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Original Research Article

Anxiolytic and anti-depressant effects of *Bombax buonopozense* in streptozotocin-induced diabetic rats

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Abstract

Introduction: Diabetes Mellitus (DM) is a metabolic disorder resulting in the presence of chronic hyperglycemia. There seems to be a bi-directional association between diabetes, anxiety and depression.
Purpose: This study evaluates the effect of *Bombax buonopozense* (BB) methanol stem- bark extract on stress, anxiety, depression in streptozotocin- induced diabetic rats
Method: Oral acute toxicity was done with the Methanol stem bark extract of *B. buonopozense* using Lorke's method Hyperglycemia was induced in rats by intraperitoneal injections of streptozotocin (STZ) at a dose of 45 mg/kg body weight. Hyperglycemic rats were subjected to behavioral test models such as forced swim test, elevated plus maze and sucrose splash test using test doses of 200, 400, and 800 mg/kg administered orally.
Result: Oral acute toxicity studies recorded no mortality at doses up to 5000 mg/kg. There was no significant difference in the duration of immobility of rats on day 1, on day 7 there was a significant increase in the duration of mobility of rats that received 400 and 800mg/kg BB (*p<0.05, **p<0.01 respectively) There was a significant increase in the duration of time spent by the animals in open arm of the elevated plus maze by animals that received 200 mg/kg BB on day 1 ****p<0.0001 and animals that received doses of 400 and 800 mg/kg significantly (**p<0.01, ****p<0.0001 respectively) on day 7. In the sucrose splash test, there was no significant difference in duration of grooming amongst animals on day 1 but on day 7, there was a significant increase in the duration of grooming in animals that received 400 mg/kg BB when compared to diabetic untreated group.
Conclusion: The extract of *Bombax buonopozense* is relatively safe; and possesses anti-depressant and anxiolytic-like properties.

Keywords: Diabetes mellitus, stress, anxiety, depression, *Bombax buonopozense*

Indexing: Index Copernicus, African Index Medicus

Introduction

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to heart, blood vessels, eyes, kidneys, and nerves [1]

Genetic and environmental factors contribute to the pathogenesis of diabetes mellitus, which involves; insufficient insulin secretion, increased glucose production, and/or abnormalities in fat and protein metabolism [2]. The resulting hyperglycaemia may lead to both acute symptoms and metabolic abnormalities. However, the major sources of the morbidity of diabetes are the chronic complications that arise from prolonged hyperglycaemia, including retinopathy, neuropathy, nephropathy and cardiovascular disease [2].

Reports have shown that there is a bi-directional association between diabetes and depression, a developing body of literature has reported that

patients with diabetes suffer twice as much from anxiety and depression as the general population [3]. Anxiety has been reported to impair metabolic processes and has also been reported to increase complications in diabetics [4].

Bombax buonopozense is distributed throughout the dense forests of Guinea zone (upper and lower) [5]. It has several local names: Igbo as *Akpe*, Yoruba as *Ponpola*, Hausa as *Gurjuya*, *Kurya*, Efik as *Ukim* and Ijaw as *Ido Undu*. It is indigenous primarily in West Africa where it is found in rainforests of east Gabon, Sierra Leone in the northwest, east Gabon.

Bombax buonopozense is well known for its therapeutic value [6]. Phytochemical screening of BB stem bark revealed alkaloid, tannins, protein, terpenoids, carbohydrate, phenols, flavonoids, saponins and oxalates [7]. Quantitative phytochemical analysis also showed that alkaloid, flavonoid, phenols, tannins and saponins had values of 0.68, 0.09, 2.35, 1.41 and 1.15 g respectively.

Steroids and glycosides were undetected. The percentage moisture content found during immediate analysis gave high value of 55.30%. Protein and carbohydrate were of relatively low values 1.04 % and 6.0% respectively. Ash content was discovered to be 15.30% and fiber was 16.80% [7].

This study evaluated the ameliorative effect of *B. buonopozense* on anxiety and depression in diabetic rats

Methods

Animals

Male albino Wistar rats weighing (200 - 350 g) were obtained from the Animal House of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria The rats were kept in plastic cages and housed at room temperature. They were allowed free access to dry rodent pellet feeds (Top Feeds Limited, Ibadan, Nigeria) and water (borehole). The bedding materials (wood shavings) of the cages were changed daily. All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of laboratory Animals (NIH Publications No. 80-23) Revised in 2002[8].

Collection of plant materials

Dried stem barks of *Bombax buonopozense* were collected, identified and authenticated by Mallam Ibrahim Muazzam of NIPRID Abuja, Nigeria. The voucher specimens were deposited in the herbarium for future reference.

Extraction of plant materials

The stem bark of *Bombax buonopuzense* (BB) was air-dried to a constant weight and milled to fine powder using a mechanical grinder. About 500 grams of the powdered plant was macerated with 2.5 L of methanol for 72 hours. The mixture was filtered using a clean piece of cloth. The filtrate was concentrated to dryness under reduced temperature and pressure in an oven. The dried whole extract was stored at 4 ºC until use.

