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Research Article

EFFECT OF *SMILAX CHINA* LINN ON TESTICULAR ANTIOXIDANT ACTIVITY AND SPERMATOLOGICAL PARAMETERS IN RATS SUBJECTED TO FORCED SWIMMING STRESS

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ABSTRACT
The aim of the present study was to investigate the potential benefits of ethanolic extract of *Smilax china* Linn. on testicular antioxidant activity and spermatological parameters in rats subjected to forced swimming stress. Animals of the experimental groups except vehicle control were subjected to forced swimming stress (FSS) 15 min/day for 52 days. Animals were pretreated with two doses of ethanolic extract of *Smilax china* rhizomes (100 and 200 mg/kg b.w., p.o.) for 15 days prior to the starting of FSS and were continued further for 52 days along with induction of stress. Testicular SOD, catalase and lipid peroxidation were determined. The cauda epididymis was isolated and sperms were released into saline and sperm count, viability, morphology and motility were analysed. Rats with forced swimming stress showed a significant increase in lipid peroxidation and decrease in testicular SOD, catalase, sperm count, viability and motility. Many abnormal forms of sperms were seen. Rats pretreated with ethanolic extract of *Smilax china* rhizomes at both doses significantly prevented the stress induced changes. Hence, ethanolic extract of *Smilax china* rhizomes at both doses showed good protection against testicular antioxidant activity and spermatological parameters in rats subjected to forced swimming stress.
Keywords: *Smilax china* rhizomes, Forced swimming, Testicular antioxidant, Spermatological parameters

INTRODUCTION

Oxidative stress participates in the autogenesis of more than 100 diseases. Reactive oxygen species formed in the oxidative stress react with biomolecules such as lipids, nucleic acids and proteins, changing their structure and thus their function, leading to cell damage and alters physiological functions[1,2]. Approximately 15% of couples attempting to conceive are clinically infertile and male infertility contributes to 10 – 30% of those cases [3]. Common causes include excessive alcohol consumption, drugs, toxicant exposure, restraint, excessive exercise and conditions like varicocele, cryptorchidism, testicular torsion, or endocrinopathy. All of which results in testicular damage, impairment of spermatogenesis and sperm function [4,5]. Oxidative stress is also a major factor contributing to male infertility.

Smilax china Linn. (Family: Liliaceae) also known as chopchini, madhusnuhi is a popular herb in Ayurveda[6]. It is imported from China and Japan. In traditional medicine the plant is used to prevent and treat a wide variety of diseases such as diuretic, liver disorders, skin diseases, seminal weakness, aphrodisiac, veneral diseases, infertility etc[7]. Experimental reports indicated that *Smilax china* possessed anti inflammatory[8], anti nociceptive[8], anticancer[9], immunomodulatory[10], anti microbial activities[11]. The various pharmacological activities were due to the presence of chemical constituents like saponins, alkaloids, flavonoids etc. The present study was aimed to evaluate the potential benefits of ethanolic extract of *Smilax china* on testicular antioxidant and spermatological parameters in rats subjected to forced swimming stress.

MATERIALS AND METHODS

Plant material

The rhizomes of *Smilax china* Linn were authenticated and the ethanolic extract was obtained from Green chem herbal extracts and formulations, Bangalore, Karnataka, India (Batch no**: SCR/RD/04**)

Animals

Experimental study was carried out using adult male Wistar albino rats weighing between170–200 g. The animals were procured from Drug Testing Laboratory, Bangalore. The animals were housed in polypropylene cages and were maintained clean and hygiene. Animals were acclimatized in a room 12–12 h dark–light cycle, maintained at temperature 25±2ºC and humidity 50±5%. The rats were fed with commercial pelleted rat feed (Gold Mohur Lipton India Ltd.) and water *ad libitum*. The animal caring and handling were done according to the CPCSEA guidelines. The Institutional Animal Ethics Committee (IAEC/NCP/17/10) approved the above study at Nargund College of Pharmacy, Bangalore.

Acute oral toxicity study

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides and Toxic Substance) guidelines following the Up and down procedure[12].

Forced swimming stress

The rats were placed individually in an acrylic plastic pool (90 cm×45 cm×45 cm) filled with water up to a depth of 37cm and was made to forced swim for 15 min/day for 52 days at ambient room temperature[13,14]. Animals were divided into four groups, six animals in each group.

Group I- Vehicle control - distilled water, orally (5 mL/kg body weight)

Group II- Forced swimming stress (15 min/day) for 52 days.

Group III and IV –Rats were pretreated with oral administration of ethanolic extract of *Smilax china* (EESC) rhizomes (100 and 200 mg/kg b.w., p.o.) respectively for 15 days prior to the starting of forced swimming stress and was continued further for 52 days along with induction of stress. From 16th day the animals were subjected to forced swimming stress for 15min/day for 52 days half an hour after administration of the extract.

