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Research Article

PROTECTIVE EFFECT OF *SOLANUM TORVUM* AGAINST TESTICULAR TOXICITY IN MALE WISTAR RATS

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ABSTRACT

Oxidative stress is the main factor in Doxorubicin (DXR) induced toxicity. Rats received either DXR (3 mg/kg, i.p.) on day 1, 7, 14, 21, 28 or *Solanum torvum* extract (100 mg/kg and 300 mg/kg, p.o.) prior to DXR or *S. torvum* (100 mg/kg and 300 mg/kg, p.o.) extract alone for 4 weeks. Testicular toxicity was assessed by recording changes in body weight, testis weight, epididymal sperm count and measuring the levels of marker enzymes-LDH, SGOT and CK-NAC; antioxidant defence enzymes SOD, CAT and GSH; membrane bound enzymes such as Na^+ K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase; total proteins and histopathological changes. *S. torvum* (100 mg/kg and 300 mg/kg) significantly ($p<0.05$) reversed the changes induced by DXR and showed a lesser degree of cellular infiltration in histopathological studies. The results suggest that *S. torvum* has the potential in preventing the testicular toxicity induced by Doxorubicin.

Keywords: *S.torvum*, Doxorubicin, Testicular toxicity.

INTRODUCTION

Doxorubicin is well known anthracycline glycoside antibiotic that possesses a potent broad spectrum antitumour activity against a variety of cancers including severe leukemias, lymphomas, human solid tumours and haematological malignancies [1-3]. However its use in chemotherapy has been limited largely due to its diverse toxicities, including kidney, renal, hematological and testicular toxicity [4-6]. Oxidative stress has been associated with DXR induced tissue damage [7]. Although the exact mechanism of DXR induced toxicity remains unknown, it is believed to be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules and membrane lipid peroxidation [8]. The production of free radicals as a byproduct of DXR metabolism is considered to be the primary mechanism of DXR induced toxicity, which can be ameliorated by the use of natural antioxidants [9].

Solanum torvum Sw. (Solanaceae), commonly known, as Turkey berry is an erect spiny shrub of about 4 m tall, evergreen and widely branched. It is native and found cultivated in Africa and West Indies [10]. The fruits and leaves are widely used in Cameroonian folk medicine. *Solanum torvum* contains a number of potentially pharmacologically active chemicals like isoflavonoid sulfate and steroidal glycosides [11,12], chlorogenone and neochlorogenone [13] , triacontane derivatives [14,15], 22-β-O-spirostanol oligoglycosides [16], 26-O- β-glucosidase [17].

Several natural compounds with antioxidant activities are known to exhibit protection against DXR induced testicular toxicities. *Citrus paradisi* [18], Grape fruit seed extract [19] and melatonin [20] was found to protect against DXR induced testicular toxicity. The antioxidant properties of flavonoids and their ability to chelate free iron could be effective in reducing toxicity of DXR [21]. In view of this, though the antioxidant activity of *S.torvum* is well known, its protection against DXR- induced testicular damage is not reported. Therefore the present study was aimed at investigating the possible protective effect of *Solanum torvum,* against DXR-induced testicular toxicity in rats.

MATERIAL AND METHODS

Animals

Albino rats (Wistar strain) of either sex weighing between 200-250 g, were obtained from Serum Institute, Pune. Animals were housed into groups of five under standard laboratory conditions of temperature 25 ± 1ºC with free access to food (Amrut rat and mice feed, Sangli, India.) and water. The experiments were performed during the light portion. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee.

Drugs and chemicals

Doxorubicin (ADRIM, 50 mg) was purchased from Dabur Pharma Ltd. Baddi, India. ATP (Adenosine-5' triphosphate, disodium salt was purchased from Hi media, Mumbai). All the chemicals used were of analytical grade and chemicals required for sensitive biochemical assays were obtained from Merck, India. CK-NAC and SGOT kits were purchased from Aspen laboratories, Baddi, India. LDH kit was purchased from Reckon Diagnostics Pvt. Ltd. Vadodara, India. *Solanum torvum* extract was dissolved in distilled water and administered orally.

