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Der Pharmacia Sinica, 2014, 5(5): 86-90

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Methanolic whole plant extract of *Biophytum sensitivum* modifies the testicular damage in Streptozotocin induced diabetic rats

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ABSTRACT

Sexual abnormalities are common in streptozotocine induced diabetic rats. In diabetic animals, histopathological and histomorphometric alterations occur in seminiferous tubules as which increases apoptosis in testicular germ cells. Values of sperm count, motility percentage and sperm vitality percentage gradually decrease in diabetic male rats. The whole plant extract of Biophytum sensitivum showing some prominent result against all the alteration of testicular damage.

Key words: streptozotocine, testicular, apoptosis, Biophytum sensitivum

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INTRODUCTION

Now days, cultivation of wild medicinal plants are started, due to the increased popularity and greater demand for medicinal plants. For the treatment of diabetic patients, herbal medicines have been used since long. Herbal remedies are currently accepted as an alternative therapy for diabetic treatment. However in the indigenous Indian system of medicine good number of plants was mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principle were isolated [1]. WHO (1980) has also recommended the evaluation of the effectiveness of plants in conditions where there are no safe modern drugs. According to ethno botanical information reports, about 800 plants may have antidiabetic potential [2]. Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research. Various diseases such as damage of eyes, kidneys, nerves, heart and blood vessels are associated with diabetes. It is associated with health complications including renal failure with risk of foot ulcers, including sexual dysfunction, heart disease, stroke and blindness [3].

Biophytum sensitivum DC(*Oxalidaceae*) is a small, sensitive annual herb. This plant growing throughout tropical Africa and Asia, especially in Philippines and the hotter parts of India and Nepal. It is commonly known as Lajjaluka in Sanskrit, as it can be observed as inward curling of its leaves in response to touch stimuli. For, people of Kerala using the flowers (Mukkutti) both for its medicinal and for its cultural and traditional values. Generally, the whole plant is frequently used for medicinal purpose [4].

Different types of diabetes have been identified and categorized as Type I Diabetes, Type II Diabetes and Gestational Diabetes. Diabetes mellitus (DM) is a quite common metabolic disease that can result in severe structural and functional complications [5]. In addition to unfavourable effects on many tissues and organs, it was reported that DM also affects adversely sexual and reproductive functions in diabetic patients and animals. Studies on diabetic males have shown impaired spermatogenesis, decreased sperm count, sperm motility and seminal fluid volume, as well as decreased testosterone levels. In diabetic animals, histopathological and histomorphometric

alterations occurs in seminiferous tubules [6]. Diabetes increases apoptosis in testicular germ cells either in mice or rats [7].

The present study aimed to investigate the effects of methanolic whole plant extract of Biophytum sensitivum (MEBS) on the testicular tissue of rats with streptozotocin (stz)-induced diabetes.

MATERIALS AND METHODS

Plant material

The whole plant of *Biophytum sensitivum* was collected locally from the forest of Midnapore, West Bengal, India. The plant materials were identified and authenticated taxonomically by an expert taxonomist at the Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103, West Bengal, India. A voucher specimen of each of the collected samples was deposited in the institutional herbarium for future reference.

Preparation of extracts by hot extraction

After thoroughly washed of the plant materials, dried under shade and powdered by a mechanical grinder to obtain a coarse powder and then passed through 20# mesh sieve. 500 g of the dried pulverized whole plant of *Biophytum sensitivum* was extracted with methanol successively in a soxhlet apparatus. Temperature keeps bellow 60 deg C. The successive methanolic extracts were concentrated at reduced temperature on a rotary vacuum evaporator and stored at 4 deg C until further use. The yield was found to be around 8.26 % (w/w) with respect to dried whole plant of *Biophytum sensitivum* [8].

Experimental animals

Healthy young Albino rats weighing between 120 g to 200 g were procured from Indian Institute of Cultivation of science, Kolkata. The animals were individually housed in polypropylene cage and the room condition was maintained at temperature of 25±5 deg C and humidity 45±5 per cent with 12 hr day and night cycle. The animals were fed with Pellet chew feed standard diet and water *ad libitum*. All experimental procedures were conducted with the approval of the Institutional Animal Ethics committee CPCSEA (Reg. No. 1458/ PO/a/11/CPCSEA) for the care and use of animals and their guidelines were strictly followed throughout the study.

Experimental

Experimentally induced diabetes mellitus

Diabetes was induced by an i.p. injection of a single dose of streptozotocin (50 mg/kg) in groups II, III, IV, V rats following overnight fasting. Control rats were injected with the buffer alone. Streptozotocin was dissolved in a freshly prepared 0.01 M citrate buffer at pH 4.5. The streptozotocin-injected animals were given 5% glucose for 24 h to prevent initial streptozotocin-induced hypoglycaemic mortality. The experiment was conducted for 4 weeks. Rats with blood glucose levels of more than 200 mg/dl were considered diabetic and included in the experiment [9].

