



Vandana Publications

IJRASB

Volume-1, Issue-3, September 2014

International Journal for Research in Applied Sciences and Biotechnology

Page Number: 6-12

## Effects of Diabetes on Sexual Behavior and Castrated Rats

Mohana Sundaram<sup>1</sup>, Surya Prakash<sup>2</sup>, Amirtha Gowri<sup>3</sup>

<sup>1,2,3</sup> Department Of Zoology, Aliah University, Kolkata, West Bengal, India

### ABSTRACT

Diabetes is a metabolic syndrome and a potentially life-threatening illness in which blood glucose levels are abnormally high. Recently, sexual impairment and infertility emerged out to be the most common complications in diabetes mellitus. Sexual impairment is an established risk factor in diabetes mellitus affecting about 75% of male diabetic population. In diabetes, along with poor metabolic status the over expression of enzyme arginase leads to decreased production of NO and erectile dysfunction. Inhibition of arginase enzyme can lead to improvement in diabetes induced sexual dysfunction. In the present investigation diabetes mellitus was induced in adult male rats by intra-peritoneal injection of single dose of alloxan monohydrate (ALX at 120 mg/kg body weight) in distilled water and after the animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. These animals were tested for diabetes after 15 days and animals with blood glucose (fasting) range 300 – 450 mg/dl were selected. Diabetic animals were divided into three groups comprising six animals in each. The 50% methanolic extract of whole plant *Tridax procumbens* (TDX) a potent anti-diabetic plant was administered at a dose of 250 mg/kg b.wt. orally to the different groups of diabetic animals for a period of 30 days. *Tridax procumbens* (TDX) exhibited significant improvement in mating behavior and serum testosterone level as compared with diabetic control rats. Glibenclamide (GLB) showed no improvement in above parameters except restoration in fasting blood glucose. The enhanced sexual performance mediated by *Tridax procumbens* (TDX) may also be anticipated to have biological significance and provide a scientific rationale for the use of *Tridax procumbens* as a sexual performance enhancer in diabetics.

**Keywords**— *Tridax Procumbens*, Mating Behavior, Testosterone, Libido

### I. INTRODUCTION

Diabetes is a metabolic syndrome and a potentially life-threatening illness in which blood glucose levels are abnormally high. The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind's health in all parts of the world.

The frequency of the diabetes will escalate worldwide, with a major impact on the population of developing countries including India (Gupta and Pathak, 2003). Diabetes has long been recognized as a major risk factor for erectile dysfunction (ED) in man. The prevalence of ED is higher in diabetic men than in age-matched non-diabetic men, and this difference increases with age (reviewed in (Hakim and Goldstein, 1996 & Robinson and Ryder, 1997)).

A variety of mechanisms have been suggested for the erectile disorders associated with diabetes. Autonomic neuropathy can account for a decreased function of erectogenic nerves or an altered balance of the proerectile and anti-erectile transmitters reaching the cavernosal smooth muscle. The vascular supply of the penis is highly sensitive to atherosclerotic changes, which are accelerated in diabetic individuals.

Animal studies with experimentally induced diabetes have also shown deficits in several indices of sexual function. In the male rats, such effects have been documented for the mating behavior by some of the research-workers (Stager *et al.*, 1989; Murray *et al.*, 1992; Hassan *et al.*, 1993; McVary *et al.*, 1997 and Ari *et al.*, 1999).

In a very recent review article, the specific impact of diabetic syndrome on sexual behavior was studied and it was concluded that the diabetes affects male reproductive functions at multiple levels as a result of its effects on the endocrine control of spermatogenesis, steroidogenesis, sperm maturation, impairment of penile erection and ejaculation (Jangir and Jain, 2014).

The mechanisms responsible for diabetes-induced sexual impairment in the animal models are controversial. A variety of mechanisms including central neuropathy (McVary *et al.*, 1997) and decreased levels of circulating androgens (Stager *et al.*, 1989; Murray *et al.*, 1992; Hassan *et al.*, 1993; McVary *et al.*, 1997 and Vernet *et al.*, 1995) have been proposed to explain the sexual deficits found in diabetic rats. Diabetes leads to vacuolization in Sertoli cells, raises apoptosis in spermatogonia cells and spermatocytes in seminiferous tubules of male rats (Kanter *et al.*, 2012).