Preparation of extract

Dried fruits of *Solanum torvum* sw. (Solanaceae) were purchased from local market and authenticated by Dr. Dasari, from Ayurvedic Seva Sangh, Panchavati, Nashik, India. Mature fruits were collected, sun dried and grounded. The powder obtained (950gm) was defatted using petroleum ether (60-80^{0}C). The marc was macerated in methanol for 3-4 days at room temperature. The filtrate was air dried and concentrated under reduced pressure to obtain 113 g, corresponding to a yield of 11.3 % w/w. Appropriate concentrations of the methanolic extract of *Solanum torvum* were made in distilled water. The chemical tests for phytoconstituents present in the crude extract such as flavonoids, alkaloids, tannins, saponins, were carried out [22].

Experimental Protocol

The animals were divided into six groups each consisting of five rats and received the following treatment.

Group I (Control): Animals received distilled water for injection.

Group II (DXR): Animals received DXR injection (3 mg/kg, i.p.) on day 1, 7, 14, 21, 28.

Group III (DXR + ST): Animals received ST (100 mg/kg /day p.o. for 28 days) and DXR injection (3 mg/kg, i.p.) on day 1, 7, 14, 21, 28. Group IV (DXR + ST): Animals received ST (300 mg/kg /day, p.o. for 28 days) and DXR injection (3 mg/kg i.p.) on day 1, 7,14,21,28. Group V (ST): Animals received ST (100 mg/kg /day, p.o. for 28 days)

Group VI (ST): Animals received ST (300 mg/kg /day, p.o. for 28 days)

Estimation of body and organ weight

In each group, body weight of rats was taken before and after DXR treatment. The testis was weighed after keeping them in ice-cold saline.

Preparation of serum and tissue homogenate

After 48 hours of the last injection of either DXR or vehicle, blood was collected retro-orbitally from the inner canthus of the eye (under light ether anesthesia) using capillary tubes for serological analyses. Serum was separated using R-24 research centrifuge (Remi Instruments Ltd., Mumbai) at 3000 rpm for 15 min. and used for estimation of CK-NAC [23], LDH [24], SGOT [25].

The animals were sacrificed under euthanasia. Epididymis was removed, after the adhering tissues were cleared. The epididymal sperm count was done immediately. One of the testes was excised in chilled Tris buffer (10 mM pH 7.4) for measurement of tissue markers of oxidative stress and the other one was collected for histopathology. The excised testes were then weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 rpm for 20 min using Remi centrifuge machine. The clear supernatant was used for the assays of lipid peroxidation (TBARS)[26]; endogenous antioxidant enzymes, superoxide dismutase (SOD)[27], catalase (CAT) [28] and reduced glutathione (GSH) [29]. The sediment after centrifugation of tissue homogenate were resuspended in ice cold Tris buffer (10mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes such as Na^+ K^+ ATPase [30], Ca^{2+} ATPase [31], Mg^{2+} ATPase [32] and total proteins [33].

Epididymal sperm count

Epididymal sperm was collected by slicing the epididymis in 5 ml phosphate buffer saline (pH 7.2). An aliquot of the epididymal sperm suspension was used for spermatozoa count using Neubauer hemocytometer [34].

Histopathological studies

The testes were fixed in 10% formalin. The specimens were then processed for standard procedure and were embedded in paraffin wax. The blocks were then sectioned according to hematoxylin and eosin method. Five-micrometer thick histological sections were obtained from the paraffin blocks. The sections were examined under the light microscope and photographs were taken under 10X using Moti camera.

Statistics

The mean ± SEM values were calculated for each group. One-way ANOVA followed by Dunnett's multiple comparison tests were used for statistical analysis. Values of $p<0.05$ was considered statistically significant.