After the last stress session, control and other group of animals were sacrificed by cervical dislocation. Testis were removed, weighed and placed in KCl (10% w/v) solution, homogenated using a homogenizer and centrifuged at 4000

rpm for 10 min. Liver homogenate was analysed for lipid peroxidation. The supernatant were separated and estimated for SOD and catalase. The cauda epididymides were isolated and spermatological parameters such as sperm motility, viability, count and morphology were performed[15,16].

Statistical Analysis

Data are expressed as mean ± SEM and were analysed statistically using one way ANOVA followed by Dunnett's *t* test. Results were considered significant when P values were <0.05.

Table 1 Effect of ethanolic extract of *Smilax china* (EESC) rhizomes on testicular antioxidant enzymes (SOD and catalase units/mg of protein) and lipid peroxidation (n mol of MDA/mg of protein) in rats

Groups	SOD	Catalase	Lipid peroxidation
Vehicle control	152.9 ± 7.73	1.11 ± 0.12	102.6 ± 1.18
FSS	94.37 ± 0.98***[a]	0.67 ± 0.01***[a]	152.1 ± 0.68***[a]
FSS + EESC (100 mg/kg b.w)	127.0 ± 0.67***[b]	0.87 ± 0.01***[b]	122.4 ± 0.15***[b]
FSS + EESC (200mg/kg b.w)	142.8 ± 0.76***[b]	1.05 ± 0.03***[b]	113.2 ± 0.96***[b]

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's *t* test. n = 6.
[a] Comparison made with vehicle control group. [b] Comparison made with FSS group. *** P<0.001

Table 2 Effect of ethanolic extract of *Smilax China* rhizomes on spermatological parameters in rats

Groups	Epididymis sperm count (× 10^6/mL)	Sperm viability (%of viable sperms)	Sperm motility (% of rapidly progressive motile sperms)	Sperm morphology
Vehicle control	107.0 ± 0.36	45.0 ± 0.81	24.0 ± 0.68	Normal
FSS	77.95 ± 0.52***[a]	13.0 ± 0.57***[a]	3.45 ± 0.12***[a]	More abnormal forms
FSS + EESC (100 mg/kg b.w)	94.10 ± 0.25***[b]	21.33 ± 0.61***[b]	16.26 ± 0.18***[b]	Few abnormal forms
FSS + EESC (200 mg/kg b.w)	98.14 ± 0.25***[b]	39.0 ± 0.36***[b]	23.50 ± 0.42***[b]	Normal

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's *t* test. n = 6.
[a] Comparison made with vehicle control group. [b] Comparison made with FSS group. *** P<0.001

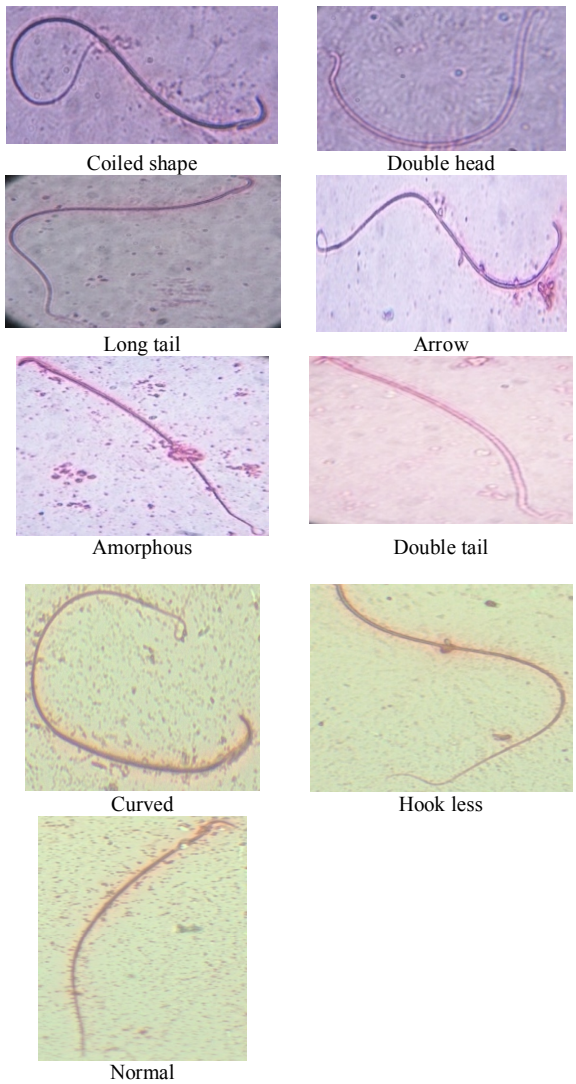

Figure 1 Abnormal forms of sperm

RESULTS

Acute oral toxicity study

EESC rhizomes showed no mortality in rat's upto a dose of 5000 mg/kg b.w. Hence 1/50th and 1/25th of the dose i.e. 100 and 200 mg/kg b.w was selected for improving antioxidant and spermatological parameters in forced swim stressed rats.

