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Research Article

Crataegus Monogyna Alleviate Testicular Damage Induced by High Fat Diet in Albino Rats

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Abstract

Crataegus monogyna has attracted particular attention of modern medicine because of its widespread use for the prevention and treatment of some human various diseases. Atorvastatin (ATV), a lipid lowering agent, are widely used in combination with drugs. However, the effect of *Crataegus monogyna* and Atorvastatin on the male reproductive system and fertility has not been clearly defined. The current investigation aimed to study the protective effect of *Crataegus monogyna* aqueous extract (CMAE) or Atorvastatin against histological damage and oxidative stress induced by High fat diet (HFD) in the testes of Wistar rats were used and divided into four groups of animal search; Groups I and II served as standard and HFD controls and had double distilled water. Groups III and IV were treated with CMAE and ATV respectively (300, 2.1mg/kg, b.w. p.o.). All of the doses were per orally (p.o.) during 12 weeks. Our result revealed that HFD-treatment induced significant testis histological damages proved by an induction of testicular dysfunction like a lower number of Leydig cells and spermatocytes as well as an oxidative stress status assessed by an increase of malondialdehyde (MDA) level and antioxidant enzyme activities as superoxide dismutase (SOD) and catalase (CAT) in testicular tissue. The administration of CMAE or ATV improved all histological injuries and biochemical parameters. These finding suggested that firstly the ability of CMAE to protect the testicular damages in rats might be related by pharmacologically active substances such phenolic compounds with higher antioxidant capacity. Secondly clearly indicates that ATV offers a significant protection against HFD-induced oxidative stress and histological damage in the testes of rat.

Keywords: *Crataegus Monogyna*; Atorvastatin; HFD; Histological Damage; Oxidative Stress; Rats

Abbreviations

CMAE: *Crataegus Monogyna* Aqueous Extract; HFD: High-Fat Diet; ATV: Atorvastatin, MDA: Malondialdehyde; CAT: Catalase; ROS: Reactive Oxygen Species

Introduction

Obesity, as a life syndrome, is a result of excessive accumulation of body fat [Body Mass Index (BMI) above 30 kg/m2], which negatively is able to affect the human health [1]. According to the International Obesity Task Force (IOTF) report, 1.1 billion adults are overweight (with 312 million obese), representing the increasing population of obese individuals worldwide [2]. Obesity is also associated with an oxidative stress state, defined by a disorder of the balance prooxidants/antioxidants in favor of oxidants, causing significant cellular damage. This imbalance may be due to overproduction of reactive oxygen species (ROS) or a deficit of antioxidants [3].

This white substance associates with various metabolic complications, including diabetes, dyslipidemia, hypertension, cardiovascular diseases, cancer, sleep apnea syndrome, and reproductive disorders [4]. Among the aforementioned obesity-induced impairments, its impact on the fertility potential has gained extra attention [5]. Many epidemiological studies have reported that obesity affects the male reproductive system, as well as sperm and testicular oxidative damage, characterized by increased lipid peroxidation [6,7]. reactive oxygen species (ROS) production and oxidative DNA damage (18; 19; 39). Sperm oxidative DNA damage is increasingly being considered as one of the potential mechanisms underpinning paternal programming of offspring health in both human and rodents [8,9]. Thus, reduction of oxidative stress by interventions could be a potential approach to mitigate obesity-induced sperm and testicular oxidative damage.

Crataegus monogyna or common Hawthorn, is a genus of the Rosaceae family found in northern temperate regions such as East Asia, Europe, and Eastern North America. *C. monogyna* specie is commonly used in traditional medicine. This medicinal plant constitutes a valuable source of bioactive phytochemicals or bio-nutrients. This plant is a species recognized by the European Pharmacopoeia (2007). Some studies have showed that extracts of *C. monogyna* (from several parts of the plant including fruits) demonstrated a high antioxidant activity attributed to flavonoids and procyanidins [10] and many of these phenolic compounds have been shown to be cytoprotective by scavenging superoxide anion, hydroxyl radical, hydrogen peroxides, and reducing lipid peroxidation [11,12,13]. This plant presents many pharmaceutical properties such as antioxidant, anti-lipoperoxidant, anticancer, anti-inflammatory, anti-hyperlipidemic, hypoglyceamic, antidiabetic, gastroprotective, hepatoprotective and immunostimulant activities [14]. However, few studies reported that *C. monogyna* stimulate the process of spermatogenesis with an increase in testosterone level to reach the normal values and a significant rise of sperm activity [15]. As it has the aptitude to stimulate the growth of testes and improve the proliferation, maturation and differentiation of spermatozoa [16]. Atorvastatin (ATV), a 3-hydroxy 3-methyl glutaryl coenzyme A reductase inhibitor, with lipid-lowering activity, acts as an antioxidant at lower doses. It possesses pleiotropic effects independent of cholesterol-lowering property usually shown at lower doses, which include antioxidant and anti-inflammatory activities [17].

