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Research Article

PROTECTIVE EFFECT OF *BAUHINIA RACEMOSA* AGAINST STREPTOZOTOCIN INDUCED DIABETES MALE INFERTILITY COMPLICATIONS

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ABSTRACT

The present study is intended to evaluate the antidiabetic activity of *Bauhinia racemosa* and its protective nature in male infertility of streptozotocin (STZ) induced diabetic male rats and compared with the standard Gliclazide. At the end of study period all the animals' serum was analyzed for testosterone levels along with blood glucose levels, and sperm was collected from the epididymis and sperm parameters analyzed, Testis were examined for antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) activity and animals were sacrificed for histopathological studies. The results of the study indicate that, *Bauhinia racemosa* possess anti-diabetic activity and in addition it possess the capacity in the reduction of diabetic complications including male infertility. The assessment superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels in testis in *Bauhinia racemosa* extract treated animals were recovered as the animals treated with the standard drug Gliclazide. Further research is in progress to identify the particular bioactive molecules responsible for the anti-diabetic activity and protection from complications of diabetes in *Bauhinia racemosa* plants' bark.

Keywords: *Bauhinia racemosa*, Streptozotocin, Diabetes, Complications, Sexual dysfunction.

INTRODUCTION

The chronic metabolic disorder, diabetes has been increasing drastically around the worldwide in recent times compared to past decades. It was characterized by the diminished insulin secretion from the islet of β cells of Langerhans of pancreas. India occupies 2nd place with more than 60 million people followed by china, U.S in the world[1]. Type II diabetes mellitus is commonly appeared diabetes in the majority of people and cause prolonged hyperglycaemia which in turn leads to severe complications such as retinal, neural, renal, cardiovascular dysfunction and sexual dysfunctions[2]. Sexual dysfunction can affect anyone at any time but it is more common in people suffering with diabetes. It has been estimated that approximately about 35 - 75% of diabetic men will experience at least a minimum degree of Sexual impotence during their life time[3]. Synthetic drugs which were used for diabetes and for its complications have many major side effects like Severe hypoglycemia, diarrhoea, metallic taste, nausea and some are heavy teratogenic, Though, there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects[4]. In this point of view, we have carried out the present work using medicinal plant *Bauhinia racemosa*. *Bauhinia racemosa* belongs to the family Fabaceae, its leaf extract having anti-inflammatory, analgesic, anti-pyretic[5], skin diseases, anti-spasmodic, anti-helmenthetic and antimicrobial activity[6]. Anti-oxidant, anti-tumor[7,8] and hepato-protective effects[9]. A bark extract is widely useful for ulcers, tooth and inflammation. *B. racemosa* have been using for sexual dysfunctions in traditional medicine. So, the present work carried out to evaluate antidiabetic activity and protective effect on sexual dysfunction in male rats.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents used for the present study were of analytical grades. Diagnostic kits were purchased from Span diagnostics Ltd, Gujarat, India. Streptozotocin (STZ) was purchased from Sigma chemicals, St Louis, USA and Gliclazide from Avantis Pharma Ltd.

Collection of plant material and preparation of extract

Bauhinia racemosa was widely grow throughout the India, China, U.S and Hawaii[10]. The branches of *Bauhinia racemosa* were collected at near Bhimavaram, Andhra Pradesh, India and it was authenticated by Dr. S.B. Padal, Department of Botany, Andhra University. The bark was removed and washed with distilled water allowed to shade dry at room temperature. The dried barks were made in to coarse powder by using dry grinder and passed through sieve no 40. The powder of *Bauhinia racemosa* bark was used for extraction using Soxhlet apparatus with water for 18 hours.

Selection of experimental animals

Adult male albino wistar rats of 200-220 g weighed rats procured from mahaveer enterprise, Hyderabad. Animals were

kept at 25-28°C, maintain clean environmental conditions, and 12hr dark and 12 hr light cycle. They were fed with normal pellet diet (NPD) and water ad libitum. This protocol was subjected to scrutiny of institutional animal ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for experimental clearance (Regd no./516/01/A/ CPCSEA).

