Research Article E-ISSN: 2707-6261 RAFT Publications

Lybian Journal of Basic Sciences

A Study on the Effect of Samoa Extract (Cleome droserifolia) on Sperm Quality in Male Albino Rats Exposed to Pyrethroid

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Received: 15 March 2021 **Accepted: 13 April 2021** **Published: 15 April 2021**

DOI: https://doi.org/10.36811/ljbs.2021.110064

Citation: Nura I. Al-Zail, Salah F. Kamies. 2021. A Study on the Effect of Samoa Extract (Cleome droserifolia) on Sperm Quality in Male Albino Rats Exposed to Pyrethroid. LJBS. 5: 39-45.

Abstract

Pyrethroid cyhalothrin (PC) is an insecticide that is used worldwide for pest control in agriculture and household use. Samoa extract (SE) is a potent antioxidant protecting cells from oxidative stress. The present study investigates the protective and therapeutic effect of SE on PC-induced changes in sperm quality in male rats. Fifty adult male albino rats were divided into five groups: group I: served as control; group II: received PC i.p. only (6.2 mg/kg b.wt.); group III: received SE only (100 mg/kg b.wt., p.o.) for eight weeks; group IV: received SE as a protective agent daily for eight weeks, then followed by the administration of PC (i.p.) three times a week for two weeks; group V: exposed to PC (i.p.) three times a week for two weeks, then treated with the SE daily for 8 weeks. Results showed that PC caused markedly impaired sperm quality (a count, viability, motility, and abnormality). Compared to PC-treated animals, SE in the protective group markedly restored the alteration of sperm indices. However, SE in the curative group was found to be less effective in restoring PC-induced alterations. In conclusion, the data of this study revealed that the SE as a protective agent is more effective than as a therapeutic agent.

Keywords: Samoa; Pyrethroid; Sperm quality; Rat

Introduction

Pyrethroid cyhalothrin (PC), a new generation type II synthetic insecticide, has extensive uses as an agro-pesticide [1]. It is widely used in Egypt and valued for its broad-spectrum control on a wide range of pests in a variety of applications [2]. PC has been found to accumulate in biological membranes leading to oxidative damage, it was reported that PC caused oxidative stress by altering antioxidant systems and

increasing lipid per-oxidation in mammals [1,3,4]. The production of reactive oxygen species (ROS) is a normal physiological event in various organs including testis controlling sperm capacitation, acrosome reaction and sperm-oocyte fusion. However, over-production of ROS can be harmful to sperm and subsequently to male fertility [5]. Although the organism has several biological defense mechanisms against intracellular oxidative stress including enzymatic and non-enzymatic antioxidant defense system, and can act to overcome the oxidative stress [6], a positive correlation has been established between dietary supplementation with certain vegetables and plant products and the reduction of toxic effects of various toxicants and environmental contaminants [7].

Samoa (*Cleome droserifolia*), family Cleomaceae, commonly grown in different areas of North Sinai, Egypt. These species are generally used in folk medicine as stomachics, rubefacients and in the treatment of scabies, rheumatic fever and inflammation [8]. The dried herb of Samoa, locally known as Afein, Reeh-El-Bard, is used by herbalists in Egypt as a hypoglycemic agent, and its decoction is widely used in Sinai by Bedouins for the treatment of diabetes mellitus [9]. Extract of leaves and stems for Samoa rich in bioactive compounds as flavonoids, flavonol glycosides, alkaloids, tannins and steroids [10]. Flavonoids from Samoa were identified as quercetin, kaempferol, isorhamnetin, rutin and luteolin-7-O-glucoside [11,12]. Evidence suggests that certain phytochemicals found in citrus sources, such as flavonoids and limonoids, play a major role in treating or retarding a wide spectrum of diseases and reported to possess anti-oxidative, antiatherosclerotic, anti-inflammatory, antitumor, antithrombogenic, antiosteoporotic, and antiviral properties [13]. Antioxidants protect deoxyribonucleic acid (DNA) and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men [14].

The present study was aimed at comparing the protective and therapeutic effects of a natural antioxidant, Samoa extract (SE) against toxic effects of PC on sperm quality in male rats.

Materials and Methods

Animals

Fifty adults male Wistar albino rats, whose average body weight was 120±10 g/animal representing 2-3 months of age, were used in the present study. The experimental animals were obtained from the animal house of the research center, Faculty of Kasr Al-Ainy Medicine, Cairo University. The animals were housed under standard conditions of temperature (23±2°C), relative humidity (55±10%), and 12h light/12h dark cycle and were given food and water ad libitum. All ethical considerations for the studies on animals were considered carefully and the experimental protocol was approved by the Ethics Committee for research on laboratory animals at Cairo University.

Pyrethroid Cyhalothrin

Pyrethroid cyhalothrin (PC) with the empirical formula ($C_{23}H_{19}ClF_3NO_3$) was used. It was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). LCT was intraperitoneally (i.p.) administrated at a dose of 1/10 LD_{50} (6.2 mg/kg/b.w.) [15].