According to research, male reproductive dysfunctions in animal models of diabetes include decreased semen quality, testicular weight, sperm count and motility, and testosterone levels in addition to increased abnormal sperm and oxidative damage in the testes (Amaral *et al.*, 2006). Previous workers have also reported the reduced levels of serum testosterone in rats with experimental diabetes (Stager *et al.*, 1989 and Hassan *et al.*, 1993).

*Tridax procumbens* Linn. (Family- Asteraceae) is common herb found in India. The whole plant and seeds are reported to be used to treat various ailments, such as bronchial catarrh, dysentery, diarrhea, preventing hair loss, and to check hemorrhage from cuts (Saraf *et al.*, 1991; Taddaei *et al.*, 2000). Pharmacological studies have shown that *T. procumbens* possess properties like- anti inflammatory, hepatoprotective, wound healing, Immunomodulatory, antimicrobial, antiseptic, and hypotensive, bradycardiac effects (Diwan *et al.*, 1989; Udupa *et al.*, 1991; Tiwari *et al.*, 2004; Salahdeen *et al.*, 2004; Ravikumar *et al.*, 2005).

In our preliminary investigation (Pareek *et al.*, 2009), the methanolic (50%) extract of whole plant of *Tridax procumbens* exhibited a high anti-diabetic action and the results prompted to undertake the present investigation. Thus, the present study was conducted with an objective to investigate the efficacy of the methanolic extract of *Tridax procumbens* (at its optimal dose) to evaluate its ameliorative action against diabetic induced sexual impairment in male rats.

## II. MATERIALS AND METHODS

### **Plant Material:**

*Tridax procumbens*, whole plant was collected from the Udaipur district during the month of September – October, and was identified and authenticated from the department of Botany, University of Rajasthan, Jaipur. A voucher specimen (RUBL No. 20534) was retained in the Herbarium of the department of Botany, University of Rajasthan, Jaipur.

### **Preparation of Extract:**

The shed-dried plant material was coarsely powdered (6.9 kg) and extracted with 50% methanol using soxhlet apparatus for 36 hrs. The resulting mixture was filtered and the filtrate was evaporated in an oven at 40°C to get the dry residue (7.86 g). The resultant residue was used as ‘drug’ in experiments.

### **Chemicals:**

Alloxan monohydrate and estradiol benzoate were obtained from Sigma Chemical Co. (St. Louis, MO., USA). Glibenclamide tablets (Daonil; Aventis Pharma. Ltd., India) and progesterone (Shalina Laboratories Mumbai, India) were procured from the authorized

distributor of the company. All other chemicals used were of analytical grade.

### **Experimental Animals:**

Adult healthy male albino rats of Wistar strain weighing 140-160g, obtained from IVARI Izatnagar, Bareilly (U.P.) were used after acclimatization for 14 days for this study. The animals were housed in polypropylene cages under standard husbandry conditions (12 hrs light/dark cycle: 25 ± 3°C). Rats were provided water and pellet diet (Hindustan Lever Ltd., Bangalore, India.) *ad libitum*. The study was conducted after the approval from the institutional ethical committee for animal care.

### **Induction of Experimental Diabetes in Rats:**

After fasting, diabetes was induced by a single *intraperitoneal injection* of 120 mg/kg body weight of ‘Alloxan monohydrate’ in distilled water. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. These animals were tested for diabetes after 15 days and animals with blood glucose (fasting) range 300 – 450 mg/dl were selected for experimentation.

### **Experimental Protocol:**

Animals were divided into seven groups of 6 rats each.

**Group I:** Rats served as normal-control and received the vehicle (0.5 ml distilled water /day/rat)

**Group II:** Rats (diabetic) served as diabetic-control and received the vehicle (0.5 ml distilled water /day/rat)

**Group III:** Rats (diabetic) were administered *T. procumbens* (250 mg/kg b.wt./day) in distilled water as a fine aqueous suspension orally.

**Group IV:** Rats (diabetic) were administered *Glibenclamide* (10 mg/kg b.wt./day) in distilled water as a fine aqueous suspension orally.

All the rats were fasted for 16hr. before experimentation, but allowed free access to water.