Acute toxicity

Acute toxicity study of the extract was carried out using Lorke's method. In the first phase, three groups of three mice each were administered 10, 100 and 1000 mg/kg of the plant extract orally. The animals were closely observed for the first 2 hours, intermittently for the next 4 hours and then overnight. Observations were made at the end of 24 hours. From the results obtained, the second phase was performed using doses of 1600, 2900 and 5000 mg/kg of plant extract administered orally to three groups of one mouse each. The animals were observed for signs of toxicity and mortality as well as their general behavior. Median lethal dose (LD50) was calculated geometrically meaning the highest non-lethal dose and the lowest lethal dose [9]

Experimental design

The animals were fasted overnight and diabetes mellitus was induced by a single dose intraperitoneal injection of streptozotocin (45 mg/kg body weight) dissolved in freshly prepared 0.1 M citrate buffer, pH 4.5. After administration, the animals were allowed free access to feed and water. After 48 h, the animals fasting blood glucose was determined using the Accu-Check® Active Glucometer (Roche, USA) and any animal with blood sugar level > 200 mg/dl was considered diabetic [10].

The animals were selected into six groups of 7 rats each and treated orally for one week as follows;

Group 1: Diabetic animals treated with glibenclamide 5 mg/kg body weight daily

Group 2: Diabetic animals treated with the extract 200 mg/kg body weight daily

Group 3: Diabetic animals treated with the extract 400 mg/kg body weight daily

Group 4: Diabetic animals treated with the extract 800 mg/kg body weight daily

Group 5: Diabetic animals given 0.2 ml distilled water daily

Group 6: Non-diabetic (healthy) animals given 0.2 ml distilled water daily

Blood samples for measuring the glucose level were collected from the caudal vein of the fasted rats (overnight) daily before 9 am for 7 days. The tail was cleaned with methylated spirit and allowed to dry. The lateral tail vein was pricked using a sterile lancet and a droplet of the blood was placed on glucose test strip and read using the Accu-chek® Active glucometer [11].

Behavioral studies

Elevated plus maze test

The animals were grouped into six groups (treated and untreated group) with 7 rats in each group. Prior to induction of diabetes animal were placed at the center of the maze, facing one of the open arms. The time spent in enclosed and open arms was recorded in 5 min. The test was repeated 24 hours after confirmation of diabetes and on day 7 before sacrificing [12]

Sucrose splash test

Sucrose splash test was conducted on the first day before inducing diabetes, a day after induction of diabetes and 24 hours after the last treatment before sacrificing the animals. The rat's dorsal coat was sprayed with 10% sucrose solution which induced grooming behavior. The time spent grooming was observed and recorded for 5 minutes as an index of self-care and motivational behavior. [13]

Forced swim test

Prior to induction of diabetes, animals were placed individually in glass cylinder (25 cm in diameter × 48 cm in height) containing water to a depth of 30 cm (25 °C ± 1°C). The procedure involved forcing animals to swim and the duration of immobility recorded for the last 4 min within 6-min duration. Immobility time is the time during which the animal floated on the surface with front paws together or making those movements which were necessary to keep afloat. Shorter immobility time is an indication of stronger antidepressant effect of the tested substance [14]. The test was repeated 2 hours after drug treatment on day 7 before sacrificing.

Results

Oral acute toxicity

Table 1 shows the acute toxicity at doses 1600, 2900 and 5000 mg/kg of methanol extracts. Physical examination showed no sign of skin, fur, eyes, writhing and behaviour. The result also showed that there were no tremor and diarrhoea in the mice. There was no record of death at the tested doses thus making the oral LD_{50} of the extract to be estimated as more that 5000 mg/kg.

Effect of treatments on depression in streptozotocin-induced diabetic rats using forced swim test model

Figure 1 shows no significant difference in the duration of immobility of rats on day 1 (day prior to induction of diabetes) whereas on day 7 there was a significant increase in the duration of mobility of rats that received 400 and 800 mg/kg BB (*$p<0.05$, **$p<0.01$ respectively) when compared to the diabetic untreated group.

Effect of treatment on anxiety in streptozotocin- induced diabetic rats using elevated plus maze model

The duration of time spent by the animals in open arm of the elevated plus maze is shown in Figure 2. The result shows a significant increase ****$p<0.0001$ in the duration of time spent in the open arm by animals that received 200 mg/kg BB on day 1. However, on day 7, doses of 400 and 800 mg/kg significantly (**$p<0.01$, ****$p<0.0001$ respectively) increased time spent in the open arm of the maze at end of the experiment.

Effect of treatment on depression in streptozotocin induced diabetic rats using sucrose splash model

Figure 3 shows no significant difference in duration of grooming amongst animals in all groups on day 1. However, on day 7, there was a significant increase in the duration of grooming in animals that received 400 mg/kg BB when compared to diabetic untreated group.