RESULTS

Testis weight and sperm count

Control animals showed 1.46 ± 0.02 gm and 44.76 ± 1.79 millions/ml as isolated testes weight and sperm count. DXR treated group (3 mg/kg, i.p., on day 1, 7, 14, 21, 28) significantly ($p<0.05$) decreased isolated testes weight and sperm count as compared to the control group. Co-treatment with methanolic extract of *Solanum torvum* (100 and 300 mg/kg/day, p.o.) in DXR treated (3 mg/kg, i.p. on day 1, 7, 14, 21, 28) animals significantly ($p<0.05$) restored the isolated testes weight and sperm count as compared to DXR treated group (Table 1)

Antioxidant enzymes

The antioxidant level of SOD, CAT, GSH and TBARS enzyme of testes in control rats was found to be 0.23 ± 0.02 U/g, 7.58 ± 0.24 U/g, 42.77 ± 0.76 nM/mg and 2.07 ± 0.11 nM/mg of wet tissue. The SOD, CAT and GSH levels were significantly ($p<0.05$) decreased and TBARS levels were significantly increased in testes tissue of DXR (3 mg/kg, i.p. on day 1, 7, 14, 21, 28) treated animals as compared to control group. Co-treatment with methanolic extract of *Solanum torvum* (100 and 300 mg/kg/day, p.o.) in DXR treated (3 mg/kg, i.p.on day 1, 7, 14, 21, 28) animals showed significant ($p<0.05$) increase in SOD, CAT and GSH levels and significant decrease in TBARS levels as compared to DXR treated animals (Table 2).

Table 1: The Effect of methanolic extract of *Solanum torvum* on body weight, testes weight and sperm count in DXR treated animals.

S. No.	Treatment groups (mg/kg)	Final Body Weight (gm)	Absolute testes weight (gm)	Sperm count (millions/ml)
1	Control	239.4 ± 2.34	1.46 ± 0.02	44.76 ± 1.79
2	DXR (3)	197.9 ± 4.93*	0.89 ± 0.01*	10.80 ± 0.44*
3	ST (100) + DXR (3)	219.8 ± 4.54#	1.09 ± 0.02#	27.44 ± 0.66#
4	ST (300) + DXR (3)	225.2 ± 4.05#	1.15 ± 0.06#	30.80 ± 0.59#
5	ST (100)	211.4 ± 5.65*	1.34 ± 0.08	36.01 ± 1.25*
6	ST (300)	220.6 ± 5.56*	1.37 ± 0.03	39.92 ± 1.21*
7	F value	8.89	22.89	118.61

N = 5, All values are expressed as mean ± SEM. One way ANOVA followed by Dunnett's test. *P< 0.05 against control group, # P< 0.05 against DXR group.

ST = methanolic extract of *Solanum torvum,* DXR = Doxorubicin.

Table 2: The Effect of methanolic extract of *Solanum torvum* on enzymes of oxidative stress in DXR treated animals.

S. No.	Treatment groups (mg/kg)	SOD (U/gm of wet tissue)	CAT (U/gm of wet tissue)	GSH (nm/mg of tissue)	TBARS (nm/mg of tissue)
1	Control	0.23 ± 0.02	7.58 ± 0.24	42.77 ± 0.76	2.07 ±0.11
2	DXR (3)	0.15 ± 0.01*	2.35 ± 0.06*	16.43 ±1.33*	7.46 ±0.11*
3	ST (100) +DXR (3)	0.19 ± 0.01#	3.22 ± 0.08#	25.53 ± 1.76#	5.79±0.24#
4	ST (300) +DXR (3)	0.23 ± 0.01#	4.43 ± 0.14#	34.85 ± 0.79#	4.31 ±0.42#
5	ST (100)	0.19 ± 0.01*	7.00 ± 0.09 *	34.68 ± 0.92 *	3.51±0.16*
6	ST (300)	0.27 ± 0.01*	6.99 ± 0.22 *	37.82 ± 0.39*	3.04±0.08*
7	F value	16.0	210.40	76.32	80.08

N = 5, All values are expressed as mean ± SEM. One way ANOVA followed by Dunnett's test. *P< 0.05 against control group, # P< 0.05 against DXR group.

ST = methanolic extract of *Solanum torvum,* DXR = Doxorubicin.

Marker enzymes

Control animals showed (12.82 ± 0.56IU/L), (11.21 ± 0.47IU/L) and (4.81 ± 0.38 IU/L) as serum marker enzyme level of LDH, CK-NAC and SGOT respectively. DXR treated group (3 mg/kg, i.p., on day 1, 7, 14, 21, 28) significantly ($p<0.05$) increased the levels of marker enzymes as compared to the control group. Co-treatment with methanolic extract of *Solanum torvum* (100 and 300 mg/kg/day, p.o.) in DXR treated (3 mg/kg, i.p., on day 1, 7, 14, 21, 28) animals showed significant ($p<0.05$) decrease in levels of marker enzymes as compared to the DXR treated group (Figure 1).