Acute toxicity studies

Acute toxicity study of aqueous bark extract of *Bauhinia racemosa* was performed as per Spearman- Karber analysis[11]. For this study, we utilized either sex of Swiss albino mice (n=6). Food was removed from 12hr before starting the experiment. 2 g/kg dose of *B. racemosa* bark extract was administered orally and observed the behaviour of animal like muscle functioning, psychological activities, skin colour and other abnormal reactions observed for 3 days, no abnormal signs and no mortality rate was observed during these days. Aqueous extract of *Bauhinia recemosa* extract was found to be safe (no mortality) even when given at the dose of 2000 mg/kg body weight with no signs of acute oral toxicity at respective dose. Hence, 1/10th of this lethal dose was taken as effective dose (Therapeutic dose) for antidiabetic activity i.e., 200 mg/kg b. w. p. o.

Antidiabetic activity
Experimental protocol

Animals were categorized into five groups, each having six rats[12].
Group I: Normal control rats administered saline daily for 28 days.
Group II: Diseased control rats administered saline daily for 28 days.
Group III: diabetic rats administered standard drug Gliclazide (1mg/kg, p.o.) daily for 28 days.
Group IV: diabetic rats administered Aqueous extract of *Bauhinia recemosa* (AEBR) (200 mg/kg, p.o.) daily for 28 days.
Group V: diabetic rats administered AEBR (400 mg/kg, p.o.) daily for 28 days.

Preparation of streptozotocin in ph 4.5 containing citrate buffer

In this study, we used 60mg/kg body weight of Streptozotocin which is dissolved in ph 4.5 containing citrate buffer[13].

Preparation of 0.1M citrate buffer (pH.4.5)

Solution-A (0.1M citric acid monohydrate): 2.1gm of citric acid monohydrate taken into a volumetric flask and dissolved with 100ml of distilled water.
Solution-B (0.1M Tri sodium citrate dihydrate): 2.94 gm of Tri sodium citrate dihydrate taken into a volumetric flask and dissolved with 100ml of distilled water.
The addition of 44.5ml of solution-A and 55.5ml of solution-B gives 100 ml of 0.1M citrate buffer with pH.4.5.

Induction of Type II diabetes mellitus

Before the induction of Non-insulin dependent diabetes mellitus (NIDDM) animals were fasted overnight, diabetes induced with 60 mg/kg body weight of STZ, 15 min after the i.p. administration of 120 mg/kg nicotinamide intraperitonially[14]. After 72 h of administration the blood glucose levels were elevated, and the blood glucose levels were reached above 300 mg/dl it indicates that all the rats were induced with diabetes. Gliclazide used as standard.

Collection of blood for estimation of blood glucose levels

Blood was withdrawn by puncturing of retro-orbital plexus under anaesthesia (Diethyl ether). These blood samples were collected into an empty eppendorf tubes. Serum was separated by centrifugation at 2000rpm for 10mins and thereafter allowed for analysis by using auto-analyzer for estimation of blood glucose levels at the end of every week. And at the end of the study period serum testosterone levels were also estimated. All the animals were scarified and allowed for Sperm analysis, *in vivo* antioxidant and histopathology studies.

Sperm Analysis

The cauda epididymis was examined for sperm analysis. The epididymis was finely minced 5.0 ml of isotonic saline in a petridish. The sperms were counted by using Neubaur chamber (Deep 1/10 nm), LABART, Darmstadt Germany) as described by Belsey[15]. Sperm motility was evaluated microscopically within 5 min following their isolation from cauda epididymis. at 37^0 C will be expressed in percentage [15]. The ratio of live to dead sperms was determined by using 1% tryptan blue as described in the method of Tabolt and Chacon[16].

To determine the normal sperms and sperm abnormalities morphologically the samples were stained with eosin-nigrosin. Sperm count data is expressed in million cells per cauda. And for all the other sperm parameters data is expressed as percentage of total sperm.

Determination of Testosterone levels

Serum sample was taken in a micro plate well and enzyme testosterone conjugate was added, then the reactant was mixed. After the completion of required incubation period (60 minutes at 37^0C) the antibody bound enzyme testosterone conjugate was separated from the unbound enzyme testosterone conjugate by decantation. The activity of the enzyme present on the surface of the well is quantitated by the reaction with tetramethylbenzidine (TMB) substrate solution with 15 min incubation and finally by adding 0.3 M H_2SO_4 as stop solution. The absorbance was read against blanking well at 450 nm within 30 minutes in Enzyme-linked immunosorbent assay (ELISA) reader.