Natural antioxidant (Samoa)

The raw material was collected from Arish, North Sinai, Egypt, and authenticated to Samoa by Agricultural Researches Center, Giza, Egypt. The Samoa herb was spread over the bench and left for drying in the shade, then reduced to a powder. Decoction of the plant material was prepared by boiling 400 g of the dry plant

material in 6 liters of tap water for 2 minutes and then filtered after 10 minutes. To minimize the volume of the decoction, it was concentrated in a rotary vacuum evaporator at a temperature below 40°C. The dried extracted material was stored at -20°C in clean vials until used [16]. For oral administration (p.o.), the dried extract was dissolved in distilled water on the day of experimental studies and administered by gavages at 100 mg/kg/b.w. [17].

Experimental Design

Rats were divided into five groups (every 10 animals) as follows:

Group I: control, which received distilled water only throughout the experiment (p.o.) daily; Group II: PC group, which was given pyrethroid cyhalothrin only (6.2 mg/kg b.w.,i.p.) three times a week for two weeks; Group III: SE group, in which the animals received Samoa extract only (100 mg/kg b.w.,p.o.) in distilled water daily for eight weeks; Group IV (Protective by SE): animals were given SE (p.o.) daily for eight weeks. On the 7^{th} week they received PC (i.p.) three times a week for two weeks; Group V (Therapeutic by SE): Rats receiving a dose of PC (i.p.) three times a week on the 1^{st} and 2^{nd} weeks, then administered dose of SE (p.o.) daily for 8 weeks.

Sperm Indices

After sacrificing rats, the caudal epididymis was dissected out and placed in 2 ml of 0.9% physiological saline. It was cut into small pieces to release the mature sperms in solution [18]. The concentration of sperm cells was determined using the haematocytometer according to the technique adopted by [19], Using eosin as a differential stain for staining dead sperm cells (it can't pass through the living cell membrane) and nigrosin as a background stain, the percentage of live sperm was determined [20]. Sperm motility was examined according to the method reported by [19]. A small drop of the cell suspension was put on the slide and the spread slides were air- dried without fixation for about 24 hours. Slides were stained with hematoxylin and eosin. Three slides were prepared for each rat [21]. Sperm smears were examined by light microscopy. For each rat, 500 sperms were examined and morphological abnormalities were recorded according to the criteria of [22].

Statistical Analysis

All data were analyzed using the SPSS for Windows software, version 17.0 statistical program. Analysis of variance which is an indication of the dispersion or difference between more than two means to the calculated standard error of this difference was assessed.

Results

Effect of SE on PC-induced alteration in sperm indices

Sperm Count

The results in table (1) recorded a very highly significant decrease in sperm count of PC-treated rats (GII) reached (-62.13%) in comparison with control (GI). In the protective rats treated with SE (GIV), the value was nearly returned to the control and reached (-6.36%). whereas, the sperm count was showed partial improvement in GV which recorded (-20.21%) as compared to the control group.

Table 1: The protective and therapeutic role of SE on sperm count (x10^6/ml) in treated groups with PC.		
Groups		***Sperm count (x10^6/ml)***
Group I	**Control**	195.98±3.06
Group II	**PC**	74.21±4.89 (-62.13%) a^{***}
Group III	**SE**	194.40±2.16 (-0.81%) b^{***}
Group IV	**Protection by SE**	183.52±3.08 (-6.36%) b^{***}
Group V	**Therapy with SE**	156.37±5.07 (-20.21%) a^{***} $b^{***}c^{***}d^{***}$
Data are expressed as means ± S.E. (n=10 in each group). a=compared to control group (GI); b=compared to GII; c=compared to GIII; d=compared to GIV. *=significant change at p<0.05; **=highly significant change at p<0.01; ***=very highly significant change at p<0.001; ( ): % difference with respect to control value.		

Sperm Viability

Data recorded for the sperm viability were presented in table (2). The sperm viability of the PC group (GII) was a very highly significant decrease compared to the control group; the percentage of change was (-34.58%). Furthermore, it is clear that there was statistically significant (p<0.001) improvement of the sperm viability percentage in (GIV and GV), with a mean value of (84.81±3.09 and 81.68±3.13), respectively compared to (61.83±2.01) for the PC group (GII).

Table 2: The protective and therapeutic role of SE on sperm viability (%) in treated groups with PC.		
Groups		***Sperm viability (%)***
Group I	**Control**	94.51±3.20
Group II	**PC**	61.83±2.01 (-34.58%) a^{***}
Group III	**SE**	95.62±3.21 (1.17%) b^{***}
Group IV	**Protection by SE**	84.81±3.09 (-10.26%) b^{***}
Group V	**Therapy with SE**	81.68±3.13 (-13.58%) a^{*} $b^{***}c^{*}$
Data are expressed as means ± S.E. (n=10 in each group). a=compared to control group (GI); b=compared to GII; c=compared to GIII; d=compared to GIV. *=significant change at p<0.05; **=highly significant change at p<0.01; ***=very highly significant change at p<0.001; ( ): % difference with respect to control value.		