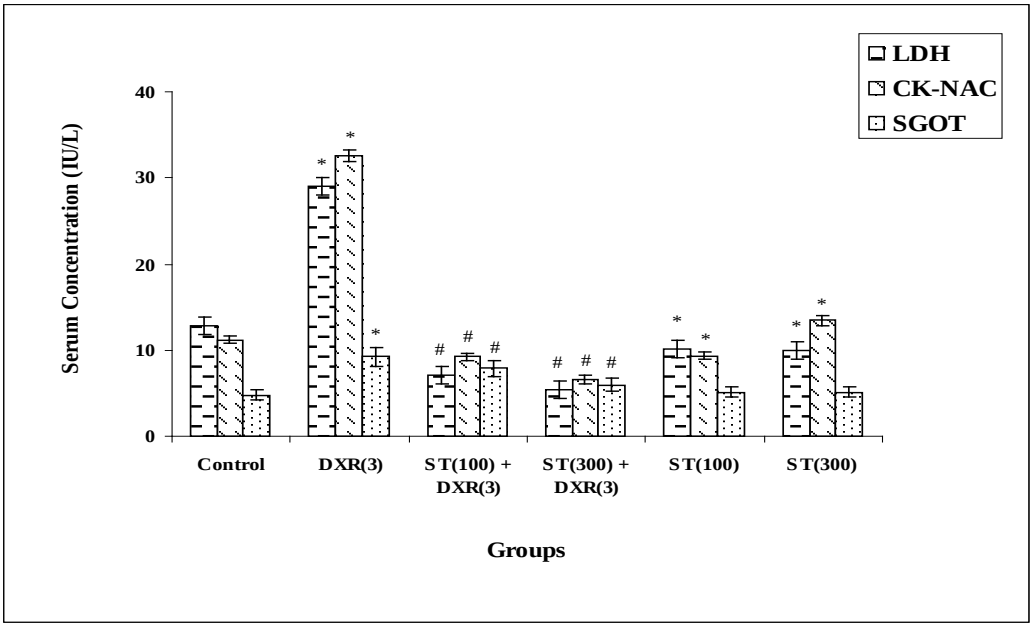

Fig. 1: The Effect of ST extract (100, 300 mg/kg/day, p.o., for 4 weeks) on biomarkers enzyme in DXR treated male rats.

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * $p<0.05$ when compared to control and # $p<0.05$ when compared to DXR group. Vertical lines represent S.E.M. ST-*Solanum torvum,* DXR-Doxorubicin.

Membrane bound enzymes

The membrane bound enzyme level of Na^+ - K^+ ATPase, Ca^{2+} - ATPase and Mg^{2+} - ATPase of testes in control rats was found to be 6.88 ± 0.07, 4.33 ± 0.08 and 7.03 ± 0.26 µmoles of inorganic phosphorus liberated/min/mg protein. These membrane bound enzyme levels were significantly ($p<0.05$) decreased in testes tissue of DXR (3 mg/kg, i.p. on day 1, 7, 14, 21, 28) treated animals when compared to control group. Cotreatment with methanolic extract of *Solanum torvum* (100 and 300 mg/kg/day, p.o.) for 4 weeks in DXR treated (3 mg/kg, i.p. on day 1, 7, 14, 21, 28) animals showed significant ($p<0.05$) increase in membrane bound enzyme levels level in testes tissue as compared to DXR treated animals (Figure 2).