***In vivo* antioxidant activity**

At the final stage of the experiment, isolate testis from animals by cervical dislocation method. Washed in ice-cold (0.1 M, pH 7.4) containing Tris–HCl buffer. The testis was again rinsed in ice cold 0.15M containing potassium chloride (KCl) and homogenized (10% w/v) using 0.05% pH 7.5 containing potassium dihydrogen phosphate (KH2PO4) buffer in 0.5 mM EDTA (Rahul Chandran et al., 2016). The cytosolic sample of testis homogenate was centrifuged at 10,000 rpm for 15 min at 5^0 C. Separate the upper lipid layer carefully and the resulting supernatant further centrifuged at 5000 rpm for 15 min at 5^0 C. The supernatant obtained was used for further in vivo antioxidant assays. The in vivo antioxidants like Superoxide dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH) were estimated[17, 18].

Histopathological studies

The testis was kept in the 10% neutral buffered formalin prior to histopathology. The organs are washed and embedded in

paraffin and subjected to microtome. Three micron section were prepared and stained by haematoxylin and eosin dye. The stained sections were observed under phase contrast microscope (Labomed) (400X magnification). Sections were analysed using computer-assisted colour image analysis system to estimate the damage of basement membrane, integrity, seminiferous tubules, decrease in spermatogonia destruction of leydig cells.

Statistical Analysis

All the results were expressed as mean±SEM. Blood glucose levels estimated by Bonferroni posttest test. All the semen parameters and tissue biochemical estimations were tested by using one way ANOVA repeated measurements followed by Tukey's t-test. $P < 0.05$ was considered as statistically significant.

RESULTS

Anti-diabetic activity

Streptozotocin induction was raises the blood glucose levels to an average of four to five folds than the normal control. However, in this study nicotinamide (NA) (120 mg/kg) injection was given intraperitonially to rats to protect the pancreatic b-cells from vast damage. On the 4th day after the administration of STZ-NA injection, the rats were having fasting blood glucose level above 300 were selected for treatment. Observed the food intake, water intake during the study period and the body weight was measured on end of 1st, 2nd, 3rd and 4th week of treatment with AEBR. After the 1st week of treatment it was found that AEBR 400 mg/ kg dose and gliclazide could help the rats in acquire weight. The diseased rats reported loss of weight from the 1st week of treatment itself. The body weight of the standard Gliclazide treated rats were increased for the 2nd consecutive week (206.5 ± 2.84 to 221.2 ± 1.90 g). Finally, weight gain on 4th week by the rats treated with AEBR (400 mg/kg) (Table 1) observed were statistically significant ($p< 0.05$)

Blood glucose levels was noted on the 0^{th} week (0^{th} day), 1^{st} week (7^{th} day), 2nd week (14^{th} day), and 4^{th} week (28^{th} day) of treatment with extract and standard drugs. The major glucose changes of the study are presented in Table 2. The blood glucose level was raised on the 4th day of Streptozotocin-nicotinamide (STZ-NA) injection and taken as the 1st day of drug administration. On the end of the forth week, AEBR at 400 mg/kg showed a significant ($p < 0.001$) reduction (109.74 ± 1.24) compared to other (200mg/kg) treatment groups have showed a significant ($p < 0.001$) reduction (124.18 ± 1.63) and standard (Gliclazide) have showed a significant ($p < 0.001$) reduction (101.74 ± 1.89). The crucial step was to notice whether the extract and standard drugs reduced the blood glucose level till the final stage of the study. On end of the last week (28th day) it was observed that the AEBR extract (400 mg/kg) was efficient to reduce the blood glucose levels (109.74 ± 1.24) to a far better compared to other groups more notably to standard gliclazide (Figure 1).

Sperm Analysis

The data on sperm characteristics showed a significant ($P < 0.05$) decrease in sperm count, sperm motility, sperm viability, and there is increase in abnormal sperms in the diabetic control group as compared to that of normal control group. In comparison between, high dose of AEBR and Gliclazide treated diabetic rats showed a significant ($P < 0.05$) elevated levels of sperm count, motility, viability with decreased abnormality of sperms when compared to that of diabetic control group. The diabetic control group showed significant ($P < 0.05$) decrease in serum testosterone levels when compared to the control group. whereas high dose of AEBR and Gliclazide treated diabetic rats showed a significant ($P < 0.05$) increase in serum testosterone levels when compared to the diabetic control group. (Table 3).