### **Mating Behavior Test Procedure:**

The male rats were trained by exposing them to sexually receptive females thrice daily for 4 consecutive days before the experiment. Each male rat was allowed a 30-min exposure (adaptation period) to a female rat in behavioral estrous for copulatory behavior, as described previously (Gopumadhavan *et al.*, 2003; Hu *et al.*, 2009). The test was conducted after oral administration of the different doses of the extract and controls.

The test was carried out and sexual behaviours were monitored in each group between 19.00 and 22.00 h under dim light. Three receptive female rats were introduced to each male rat in a metabolic cage for 30 min (adaptation period) after drug administration.

The female was made receptive by the sequential administration of estradiol benzoate (10 µg/100 g body

weight) and progesterone (0.5 mg/100 g body weight) by subcutaneous injections at 48 h and 6 h, respectively.

This treatment assures intense proceptivity and receptivity (Agmo, 1997). Male rats from each group were monitored for sexual behavior for 2 h using video recording observations after their daily drug doses on days 0, 7, 15, and 30 (Gauthaman *et al.*, 2002).

#### **Measurement of Sexual Performance Parameters:**

The assessed sexual parameters were latencies and frequencies of mounting, intromission and ejaculation. Adopting the standard procedures of Agmo (1997) and Gauthaman *et al.*, (2002) the following male sexual behaviour indices were recorded or calculated for the observatory period: mount frequency (MF), (the number of times the male assumed copulatory position but failed to achieve intromission-characterized by lifting of the male's forebody over the hindquarter of the female and clasping her flanks with his forepaw); intromission frequency (IF), (the number of vaginal penetration made by the male); ejaculation frequency (EF), (the number of times there was expulsion of semen by the males after vaginal penetration characterized by rhythmic contraction of the posterior abdomen). The female rats were also observed for the presence of vaginal plug.

In addition, other standard parameters of sexual behaviour obtained through manual data acquisition using stopwatch included mount latency (ML), (the time from the introduction of the female until first mount by the male); intromission latency (IL), (the time from the introduction of the female until the first intromission by the male-usually characterized by pelvic thrusting and springing dismount); ejaculation latency (EL), (the time from the first intromission until ejaculation usually characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of reduced activity; and post ejaculatory interval (PEI), (the time interval from ejaculation to intromission of the next series).

Some additional male sexual behaviour parameters computed include: % index of libido = (number mated/number paired)  $\times$  100; % mounted = (number mounted/number paired)  $\times$  100; % intromitted = (number of rats that intromitted/number paired)  $\times$  100; % ejaculated = (number of rats that ejaculated/number paired)  $\times$  100; copulatory efficiency = (number of intromissions/number of mounts)  $\times$  100; intercopulatory efficiency = average time between intromissions.

#### **Determination of Serum Testosterone Concentration:**

The blood was collected from the tail vein and the serum testosterone concentration of the animals was determined using the procedure based on the principle of competitive binding between testosterone in the test specimen (serum) and testosterone HRP conjugate for a constant amount of rabbit anti-testosterone (Tietz, 1995).

The assay procedure entailed dispensing 10  $\mu$ L of testosterone reference standards (0, 0.1, 0.5, 2.0, 6.0 and 18.0 ng/mL), serum (diluted  $\times$ 5) and testosterone controls 1 and 2 into a Goat Anti-Rabbit IgG-coated microtitre wells (96 wells) after which 100  $\mu$ L of testosterone-HRP conjugate reagent (blue colour) and 50  $\mu$ L of rabbit antitestosterone reagent were separately dispensed into each well. The resulting solution was thoroughly mixed for 30 seconds and incubated at 37°C for 90 minutes. This was to allow a fixed amount of HRP-labelled testosterone to compete with the endogenous testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific testosterone antibody (since the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen increases).

The microwells were rinsed and flicked 5 times with distilled water (to remove the unbound testosterone peroxidase conjugate) before dispensing 100  $\mu$ L of TMB reagent into each well. The resulting solution was mixed gently for 5 seconds. This was later incubated at room temperature for another 20 minutes for blue colour to develop.

The colour development was stopped with the addition of 100  $\mu$ L of Stop Solution (1N HCl) to each well and when gently mixed, the colour changed from blue to yellow. The absorbance was read within 15 mins at 450 nm with a microtitre well reader.