Experimental design

Male wistar albino rats were divided into five groups of ten animals in each group as follows:
Group-I: Vehicle control received Normal saline orally once daily (10 ml/kg, b.w)
Group-II: STZ induced diabetic rats received Normal saline orally once daily (10 ml/kg, b.w)
Group-III: STZ induced diabetic rats received glibenclamide orally once daily (10 mg/kg, b.w)
Group-IV: STZ induced diabetic rats received MEBS orally once daily (250 mg/kg, b.w.).
Group-V: STZ induced diabetic rats received MEBS orally once daily (500 mg/kg, b.w.).
Glucose concentration in the blood of the tail vein of the rats was measured with an Accu-check active blood glucose monitor test strip.

Evaluation of sperm count, sperm motility and sperm viability

By cutting the cauda region of the epididymis into small pieces, epididymal spermatozoa were collected. Sperm was forced out of the cauda epididymis using fine forceps by putting pressure on the lower region of the cauda epididymis, not forcing out excess material i.e. immature cells. In this study, sperm motility, count, and viability were evaluated by using conventional methods [10]. Progressive sperm motility was measured immediately after the collection of the sperm. The number of motile spermatozoa was calculated per unit area and expressed as percentage of sperm motility. Sperm counts were done using a haemocytometer and the results were expressed as millions/ml of suspension. Sperm viability was measured using Eosin and Nigrosin stain. The dead sperm to up the stain. Hundreds of sperm cells were counted in order to obtain the percentage of live/ dead ratio.

Histological examination:
Animals from each group were randomly selected after four weeks of the experiment, and a portion of the testis was excised from the ether anaesthetized rat, fixed in 10% formalin and processed for histological studies. Using 70%, 90%, and 100% alcohol, tissues are dehydrated and embedded in low melting point paraffin wax. Sectioning the tissues about 5 µm thicknesses. Placed the tissues on glass slide serially. The sections were deparaffinised in xylene and rehydrated through 100%, 90%, and 70% alcohol. Three continuous sections were made from each testis tissue and stained with haematoxylin and eosin for histological evaluation using light microscopy. Spermatogenesis was assessed histopathologically using Johnsen's mean testicular biopsy score criteria [11], [12].

RESULTS AND DISCUSSION

The whole plant extract of biophytum sensitivum showed the anti diabetic activity in our previous experiment[4]. According to that experiment 500mg/kg of the plant extract, reduces more blood glucose in compare to 250 mg/kg of Biophytum sensitivum extract after four weeks of experiment.

Sperm parameters
In diabetic control group Sperm count, sperm motility and sperm vitality were significantly reduced in comparison with other experimental groups. Values of sperm count, motility percentage and sperm vitality percentage were given in Table 3 . All the above parameters were significantly increased in the case of all the treated groups. MEBS (500 mg/kg) treatment significantly increased the sperm count, motility and viability as compared to MEBS (250 mg/kg) treatment.

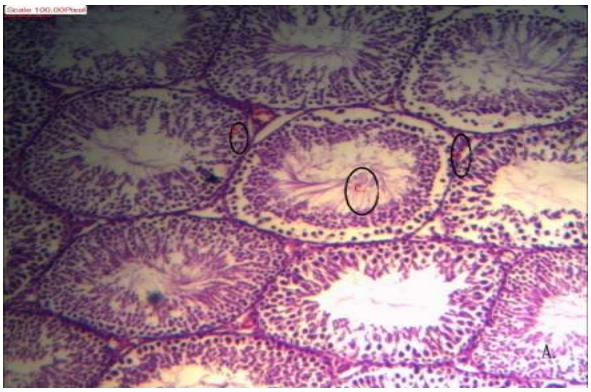
Table 1. Effect of *B. Sensitivum* on sperm parameters (Sperm motility, viability and Count) and counting of spermatogenic cells for each group

Parameters	Normal control	STZ control (50 mg/kg)	MEBS (250 mg/kg)	MEBS (500 mg/kg)
Sperm motility (%)	63.90±1.13*	22.25±0.98	33.60±0.93*	54.66±0.96*#
Sperm viability (%)	70.40±1.16*	25.20±0.82	34.30±1.31*	66.80±0.87*#
Sperm count (106/ml)	27.19±0.67*	8.78±0.97	12.14±0.60*	23.60±0.84*#
Spermatogonia	14.22±0.81*	8.22±0.65	12.01±0.77*	14.44±0.81*#
Spermatocytes	119.01±1.6*	34.28±2.76	87.32±1.7*	92.56±0.88*#
Spermatids	121.0.4±2.44*	33.55±2.66	92.12±2.1*	94.65±1.2*#
Sertoli cells	14.65±0.87*	5.28±0.98	09.66±0.68*	12.77±0.88*#

