. Furthermore, the vitamin E in *C. monogyna* [45] can act as chain-breaking antioxidant [50], and prevents lipid peroxidation and tissue damage [42]. The occurrence of linoleic acid (omega-6); oleic acid (omega-9); oxalic acid bis (trimethylsilyl) ester; palmitic acid; and tetramethylcyclodecasiloxane that show powerful antioxidant activity [46] in *C. monogyna* might explain the lower levels of MDA and the higher levels of both GSH and GPx in the CuF and CuL groups. Previous results showed that omega-6 fatty acids can improve sperm motility [51], which may have contributed to the improvement of VCL, VSL, VAP, ALH and BCF levels in this study.

Quercetin was found in hawthorn, has been reported to act as a growth inhibitor for several malignant tumor cell lines, such as human epididymal cancer [52] by scavenging free radicals and chelating divalent cations [53]. Such a compound might have a role in reducing hydrogen peroxide, increasing sperm antioxidant defenses and preventing DNA damage induced by oxidative stress [54]. Moreover, quercetin may improve sperm quality by preserving the sex organs' functions [55]. Similarly, catechin polyphenols from *C. monogyna*, on the other side, were shown to reduce ROS by quenching free radicals and chelating transition metals [56], while catechins from green tea were shown to boost reproductive parameters [57]. This may explain the observed improvement of sperm concentration, motility, live sperm, velocity, ALH and BCF in the present study, especially in the CuL group.

In conclusion, copper induced oxidative stress, affecting testicular MDA, GSH, and GPx levels, and sperm quality parameters. The co-administration of extracts of *C. monogyna* fruits and leaves kept oxidative stress markers to almost their normal physiological ranges and improved semen quality, perhaps by mitigating the copper toxicity.

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