The principal aim of the current study is the evaluation of the possible protective effect of *C. monogyna* fruit aqueous extract with the determination of its antioxidant properties. In addition, the possible protection exerted by Atorvastatin against oxidative stress as well as histological damage induced reproductive toxicity during High -Fat diet treatment in a rat model were investigated.

Materials and Methods

Reagents

Trichloroacetic acid (TCA), acetylcholine iodide, S-butyrylcholine, butylhydroxytoluene (BHT), KOH, ethanol, bovine serum albumin (BSA), acetylthiocholine iodide, 5.5'-dithiobis-(2-nitrobenzoic acid) (DTNB), hematoxylin and eosin were purchased from Sigma-Aldrich. All other chemicals were of the commercially available highest grade.

Sample collection and fruit aqueous extract preparation

C. monogyna fruits were collected from the area of Beja, northwest of Tunisia, during the months of Octobor and November 2021 and identified by the botanic coordinator, Institute of Biotechnology of Beja, University of Jendouba. Thereafter, the fruit material (all the pericarp for the *C. monogyna* plant) were dried in an incubator at 40°C for 5 days with air circulation and later rigorously crushed in a traditional metallic mortar and then with an electric blender. The aqueous extract was then prepared by adding the powder to boiled doubly distilled water (70 °C), and the mixture was stirred for 1h. The mixture was filtered, and the filtrate were lyophilized, aliquoted and stored at -80°C until use.

High-fat diet (HFD) preparation

The high-fat diet was prepared according to the protocol described by [18]. The HFD consisting of the usual food that has been previously saturated with melted lamb fat. This fat was melted by heating to 100°C, then the plugs were soaked for 15 min in the melted fat. The HFD food was administered after drying at room temperature.

Animals and treatment

Healthy adult male Wistar rats (200–250 g) were purchased from SIPHAT (Ben-Arours, Tunisia) and used in accordance with the local ethics committee of Tunis University for the use and care of animals in accordance with the NIH recommendations (NIH, 1985). They were kept in separate metallic cages under standard

temperature (24±2°C), humidity (55±5%) and lighting (12h: 12h Light: Dark) conditions. They were provided with standard food (standard pellet diet- Badr Utique-TN) and water *ad libitum.* Rats were divided into four groups of 6 animals each. The animals were fed with a standard diet (group I) or a high-fat diet (HFD) (group II) for 12 weeks.

Groups I and II served as standard and HFD controls and had double distilled water. Groups III and IV were treated with CMAE and Atorvastatin respectively (300, 2.1mg/kg, b.w. p.o.) for 12 weeks. The treatments were just once orally carried out every morning, at the same time.

After 12 weeks, the animals were decapitated, and the testis as well as the epididymis were rapidly excised and homogenized in phosphate-buffered saline (pH 7.4). After centrifugation at 10.000xg for 10 min at 4°C, supernatants were processed for biochemical parameter determinations.

Oxidative indicators

Protein determination

Protein concentration in the epididymis and testis supernatant was determined according to the method of Bradford (1976) by using bovine serum albumin as a standard.

Lipid peroxidation (MDA)

Lipid peroxidation in the supernatant of each tissue (testis and epididyms) was measured by the double heating method. Briefly, after precipitation of tissue proteins with trichloroacetic acid (TCA), MDA from supernatant was allowed to react with TBA. Spectrophotometric measurement of the color produced was measured at 532 nm, and MDA concentration was calculated using the absorbance coefficient of MDA-TBA complex: 1.56×10^5 $M^{-1}cm^{-1}$(15).