Sperm Motility

The results obtained in table (3) recorded a very highly significant (p<0.001) decrease in the sperm motility level in rats administered by PC (GII) and recorded (37.48±5.31) compared to control (95.31±3.14), with a percent of change (-60.68%). After protection with SE (GIV), the sperm motility level was nearly similar to that in the control group and reached (80.49±4.41), with a percent of change (-15.55%) and (114.75%) in relation to (GI) and (GII), respectively. In therapeutic rats by SE [23], a significant (p<0.01) elevation in the sperm motility level as compared to PC rats, were recorded (68.74±6.37). The percentage of increase was (83.40%) as compared to the PC group.

Table 3: The protective and therapeutic role of SE on sperm motility (%) in treated groups with PC.		
Groups		***Sperm motility (%)***
Group I	**Control**	95.31±3.14
Group II	**PC**	37.48±5.31 (-60.68%) a^{***}
Group III	**SE**	96.57±3.21 (1.32%) b^{***}
Group IV	**Protection by SE**	80.49±4.41 (-15.55%) b^{***}
Group V	**Therapy with SE**	68.74±6.37 (-27.88%) a^{**} b^{***} c^{***}
Data are expressed as means ± S.E. (n=10 in each group). a=compared to control group (GI); b=compared to GII; c=compared to GIII; d=compared to GIV. *=significant change at p<0.05; **=highly significant change at p<0.01; ***=very highly significant change at p<0.001; ( ): % difference with respect to control value.		

Sperm Abnormality

Table (4) showed the sperm abnormality level in both normal and experimental groups .A very highly significant increase (p<0.001) in the sperm abnormality level was observed in the PC group (36.20±3.28) compared to the control group (8.83±1.08), the percentage of change was (309.97%). In relation to the control group, (GIV and GV) showed a great reduction in the percentages of change of sperms deformation. The percentages of change were (28.09% and 71.91%), respectively.

Table 4: The protective and therapeutic role of SE on sperm abnormality (%) in treated groups with PC.		
Groups		***Sperm abnormality (%)***
Group I	**Control**	8.83±1.08
Group II	**PC**	36.20±3.28 (309.97%) a^{***}
Group III	**SE**	8.69±1.04 (-1.59%) b^{***}
Group IV	**Protection by SE**	11.31±2.22 (28.09%) b^{***}
Group V	**Therapy with SE**	15.18±2.30 (71.91%) b^{***}
Data are expressed as means ± S.E. (n=10 in each group). a=compared to control group (GI); b=compared to GII; c=compared to GIII; d=compared to GIV. *=significant change at p<0.05; **=highly significant change at p<0.01; ***=very highly significant change at p<0.001; ( ): % difference with respect to control value.		

Discussion

This study revealed that rats treated with PC (GII) had markedly impaired sperm quality. PC significantly lowered sperm count, viability, and motility percent and significantly increased sperm abnormalities. These findings may be due to an adverse effect of PC on spermatogenesis by affecting testosterone secretion which essential to maintain the structure and function of the male accessory sex gland, thus a lack of testosterone disrupts spermatogenesis. The results of the present study are in accordance with the findings of [23,24]. Sperm is highly susceptible to LPO as a result of the abundance of unsaturated fatty acids in the sperm plasma membrane and a very low concentration of cytoplasmic antioxidants [24]. The increased LPO can lead to oxidative damage to sperms DNA, alter membrane functions, impair motility and possibly have a significant effect on the development of spermatozoa [25], so the reduction in sperm quality subsequently reduction infertility according to the retardations of [26].

The administration of SE in the present investigation (GIV and GV) indicated significant improvement as regards sperm count, viability, motility, and sperm abnormality especially in the protective rat's group (GIV) in relation to PC rats (GII). It has been postulated that vitamin C which has proved its presence in

the Cleome species including Samoa minimizes testicular cytotoxic effects by prevention the production of the mutagenic electrophilic metabolites and stimulation of 7-X-hydroxylation of lipids and cholesterol nuclei, thus enhancing their degradation to bile acids which could be excreted from the body, such antioxidant action of vitamin C could relieve the germ cells from oxidative damage thereby decreasing the percentage of abnormal sperms. These findings are in agreement with the results observed by [27,28]. Also, [29], implicated the role of ascorbic acid (vitamin C) in the physiology of testis in regard to protein metabolism. Many enzymatic functions of ascorbic acid are believed to be essential for the normal integrity and function of testis i.e. synthesis, development, and maintenance of normal sperm [30], thus regulate protein metabolism and repair activities in the germinal cells.

Conclusion

The results of this study indicated that the protective effect of SE was more effective than therapeutic effects for SE in ameliorating PC-induced alterations and improvement of sperm quality.

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