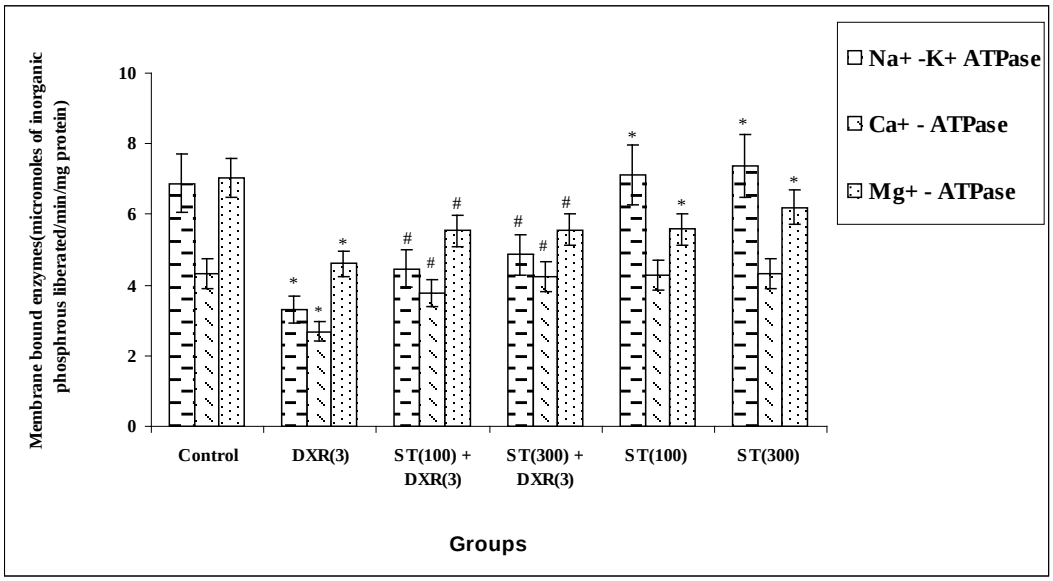

Fig. 2: The Effect of ST extract (100, 300 mg/kg/day, p.o., for 4 weeks) on membrane bound enzyme in DXR treated male rats.

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * $p<0.05$ when compared to control and # $p<0.05$ when compared to DXR group. Vertical lines represent S.E.M. ST-*Solanum torvum,* DXR-Doxorubicin.

Histopathology

Control group treated with vehicle showed normal architecture, normal feature of seminiferous tubules and interstitial tissue. Spermatocytes, sertoli cells, mature sperms are were seen. Group treated with DXR (3 mg/kg, i.p.on day 1, 7, 14, 21, 28) showed degenerative changes in the seminiferous tubules, the polarity of spermatocyte and basement membrane were disturbed. No mature sperms were present and the normal architecture of cells was disturbed.

Cotreatment with methanolic extract of *Solanum torvum* (100 and 300 mg/kg/day, p.o.) for 4 weeks in DXR treated (3 mg/kg, i.p. on day 1, 7, 14, 21, 28) animals showed near normal architecture (Figure 3).