The intensity of the colour formed was proportional to the amount of enzyme present and was inversely proportional to the amount of unlabelled testosterone in the sample. The testosterone concentration in the serum of the animals was calculated from a calibration curve (obtained by plotting the concentration of the standard against the absorbance) using the following expression:

$$\text{Testosterone concentration ng/dL} = C_s \times F, (1)$$

Where  $C_s$  is Corresponding testosterone concentration from the calibration curve and  $F$  is Dilution factor.

#### **Statistical Analysis:**

All the data were calculated and statistically analyzed with SPSS/PC<sup>TM</sup> V. 13.0 computer software. The data were expressed as mean  $\pm$  SEM and tested for variance. When variance was homogeneous, a one way analysis of variance (ANOVA) was carried out; when any significant difference was detected, the data were further analyzed with *post hoc test*. A probability (P) of < 0.05 was accepted as statistically significant.

The percentage values were converted by arcsine transformations before the above analysis. All the other data were statistically analyzed with one way ANOVA for the comparison between groups, followed by Tukey multiple comparison as a *post hoc test*.

### III. RESULTS

The results of the present investigation are summarized and exhibited in the form of table (1) and figures (1-8). *Sexual Performance Parameters* Figure (2-8) and Table 1 summarizes the data obtained with the sexual behavior study. At day 0, the sexual performance parameters were recorded, which indicates the slight lower sexual performance (non-significant) in the diabetic rats of all the three groups. Several female pro-ceptive and male pre-copulatory behavior parameters were also observed, when the extract-treated male rats were introduced to the receptive female rats.

The male rats, upon introduction, responded with immediate advances towards the females and displayed pre-copulatory behavior such as chasing, ano-genital sniffing which eventually culminated into mounting. The copulatory activity recorded and exhibited in Figure 2-4 (including mounting (MF), intromission (IF) and ejaculation frequencies (EF) data at different intervals. At day 7, 15 and 30, the extract TDX 250 investigated significantly ( $P < .05$ ) increased EF in the animals (Figure 4). There was vaginal plug in the female's vagina after ejaculation.

The number of male rats displaying copulatory activity, gradually declined in a significant manner in the diabetic control group in comparison to the normal control group; this value increased considerably in the extract (TDX-250) treated animals ( $P$  not significant vs. control). Furthermore, those diabetic animals still copulating showed substantial deficits in all of the standard measures of the mating test, and these deficits were reversed by TDX-250 treatment.

Also, the computed data clearly showing reversal of sexual behavior in diabetic rats treated with *T. procumbens* 250 mg/kg b.wt. The data pertinent to libido (%) index, percent mounting & intromitted, ejaculatory and copulatory efficiency showed non-significant differences in normal and TDX-250 treated rats, however the standard drug glibenclamide could lower the blood glucose level but not the sexual performances of male rats.

The extract produced contrasting effects on the EL and PEI of the male rats. The ICP: inter-copulatory performance (assessed by virtue of latency periods viz. ML, IL, EL & PEI are shown in Figure 5-8) response was also resulted to be decreased in the diabetic groups relative to the normal control group; in contrast, the ICP response in the TDX 250-treated diabetic group was similar to that in the control group, whereas, the GLB 10 treated group animals didn't exhibited any significant reversal.

**Table 1: Effect on Computed Sexual Behavior Parameters after 30 Days of Oral Administration of *T. Procumbens* in Normal and Alloxan-Induced Diabetic Rats**

Determination	Normal Control	Diabetic Control	Diabetic+ <i>T. Procumbens</i> (250mg/kg)	Diabetic+ Glibenclamide (10mg/kg)
Index of Libido (%)	90	36	81	45
% Mounted	80	40	80	60
% Intromitted	60	10	40	20
% Ejaculated	40	5	20	10
Copulatory Efficiency (%)	62.50	24.56	50.89	30.29
Inter-Copulatory Interval (sec)	439.68 ± 16.84 <sup>a</sup>	851.66 ± 26.44 <sup>b</sup>	485.10 ± 21.36 <sup>a</sup>	799.45 ± 18.48 <sup>b</sup>

\*Values for a given group in a row followed by same letter as superscript are not significantly different according to Tukey's multiple comparison procedure (at  $P < 0.05$ ).