In vivo antioxidant activity

Tissue antioxidant enzyme activities of all the groups were presented in Table 4. Streptozotocin induction resulted in a significant decrease in SOD, CAT and GSH activities when compared to normal control group ($P < 0.05$). However, simultaneous administration of aqueous bark extract of *Bauhinia racemosa* restored the SOD, CAT and GSH activities near to the normal levels, which was significant when compared STZ injected diabetic group ($P < 0.05$).

Table 1: The effect of aqueous bark extract of *Bauhinia racemosa* on body weight in different treatment groups due to Nicotinamide-STZ (60mg/kg) induced diabetes in rats

Groups	0^{th} day	7^{th} day	14^{th} day	28^{th} day
Normal	195.4 ±2.75	200.3 ± 5.31	204.8 ± 2.8	208.4 ±2.89
Diseased	203.50 ± 2.8	172.00 ± 2.59	158.00 ± 2.51	$145.0 \pm 1.72^{\#}$
Gliclazide	206.5 ± 2.84	196.5 ± 2.02	203.4 ± 2.52	$221.2 \pm 1.90^{***}$
AEBR (200mg/kg)	202.50 ± 2.4	188.0 ± 2.07	194.00 ± 1.69	$206.4 \pm 1.50^{**}$
AEBR (400mg/kg)	208.5 ± 2.32	192.00 ± 2.1	198.5 ± 2.28	$217.52 \pm 1.90^{**}$

Values are expressed in MEAN ± SEM

$P<0.05^{*}$, $P< 0.01^{**}$, $P< 0.001^{***}$ significantly increased body weight when compared with disease control and $P<0.001^{\#}$ significantly decreased when compared with normal control when analyzed by Bonferroni posttest when compared with normal control.

Table 2: The effect of aqueous bark extract of *Bauhinia racemosa* on blood glucose levels in different treatment groups due to Nicotinamide-STZ (60mg/kg) induced diabetes in rats

Groups	0^{th} day	7^{th} day	14^{th} day	28^{th} day
Normal	80.67 ± 0.61	93.21 ± 1.25	88.54 ± 1.65	91.84 ± 2.45
Diseased	412.14 ± 4.15	419.86 ± 2.81	433.61 ± 1.89	$441.27 \pm 0.89^{\#}$
Gliclazide	402.23 ± 3.86	356.16 ± 3.29	136.64 ± 3.45	$101.74 \pm 1.89^{***}$
AEBR (200mg/kg)	389.83 ± 6.97	306.72 ± 3.89	156.29 ± 3.25	$124.18 \pm 1.63^{**}$
AEBR (400mg/kg)	375.33 ± 4.24	311.85 ± 2.5	146.22 ± 1.58	$109.74 \pm 1.24^{***}$

Values are expressed in MEAN±SEM

P<0.05*, P< 0.01**, P< 0.001*** significantly decreased the serum blood glucose levels when compared with disease control and P<0.001# significantly increased when compared with normal control when analyzed by Bonferroni posttest when compared with normal control.

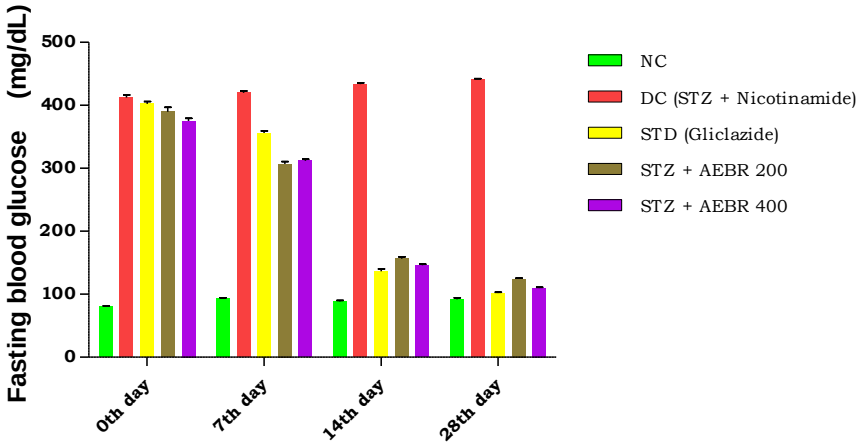

Figure 1: The effect of aqueous bark extract of *Bauhinia racemosa* on blood glucose levels in different treatment groups due to Nicotinamide-STZ (60mg/kg) induced diabetes in rats