Antioxidant activities assays

The activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich [19]. Briefly, epididymis and testis homogenate was added to 2 mL reaction mixture containing 20 µL of epinephrine (5 mg/mL), 10 µL of bovine catalase (0.4 U/µl) and 62.5 mM of sodium carbonate/bicarbonate buffer pH 10.2. Changes in absorbance were measured at 480 nm. The activity of catalase (CAT) was determined following the method described by Aebi (Aebi, 1984). The reaction mixture contained 33mM H2O2 in 50mM phosphate buffer pH 7.0 and CAT activity was calculated using the extinction coefficient of 40 $mM^{-1}cm^{-1}$ for H_2O_2.

Histological examination

For histological examination, the testis segments were harvested and washed with ice-cold saline. Tissue fragments were then fixed in a 10% neutral buffered formalin solution, embedded in paraffin, and used for histopathological examination. From this, 5 µm thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin eosin. Tissue preparations were observed and micro-photographed under a light BH2 Olympus microscope.

Data analysis

The data was analyzed as mean ±S.E.M and presence of significant differences among means of the groups was determined using one way Statview ANOVA for significance. Values were considered significant when $P < 0.05$*.

Results

Lipid peroxidation content

The effects of HFD, CMAE and ATV treatments on oxidative stress are reported in Figure 1. The HFD administration significantly increased testis and epididymis MDA levels which explain the proteins oxidation process. Treatment with CMAE and ATV protected against lipoperoxidation increase induced by HFD-treatment.

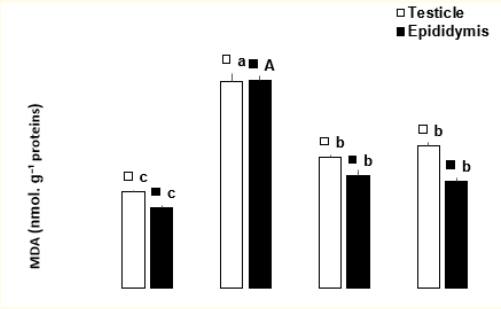

Figure 1: Effect of *Crataegus monogyna* aqueous extract and Atorvastatin on HFD-induced changes in reproductive system (testis and epididymis) MDA and protein levels. Different superscript letters: the difference is significant ($P < .05$). Similar superscript letters: the difference is not significant ($P > .05$). Values are mean –SEM (n = 6).

Antioxidant enzymes activities

We further looked at the effect of HFD, aqueous extract of *Crataegus monogyna* and Atorvastatin on antioxidant enzymes activities (Figure 2). High-fat diet treatment significantly increased testis and epididymis antioxidant enzymes activities such as SOD and CAT. CMAE and Atorvastatin treatment significantly restored the depletion of these enzymes. The aqueous extract of *Crataegus monogyna* alone decreased antioxidant enzymes activities as compared to control group.