Hormone Assay :

Figure 1 showing the level of serum testosterone after 30 days in control and treated groups. The levels of testosterone in diabetic animal were significantly low and in TDX 250 treated group it was non-significantly different from the normal group. In GLB 10 treated animals also the testosterone level was significantly lower than the normal animals but higher than the diabetic group of animals.

#### IV. DISCUSSION

In present investigation, we examined the effect of methanolic (50%) extract of *Tridax procumbens* L. whole plant (250 mg/kg body weight) on male sexual competence in alloxan-induced diabetic rats, with Glibenclamide (10 mg/kg body weight) as a positive reference drug. To the best of our knowledge, this is the first study to report the effect of methanolic (50%) extract of *Tridax procumbens* L. whole plant in diabetic male rodents rather its first ever study regarding the impact on male sexual performance. The oral administration of extract enhanced the sexual behavior of diabetic male rats compared with controls administered DW. According to Hakim & Goldstein (1996) more than 50% of diabetics are likely to suffer from sexual dysfunction.

The results of this (and earlier) investigation scientifically support the use of *Tridax procumbens* L for treating diabetes (Pareek *et al.*, 2009) and enhancing male sexual ability among the diabetics. The mating behavior test revealed that TDX extract significantly increased MF and IF, compared with the control group, the effect was much higher than that of standard anti-diabetic drug Glibenclamide. The extract of *Tridax procumbens* L. also caused significant reductions in ML and IL, compared with control animals. The MF and IF are considered to be indices of libido (sexual desire) and potency, while ML and IL are also indicators of sexual arousal. The significant increases in MF and IF and the decreases in ML and IL indicate that libido and potency were enhanced by the extract (Ratnasooriya *et al.*, 2000; Tajuddin *et al.*, 2005; Yakubu *et al.*, 2008).

Furthermore, the prolongation of EL by methanolic (50%) extract of *Tridax procumbens* L. whole plant is an indicator of prolonged duration of coitus. PEI is considered to be an index of potency, libido, and the rate of recovery from exhaustion after the first series of mating. The decreased PEI observed with plant extract may have been the result of enhanced potency and libido, and/or reduced exhaustion in the first series of mating. In addition, the higher values of the computed male rat sexual behavior parameters following treatment with the extract when compared with the distilled water-administered control animals are indications of significant and sustained increase in sexual activity.

The enhancement of the sexual behavior observed in diabetic rats after *T. procumbens* administration

(increase in sexual performance associated to a decrease of the motivation parameters) is indicative of the aphrodisiac potential of this plant (Sandroni, 2001; Shamloul, 2010; Suresh *et al.*, 2012).

Many plants with medicinal properties are effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins (Mills *et al.*, 1996). Treatments that alter the concentration of circulating sex hormones may also modify sexual behavior. Clinical data on testosterone also suggest that a slight increase in the levels of the hormone in adult males results in a moderate but significant increase in sexual desire and libido (Thakur & Dixit, 2007). Therefore, the increase in serum testosterone concentration by the plant extract might be responsible for the enhanced sexual behavior in the animals.

Thus, the improvement in the sexual ability of the alloxan-diabetic rats might be through an androgenic pathway. This indicates that the methanolic (50%) extract of *Tridax procumbens* L. whole plant also improves potency in diabetic male rats. The delay in EL treated male rats indicated the involvement of NO in the intervention centered on ejaculatory function centered on ejaculatory function (Du *et al.*, 1999; Hughes *et al.*, 1987). Therefore, we can conclude from these observations that the *Tridax procumbens* L. whole plant extract support in ameliorating the sexual functions in male diabetic rats.

#### V. CONCLUSION

In conclusion, the aqueous extract of *angifera* indicareduced the concentration of glucose, cholesterol, and triglyceride in experimentally induced diabetes rats. Therefore, the plant has a hypoglycaemic and hypolipidaemic effects on alloxan-induced diabetic rats. The study also showed that *Mangifera indica* extract may not have toxic effect on the liver at the ployed dosage, seen in the lowered concentration of AST, ALT and ALP as biochemical enzymes makers of liver damage. Based on these findings, its use should be encouraged in the management of diabetes mellitus.