Table 3: Effect of Aqueous bark extract of *Bauhinia racemosa* on sperm parameters

Sperm parameters	Normal control	Diabetic control	Standard (Gliclazide)	AEBR 200mg/kg	AEBR 400mg/kg
Sperm count (millions/ml)	73.2±2.5	38.90±3.6*	68.1±2.1**	54.7±3.2**	57.8±4.6**
Sperm motility (%)	71.5±4.50	32.7±2.45*	64.5±2.12**	52.4±2.9**	55.25±4.2**
Sperm viability (%)	78.1±4.92	46.4±4.82*	68.4±2.13**	53.1±5.4**	61.7±4.8**
Sperm morphology (%)	3.48±0.42	46.24±5.24*	16.79±1.58**	31.63±2.3**	25.4±2.54**
Testosterone levels (ng/ml)	6.42±0.84	1.64±0.54*	4.84±0.51**	3.21±0.18**	3.97±0.28**

*P <0.05, compared with normal control rats; **P<0.01 compared with disease control rats.

Table 4: Testicular SOD, CAT and GSH levels in normal and STZ induced diabetic rats

Parameters	Normal control	Diabetic control	Standard(Gliclazide)	AEBR 200mg/kg	AEBR 400mg/kg
SOD (units/mg protein)	17.14 ± 0.74	8.66 ± 0.59*	15.4 ± 0.72**	13.1 ± 0.56**	14.2 ± 0.68**
CAT (nmol/mg protein)	19.64 ± 0.56	9.84 ± 0.48*	16.48 ± 0.87**	14.8 ± 0.84**	17.9 ± 0.6**
GSH (nmol/mg of protein)	19.42 ± 0.64	8.22 ± 0.34*	17.64 ± 0.41**	12.9 ±0.48**	14.3 ± 0.15**

*P <0.05, compared with normal control rats; **P<0.01 compared with disease control rats.

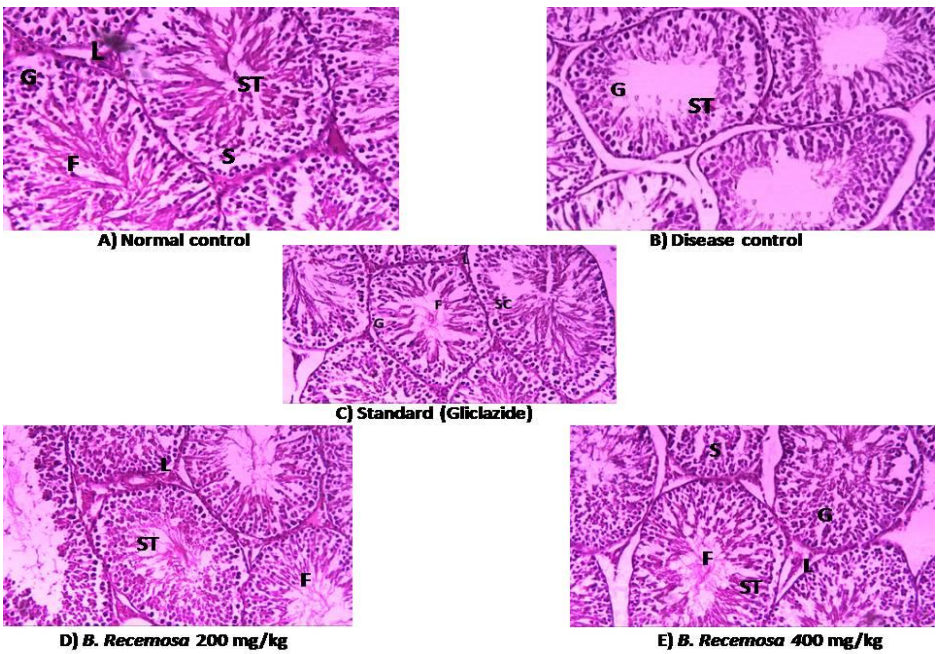

Figure 2: The histopathological study of testis of different groups male rats due to the effect of aqueous bark extract of *Bauhinia racemosa* in Nicotinamide-STZ (60mg/kg) induced diabetes in rats