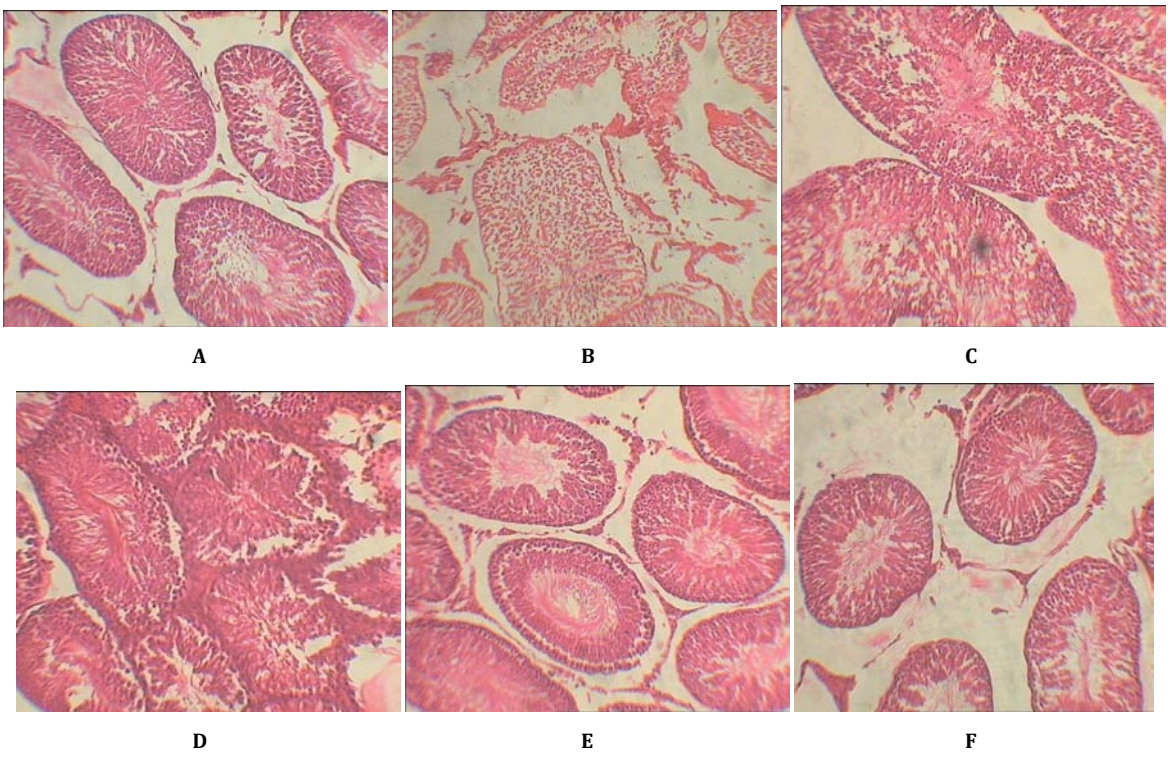

Fig. 3: The Photomicrographs of histopathological examination (10 X) of testes tissue.

A) Control group treated with vehicle shows normal architecture, normal feature of seminiferous tubule and interstitial tissue. Spermatocytes, sertoli cell,mature sperms are present .

B) Group treated with DXR (3 mg/kg) shows degenerative changes in the seminiferous tubule. It disturbs the polarity of spermatocyte. Disturption of basement membrane. No mature sperms are present. Normal architecture of cell gets disturb.

C) Group treated with ST (100mg/kg) and ST (300mg/kg) shows normal architecture, shows normal feature of seminiferous tubule and interstitial tissue. Spermatocytes, sertoli cell, mature sperms are present similar to control group.

D) Group treated with ST (100mg/kg) and DXR 3 mg/kg) and ST (300mg/kg) and DXR (3 mg/kg) shows significant increase in mature sperms, try to gain the basement membrane as compare to DXR, restored these changes towards normalcy.

DISCUSSION

Doxorubicin a widely used anticancer drug is associated with multiple organ toxicity. A stratergy to preserve the anticancer properties with minimum side effects is desired. Doxorubicin is known to disturb spermatogenesis in a dose dependent manner in animal studies [6]. Ward and his co-workers have also reported doxorubicin induced reductions in testicular sperm count [35]. Administration of DXR to rats significantly increased epididimal sperm count, TBARS levels, and levels of LDH, SGOT and CK-NAC; and significantly decreased testes weight, antioxidant defence enzymes SOD, CAT,GSH; membrane bound enzymes such as Na^+ K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase; and total proteins. Our results showed that DXR induced biochemical and histopathological changes which are indicative of toxicity are in good agreement with those previously reported [20, 36]. However treatment with *S. torvum* extract (100 mg/kg and 300 mg/kg) has resulted in significant ($p<0.05$) change in all parameters as observed in DXR treated animals thus offering considerable protection against testicular toxicity. Free radical scavenging enzymes such as catalase, superoxide dismustase are the first line cellular defense enzymes against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical ($OH\cdot$). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles [37]. In our study, a decrease in concentration of antioxidant levels in DXR treated group was observed. Treatment with *S. torvum* extract (100 mg/kg and 300 mg/kg) significantly increased the antioxidant levels. There is a general agreement that flavonoids act as scavengers of reactive oxygen species [38]. The antioxidant properties of *Solanum torvum* could be attributed to the presence of flavonoid phytoconstituent in it. Elevated serum levels of CK-NAC, LDH and SGOT are indicative of toxicity as seen in DXR group, which was ameliorated by concurrent treatment with *S. torvum* extract. The restoration of membrane bound enzymes like in *S. torvum* treated rats was due to the membrane stabilizing protective effect of *S. torvum.* Moreover, the histopathology data has revealed that treatment with *S.torvum* has protected the testis from degenerative changes in the seminiferous tubule, disruption of basement membrane and normal architecture induced by DXR. Thus, this study revealed that pretreatment with *Solanum torvum* protected against DXR induced testicular toxicity.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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