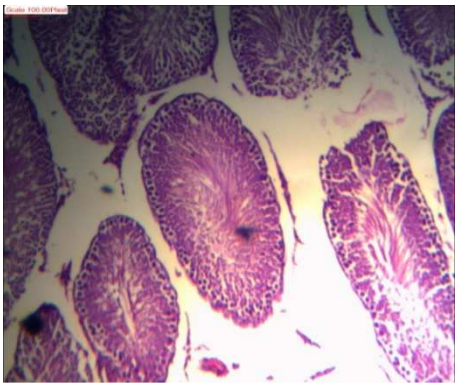
*Values are given as mean ± SEM for groups of 10 animals each. * Statistically significant as compared with streptozotocin group. # Statistically significant as compared with MEBS(250 mg/kg) treated diabetic rat.*

Histological evaluation
Light micrograph of testicular tissue of rats from the normal control group showing the seminiferous tubules have regular shape, their epithelium is structurally intact and shows normal association of germs cells(A). The diabetic control group showing part of seminiferous tubules having irregular shape and the germinal cells and epithelium structures are disorganized. Depletion of germ cells is seen(B). The testicular tissue of rats from the standard drug (glibenclamide) treated group showing histological changes, where these are reduced and the seminiferous tubules have normal structure. The interstitial oedema was seen with less intensity(C). In the MEBS treated groups, the cross section of testicular tissue showing a part of seminiferous tubules having almost regular shape and epithelium structure are organized (D,E). It was observed from histological examinations that MEBS (250 mg/ kg and 500mg/kg) treatment to diabetic rats somehow corrects the histological changes and there was an improvement in the seminiferous tubule structure compared with the diabetic group.

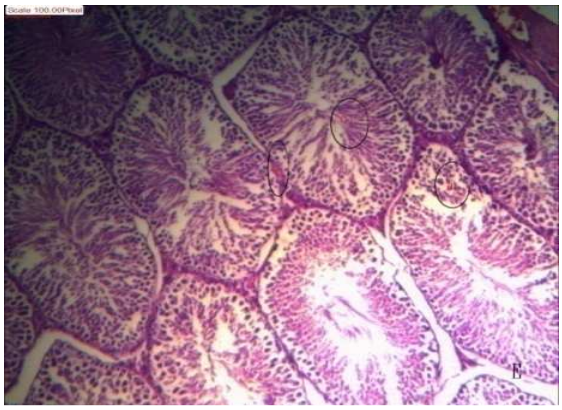
Figure 1. Histological Profile Of Representative Testis Tissue Section In Experimental Animals



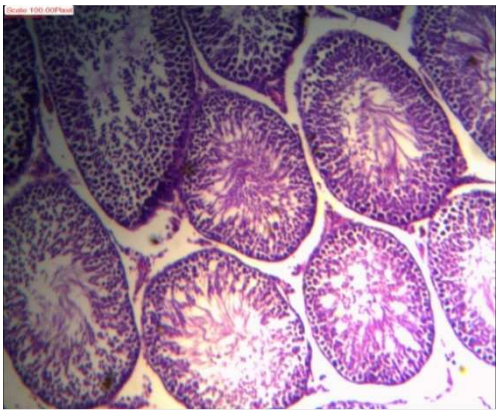
A. Normal control



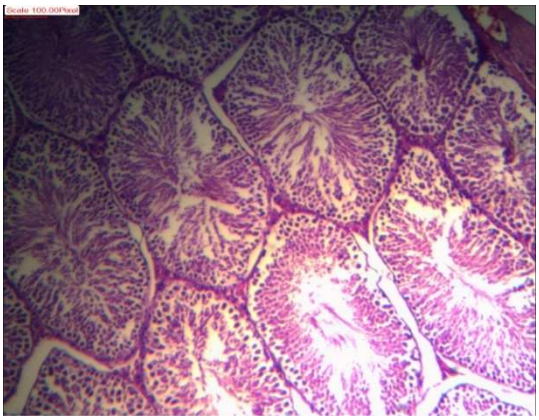
B. STZ control



C. Diabetic+glibenclamide



D. Diabetic+ MEBS(250 mg)



E. Diabetic+MEBS (500 mg)

CONCLUSION

From this result of the study, we can concluded that methanolic whole plant extract of B. Sensitivum modifies the testicular damage in diabetic rats.

Acknowledgement
The authors gratefully acknowledge Mr.Supriya Manna for his assistance in preparing and evaluating histopathological slides. We would like to thank NSHM College of Pharmaceutical Technology for providing the laboratory oriented research facilities.

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