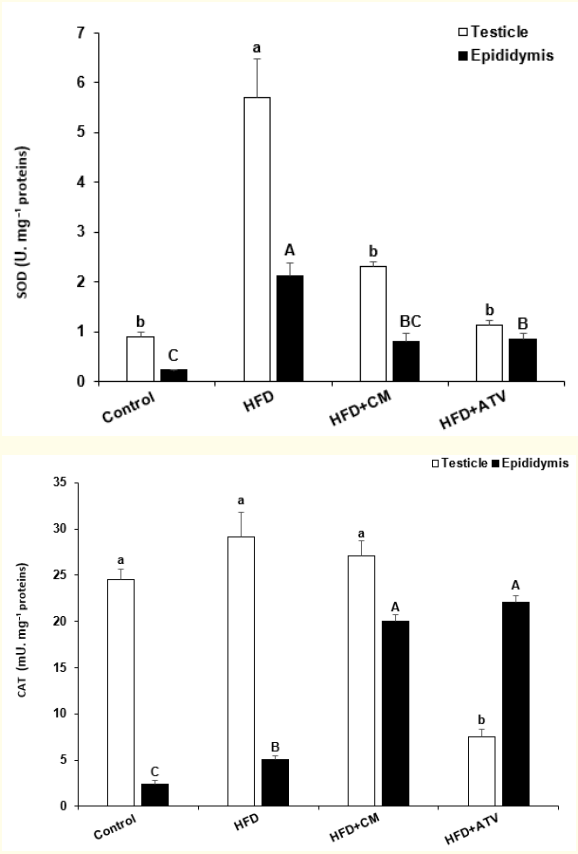

Figure 2: Effect of *Crataegus monogyna* aqueous extract and Atorvastatin on HFD-induced depletion in reproductive system enzymatic antioxidant activities. Different superscript letters: the difference is significant (P < .05). Similar superscript letters: the difference is not significant (P > .05). Values are mean–SEM (n = 6).

Histological evaluation

The biochemical parameters of rat groups were confirmed by the histological study. Indeed, morphological alterations of seminiferous tubules were observed in all treated rats (Figure 3). A significant increase in the percentage of empty seminiferous tubules was observed after treatment with extract and Atorvastatin.

Histological evaluation of testicular tissues, sections of the testis stained in control group (Figure 3 A and B) showed a normal number of adjacent seminiferous tubules separated by conjunctive tissue and interstitial cells. The tubules were lined by stratified germinal epithelium, which consists of two distinct populations of cells, the spermatogenic cells and the Sertoli cells. The spermatogenic cells presented the different stages of spermatogenesis, with the spermatogonia resting on the basal lamina and having small and dark nuclei. Spermatocytes appeared as large cells with large oval nuclei. The spermatids were detected at their different steps of spermatogenesis and gradually, they became elongating spermatids that form spermatozoa with their characteristic shape.

However, in treated groups fed with High- fat diet (Figure 3 C and D), stained testis sections showed histological changes compared to control testes. A disorganization and disruption of the germinal epithelium, with loss of the spermatogenic cells specially spermatocytes and spermatids were also observed in most tubules. A degeneration and/or necrosis were seen in the spermatogenic cells. Numerous abnormal cells were clustered in the lumen of seminiferous tubule sections of rats fed with High fat diet.

Discussion

The present study was designed to evaluate the therapeutic effects of *C. monogyna* against High-fat diet induced reproductive toxicity and testicular damage in adult male rats. The evaluation of CMAE analysis revealed the presence of phytochemicals, such as polyphenols and flavonoids, with strong antioxidant properties as disclosed by ABTS and DPPH assays. We found that *C. monogyna* is rich in total polyphenols and flavonoids respectively (23.92 ± 1.2 mg GAE/g, DW) and (05.5 ± 6.8 mg CE/g, DW) [23]. Previous

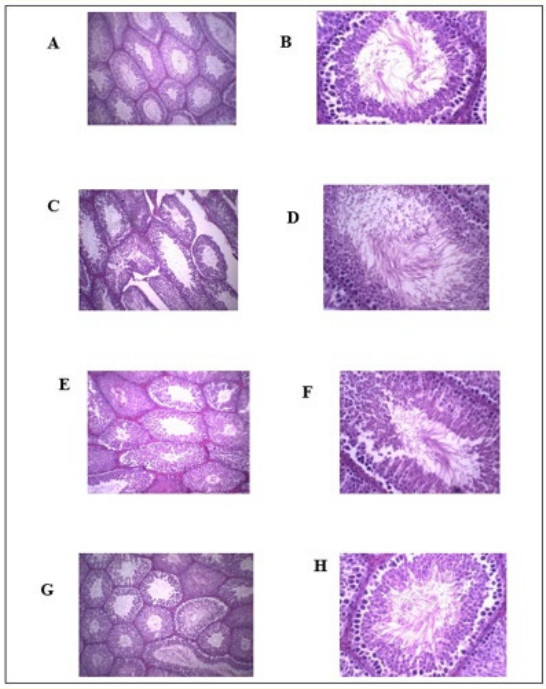

Figure 3: Histological examination of reproductive system (testis): resection of control (A x10, B x40) testis control rat (Control) depicting normal stages of spermtaogenesis, alteration of histological structures in HFD-induced reproductive damage (C) and HFD-induced perturbation in rats (D) presented by degeneration and/or necrosis in the spermatogenic cells. *Crataegus monogyna* aqueous extract and Atorvastatin consumption (E, H) protected testis organ against different destructions. Values are means ± SEM (n = 6).