Forced swimming stress

Forced swimming stressed rats showed a significant decrease (P<0.001) in the SOD, catalase and increase in lipid peroxidation levels when compared with vehicle control group. Prior and continued treatment with EESC rhizomes at both doses of 100 and 200 mg/kg b.w prevented the fall of testicular antioxidant enzymes SOD and catalase levels (P<0.001). The lipid peroxidation damage was also reduced (P<0.001) against stress induced changes (Table 1)

A significant decrease (P<0.001) in the epididymis sperm count, sperm viability and sperm motility was seen in stressed rats. More number of abnormal forms such as banana, curved, double tail, and hookless, amorphous and double head were also seen. Prior and continued treatment with EESC rhizomes at doses 100 and 200 mg/kg b.w showed a significant improvement (P<0.001) in the epididymis sperm count, sperm viability and sperm motility when compared with forced swimming stress group (Table 2). Very few abnormal forms were seen at 100 mg/kg and at 200 mg/kg b.w almost normal forms were seen.

DISCUSSION

Forced swimming, a good physical exercise model, is also considered as a physical stressor. During physical exercise, oxygen utilization increases 10-15 folds and it is well established that reactive oxygen species (ROS) generation is a direct function of the rate of oxygen utilization and oxygen reperfusion is another process of ROS imposition. From review of literature, it has been indicated that remarkable dysfunctions are noted in male reproductive system, inhibiting spermatogenesis due to intensive exercise [14]. Ethanolic extract of *Smilax china* rhizomes was evaluated for

improving male fertility parameters in stressed rats using forced swimming stress model.

SOD is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide (O_2-) to hydrogen peroxide. CAT is a peroxisomal haem protein that catalyses the removal of H_2O_2 formed during the reaction catalysed by SOD. Thus, SOD and CAT acts mutually supportive antioxidative enzymes, which provide protective defense against reactive oxygen species. These ROS are very unstable and highly reactive. They become stable by acquiring electron from nucleic acids, proteins, carbohydrates and lipids, there by a cascade of chain reaction are initiated resulting in cellular damage and causes lipid peroxidation [17]. Testes of forced swimming stressed rats showed a significant decrease in the SOD and catalase enzyme levels may be due to excessive generation of free radicals or low levels of testosterone since testosterone promotes the synthesis of antioxidant enzymes in sex organs [18,19]. EESC rhizomes at both doses showed significant protection in antioxidant enzyme levels of SOD and catalase and showed normal basal levels.

Malondialdehyde a secondary product of lipid peroxidation is a major reactive aldehyde; higher levels leads to peroxidation of biological membranes [20] or due to high rate of catecholamine secretion that generate free radicals either through auto oxidation or through metal ion or superoxide-catalyzed oxidation [14]. The present data revealed significant increase in lipid peroxidation level which may be due to oxidative stress resulting in cellular damage. Previous studies also have shown that intensive exercise is linked with testicular oxidative stress [21]. The EESC extracts at both doses were able to prevent the lipid peroxidation due to free radical scavenging properties.

Spermatogenesis is the biological process of gradual transformation of germ cells into spermatozoa [22]. Forced swimming stress rats showed a significant decrease in the sperm count, sperm motility and sperm viability which may be due to oxidative stress resulting in decreased spermatogenesis. Low level of cholesterol and testosterone also inhibits spermatogenesis. Since testosterone is required for the growth and development of male reproductive organs, in association with FSH, acts on seminferous tubules to initate and maintain spermatogenesis [23]. Reduced glycogen levels also inhibit the transformation of germ cells into spermatozoa[24,25]. Sperm motility was reduced may be due to decreased fructose levels [26], inhibiting energetic transferase or non protein substances in the epididymis [27].

Abnormal forms of sperms (Fig 1) were seen in forced swimming rats which may be due to oxidative stress, non availability of hormones or any other substances required for maintaining the shape and structure [28]. Similar results were seen from previous studies, where intensive swimming causes reduction in sperm count and motility [29]. EESC rhizomes at both doses showed normal sperm count, sperm motility, sperm viability and less or no changes in the sperm morphology may be due to free radical scavenging properties promoting spermatogenesis.

From the experimental studies carried out, ethanolic extract of *Smilax china* rhizomes at two different doses 100 and 200 mg/kg b.w showed free radical scavenging activity and restoration to normal spermatological parameters. These activities may be due to the presence of various phyto-chemical constituents like saponins, flavonoids, tannins, terpenes, alkaloids and other unknown chemical constituents present in the ethanolic extract of *Smilax china* rhizomes. Further studies are required to be carried out in isolating the potential chemical constituents present in ethanolic extract of *Smilax china* rhizomes and to find its mechanism of action in treating male reproductive dysfunctions.

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