#### REFERENCES

- [1]Agmo A (1997). Male rat sexual behavior. Brain Research Protocols 1 203–209.
- [2]Amaral S, Moreno AJ, Santos MS, Seica R and Ramalho-Santos J (2006). Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. Theriogenology 66(9) 2056-2067.
- [3]Ari G, Vardi Y and Finberg JP (1999). Nitric oxide and penile erection in streptozotocin-diabetic rats. Clinical Science (Colch) 96 365–372.

- [4]Chauhan NS and Dixit VK (2010). Effects of Bryonia laciniosa seeds on sexual behaviour of male rats International. Journal of Impotence Research 22 190-195.
- [5]Chauhan NS, Rao ChV and Dixit VK (2007). Effect of Curculigo orchioides rhizomes on sexual behavior of male rats. Fitoterapia 78 530-534.
- [6]Chauhan NS, Sharma V and Dixit VK (2011). Effect of Asteracantha longifolia on sexual behaviour of male rats. Natural Products Research 25 1423-1431.
- [7]Diwan PV, Karwande I, Margaret I and Sattur PB (1989). Pharmacology and biochemical evaluation of Tridax procumbens on inflammation. Indian Journal of Pharmacology 21 1-7.
- [8]Drewes SE, George J and Khan F (2003). Recent findings on natural products with erectile-dysfunction activity. Phytochemistry 62 1019-1025.
- [9]Du J and Hull EM (1999). Effects of testosterone on neuronal nitric oxide synthase and tyrosine hydroxylase. Brain Research 836 90-98.
- [10]Gauthaman K, Adaikan PG and Prasad RNV (2002). Aphrodisiac properties of Tribulus terrestris extract (protodioscin) in normal and castrated rats. Life Sciences 71(12) 1385- 1396. [doi:10.1016/S0024-3205(02)01858-1
- [11]Gopumadhavan S, Mohamed R, Venkataranganna MV, Kulkarni KS and Mitra SK (2003). Assessment of 'Tentex royal' for sexual activity in an experimental model. Indian Journal Clinical Practice 13 23-26.
- [12]Gupta OP and Phatak S (2003). Pandemic Trends in Prevalence of Diabetes Mellitus and Associated Coronary Heart Disease in India - Their Causes and Prevention. International Journal of Diabetes in Developing Countries 23 37-50.
- [13]Hakim LS and Goldstein I (1996). Diabetic sexual dysfunction. Endocrinology and Metabolism Clinics of North America 25 379-400.
- [14]Hassan AA, Hassouna MM, Taketo T, Gagnon C and Elhilali M (1993). The effects of diabetes on sexual behavior and reproductive tract function in male rats. Journal of Urology 145 148-154.
- [15]Hu GH, Lu YH, Mao RG, Wei DZ, Ma ZZ and Zhang H (2009). Aphrodisiac properties of Allium tuberosum seeds extract. Journal of Ethnopharmacology 122 579-582.
- [16]Hughes AM, Everitt BJ, Lightman SL and Todd K (1987). Oxytocin in the central nervous system and sexual behavior in male rats. Brain Research 414 133-137.
- [17]Jangir RN and Jain GC (2014). Diabetes mellitus induced impairment of male reproductive functions: a review. Current Diabetes Review 10(3) 147-157.
- [18]Kanter M, Aktas C and Erboga M (2012). Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis. Food and Chemical Toxicology 50(3-4) 719-725.
- [19]Mbongue FGY, Kamtchouing P, Essame OJL, Yewah PM, Dimo T and Lonsi D (2005). Effect of the aqueous extract of dry fruits of Piper guineense on the reproductive function of adult male rats. Indian Journal of Pharmacology 37 30-32.
- [20]McVary KT, Rathnau CH and McKenna KE (1997). Sexual dysfunction in the diabetic BB/WOR rat: a role of central neuropathy. American Journal of Physiology 272 R259-R267.
- [21]Mills TM, Reilly CM and Lewis RW (1996). Androgens and penile erection: a review. Journal of Andrology 17(6) 633-638.
- [22]Murray FT, Johnson RD, Sciadini M, Katovich MJ, Rountree J and Jewett H (1992). Erectile and copulatory dysfunction in chronically diabetic BB/ WOR rats. American Journal of Physiology 263 E151-E157.
- [23]Pareek H, Sharma S, Khajja BS, Jain K and Jain GC (2009). Evaluation of hypoglycemic and anti-hyperglycemic potential of Tridax procumbens (Linn.). BMC Complementary and Alternative Medicine 9 48 doi:10.1186/1472-6882-9-48.
- [24]Ratnasooriya WD and Dharmasiri MG (2000). Effects of Terminalia catappa seeds on sexual behaviour and fertility of male rats. Asian Journal of Andrology 2 213-219.
- [25]Ratnasooriya WD and Fernando TSP (2008). Effect of black tea brew of Camellia sinensis on sexual competence of male rats. Journal of Ethnopharmacology 118 373-377.
- [26]Ravikumar V, Shivashangari KS and Devaki T (2005). Hepatoprotective activity of Tridax procumbens against d-galactosamine/lipopolysaccharide-induced hepatitis in rats. Journal of Ethnopharmacology 101 55-60.
- [27]Robinson AM and Ryder REJ (1997). Impotence in diabetes. Trends in Endocrinology and Metabolism 98-101.
- [28]Salahdeen HM, Yemitan OK and Alada ARA (2004). Effect of aqueous leaf extract of Tridax procumbens on blood pressure and heart rate in rats. African Journal of Biomedical Research 7 27-29.
- Sandroni P (2001). Aphrodisiacs past and present: a historical review. Clinical Autonomic Research 11(5) 303-307.
- [29]Saraf S, Pathak A and Dixit VK (1991). Hair growth promoting activity of Tridax procumbens. Fitoterapia 62 495-498.
- [30]Shamloul R (2010). Natural aphrodisiacs. Journal of Sexual Medicine 7 39-49.
- [31]Steger RW, Amador A, Lam E, Rathert J, Weis J and Smith MS (1989). Streptozotocin-induced deficits in sex behavior and neuroendocrine function in male rats. Endocrinology 124 1737-1743.
- [32]Suresh S and Prakash S (2012). Effect of Mucuna pruriens (Linn.) on sexual behavior and sperm parameters in streptozotocin-induced diabetic male rat. Journal of Sexual Medicine 9 3066-3078.
- [33]Taddei A and Rosas-Romero AJ (2000). Bioactivity studies of extracts from Tridax procumbens. Phytomedicine 7 235-238.
- [34]Tajuddin A, Ahmad S, Latif A and Qasmi IA (2004). Effect of 50% ethanolic extract of Syzygium aromaticum