studies showed the presence of a number of bioactive molecules including, flavonoid, vitamin C, glycoside, anthocyanin, saponin, tannin to which its antioxidant activity may be ascribed in the form of free radical scavenging activity and protection against oxidative stress [20,21]. and phenolic compounds [22,23]. On the other hand, using the DPPH and ABTS radical-scavenging assay, we found that *C. monogyna* presents a high scavenging capacity respectively (225,97 µg/mL and 201.54 µg/mL) [24]. The presence of flavonoids and tannins in the fruit is likely to be responsible for the free radical scavenging effects observed. The antioxidant activity of anthocyanins can implicate free radical scavenging activity [25]. However, effectively scavenged ROS including superoxide anion (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2). Free radical scavenging activity of *C. monogyna* fruit extract may be attributed to phenolic mixture [26]. These results confirmed that *C. monogyna* might be a natural source of bioactive metabolites [27]. Recent and less recent studies have shown that extracts of leaves, flowers and fruits have high antioxidant activity [28,29].

We secondly demonstrated that a high-fat diet is one of the major causative factors in the development of male reproductive impairment [30], which is mostly associated with generalised oxidative stress because of the large amounts of free radicals produced in accumulated fats in the body [31,32]. Our results revealed that the consumption of HFD induced reproductive toxicity and testicular damage [33]. In fact, he histological observations showed that the HFD induce severe damages in the testis; including the reduction of the quantity of spermatogenic cells, deformity in the seminiferous tubules, rupture of the germinal membrane surrounding the tubule. Vacuolization in tubules and empty lumen was observed in most of the tubules. Interlobular spaces were increased in between the tubules. Our finding was also in accordance with other studies that showed the similar histological defects and deteriorated testes function at high-fat diet were observed in the testis of rats [34].

The CMAE treatment induces a significant increase in the diameter in the seminiferous tubules, spermatids and leydig cells. The decreased interstitial space was observed. This increase may be due to the ability of *C. monogyna* extract to stimulate the growth of testis and enhance the maturation, proliferation, and differentiation of sperms as compared to the control group [35]. Whereas treatment with ATV significantly depicted nearly normal histological morphology [36].

The current study revealed that the HFD group induced reproductive function damage in both testes which associated to an oxidative stress as assessed by an increase of lipid peroxidation content, as well as a depletion of antioxidant enzyme activities as SOD and CAT. Previous study revealed that the rats fed with HFD were under higher levels of oxidative stress associated with a significant reduction in the sperm quality parameters (34; 26). Spermatozoa are considered to be highly sensitive to oxidative stress because of the presence abundant polyunsaturated fatty acids [37]. In this context, HFD can induce a decrease in the activity of the antioxidant system causing an increase in the generation

of ROS [38]. In this respect, we found that CMAE treatment induce increase a sperm concentration, this may be attributed to the antioxidant effects of *C. monogyna* which is responsible for its protective effect against hydrogen peroxide toxicity, so spermatozoa are protected by various antioxidant agents belongs to the phytochemical constituent of extract [39]. Other researchers concluded the capability of *C. monogyna* to improve healthy sperm characteristics and fertility [40]. However, a significant reduction in the oxidative stress was found as evident from a significant decrease in the MDA levels in the testes after long-term treatment of ATV. This is in agreement with earlier reports where low dose of ATV reduced oxidative stress *in vitro* and *in vivo* [41].

However, very few reports investigated the mechanism of ATV in the amelioration of oxidative stress is not fully explored. To this regard, a few authors suggested and proved that lipid independent inhibition of isoprenoids and their underlying molecular events were responsible for ATV mediated amelioration of oxidative stress and inflammation in experimental diabetic conditions [42].

Conclusion

The present study indicates clearly that High-fat diet can adversely damage the testicular tissue by imposing oxidative stress. However, *C. monogyna* fruits aqueous extract alone or in combination with ATV ameliorated testicular structure and sperm parameters. Our results may be particular interest for providing information about the effects of commonly used multidrug therapy on male reproductive system.

Conflict of Interest

The authors declare that they have no competing interests

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