Testicular morphology by H–E staining is changed significantly in STZ + Nicotinamide induced diabetic rats after 4 weeks. The changes were relieved by treatment with standard drug and plant extracts at 2 doses (200 and 400 mg/kg) in rats. **(A)** Represents normal control rat testis showing spermatogonia (G), seminiferous tubules (ST), Leydig cells (L) sertoli cells (SC), Basement membrane (B) and sperm flagella (F) ; **(B)** Diabetic control group showing testicular damage with absence of sperm flagella; **(C)** DM+ Gliclazide showing restoration of all the cells **(D)** DM+AEBR 200 showing faintly appearance of seminiferous tubules and restoring aii the structures partially; **(E)** DM+AEBR 400 showing completely recovered seminiferous tubules, spermatogonia, sertoli cells, and presence of sperm flagella.

DISCUSSION

Diabetes mellitus is a heterogeneous, multifactorial disorder characterized by hyperglycaemia and gradual decline in insulin action (Insulin resistance), followed by the inability of β- cells to compensate for insulin resistance (pancreatic β-cell dysfunction)[19]. The present study address the antidiabetic effect of Aqueous bark extract of *Bauhinia racemosa* and its protective role in STZ induced diabetic male rats evidenced by remarkable reduction of elevated blood glucose levels, improvement in sperm count, Sperm motility and reduced Sperm abnormal changes and increased testicular antioxidant activities like SOD, CAT and GSH. Right from the beginning acute toxicity studies, it was found that aqueous bark extract of *Bauhinia racemosa* was highly safe and free from toxic effects in mice. A single dose of 5 g/kg *Bauhinia racemosa* bark extract was administered orally[11]. A single dose of STZ 60mg/kg and Nicotinamide 120mg/kg was selectively destroy the pancreatic β cells leading to type II diabetes mellitus. Administration of standard drug Gliclazide and aqueous bark extract of *Bauhinia racemosa* in treatment groups results increase in body weight and decreases the elevated blood glucose levels confirms the antidiabetic activity. STZ induced rats have marked decrease in testosterone levels which were enhanced by Gliclazide and aqueous bark extract of *Bauhinia racemosa.* The reduced levels of testosterone may also due to decreased levels of serum insulin in STZ induced diabetic rats[20].

The histopathological alterations in the STZ induced diabetic rat testis, contorted structures were significantly showing distorted and decreased layers of reproductive germ cells and spermatozoa were absent leaving a large cavity at the centre of lumen. The skeleton of multilayered epithelial cells and extracellular matrix was seriously disturbed and a gap can be seen between reproductive cells and the basement of the seminiferous tubules in the diabetic testis. The density of Leydig cells was greatly reduced in the space among the tubules. These changes represented a picture of less activity of both androgen and sperms in the diabetic testes and were markedly attenuated by interventions with Gliclazide and AEBR 400 mg/kg, respectively (Figure 2).

CONCLUSION

The results of the present study demonstrated that induction of STZ along with Nicotinamide to rats induces testicular dysfunction due to oxidative stress, resulting in structural changes and functional characteristics of spermatozoa and alteration in testicular histoarchitecture. Aqueous bark extract of *Bauhinia racemosa* consists of chemical constituents such as alkaloids, flavonoids, and glycosides plays an important role in preventing sexual dysfunction induced by STZ in rats by its direct effect on the germinal organs and their physiological roles. All the diabetic rats were significantly attenuated by Gliclazide and aqueous bark extract of *Bauhinia racemosa* by restoring the cellular antioxidant levels, there by preserving normal testicular spermatogenesis. The results ensured that beneficial effects of antioxidants against testicular dystropathy and emphasising the protective potential of *Bauhinia racemosa* bark as a natural antioxidant.

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