(L.) Merr. & Perry. (clove) on sexual behaviour of normal male rats. *BMC Complementary and Alternative Medicine* 4 17–24.

[35]Tajuddin Ahmad S, Latif A, Qasmi IA and Amin KMY (2005). An experimental study of sexual function improving effect of *Myristica fragrans* Houtt. (nutmeg). *BMC Complementary and Alternative Medicine* 5 16, doi: 10.1186/1472-6882-5-16.

[36]Thakur M and Dixit VK (2007). Aphrodisiac activity of *Dactylorhiza hatagirea* (D. Don) Soo in male albino rats. Evidence based *Complementary and Alternative Medicine* 4(1) 29–31.

[37]Tietz NW (1995). *Clinical Guide to Laboratory Tests*, (W. B. Saunders, Philadelphia, Pa, USA) 3 395-552.

[38]Tiwari U, Rastogi B, Singh P, Saraef DK and Vays SP (2004). Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals. *Journal of Ethnopharmacology* 92 113-119. Udupa SL, Udupa AL

and Kulkarni DR (1991). Influence of *Tridax procumbens* on lysyl oxidase activity and wound healing. *Planta Medica* 57 325–327.

[39]Vernet D, Cai L, Garban H, Babbitt ML, Murray FT, Rajfer J and Gonzalez-Cadavid NF (1995). Reduction of penile nitric oxide synthase in diabetic BB/WORdp (type I) and BBZ/WORdp (type II) rats with erectile dysfunction. *Endocrinology* 136 5709–5717.

[40]Yakubu MT, Akanji MA and Oladiji AT (2005). Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian Journal of Andrology* 7 399-404.

[41] Yakubu MT, Akanji MA, Oladiji AT and Adesokan AA (2008). Androgenic potentials of aqueous extract of *Massularia acuminata* (G. Don) Bullock ex Hoysl. Stem in male Wistar rats. *Journal of Ethnopharmacology* 118 508-513.