Table 1: Oral acute toxicity of the methanol extract in albino mice

	Dose (mg/kg)	Writhing	Diarrhoea	Tremor	Death	Others
Phase 1	10	0/3	0/3	0/3	0/3	0/3
	100	0/3	0/3	0/3	0/3	0/3
	1000	0/3	0/3	0/3	0/3	0/3
Phase 2	1600	0/1	0/1	0/1	0/1	0/1
	2900	0/1	0/1	0/1	0/1	0/1
	5000	0/1	0/1	0/1	0/1	0/1

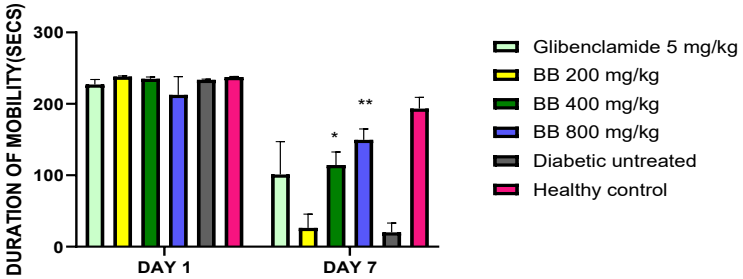

Figure 1: Effect of BB treatment on mobility in streptozotocin- induced diabetic rats using forced swim test model. Values are expressed as mean ± SEM, *p<0.05, **p<0.01 compared to diabetic untreated animals, n=7 animals per group, BB= *Bombax buonopuzense*

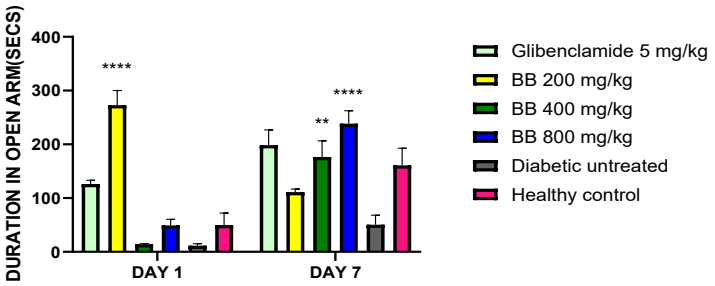

Figure 2: Effect of BB treatment on duration in open arm in streptozotocin -induced diabetic rats using elevated plus maze model. Values are expressed as mean ± SEM, **p<0.01, ****p<0.0001 compared to diabetic untreated animals, n=7 animals per group, BB= *Bombax buonopuzense*

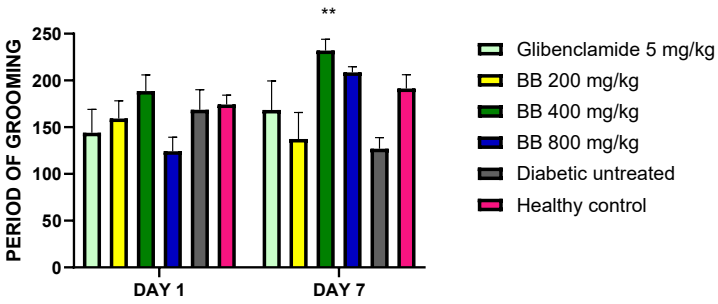

Figure 3: Effect of BB treatment on period of grooming in streptozotocin- induced diabetic rats using sucrose splash model. Values are expressed as mean ± SEM, **p<0.01 when control was compared to diabetic untreated animal, n=7 animals per group, BB= *Bombax buonopuzense*.

Conflict of Interest

The current study demonstrates the neuro-behavioral effect in relation to depression, stress and anxiety of stem bark extract of *Bombax buonopenzese* on streptozotocin-induced diabetic rats. Acute toxicity test revealed no physical sign of toxicity after first and second phase acute toxicity test using Lorke's method; this suggests that the LD_{50} in the rats is beyond 5000 mg/kg.

The principle by which Forced swim test work is predicated on is the subjection of rats to unavoidable and inescapable stress which makes

rats adopt escape-oriented behaviors with intermittent moments of despair most times in the form of immobility [15]. The rats having been subjected to the procedure are evaluated for periods of immobility which models depressive symptoms and mobility with suggests reversal of depression.

This study showed that there was reduction in the duration of mobility on Day 7 compared to Day 1 there was a significant increase in the duration of mobility in animals that received higher doses of the extract when compared to untreated diabetic animals. This is confirmatory of the antidepressant effect of the drug extract at the dose of 400 mg/kg and 800 mg/kg.

The elevated plus maze test assesses the behavior of rats in a conflict situation; this is demonstrated by assessing their hostility to open spaces and heights [16]. Literature and behavioral studies generally prove that mice avoid open unprotected arenas and occupy protected spaces [17]. They inherently prefer the closed arms of the maze as opposed to the open arms of the elevated plus maze.

However, increases in the percentage of time spent in the open arms of the maze indicate anxiolytic effects. It is known that anxiolytic agents like diazepam increase the frequency of entries and the time spent in open arms of the elevated plus maze [18]. Our study showed that higher doses of BB caused rats to spend more time in the open arm compared to diabetic untreated animals revealing that the plant possesses anxiolytic properties. Anxiety and depression are intimately linked together and they appear as comorbidities in diabetic patients.

The anti-depressant effect of the plant was further assessed using sucrose splash model where rat's dorsal coat was sprayed with 10% sucrose solution. The solution will dirty the rat's coat and induce grooming behavior. From the result of the experiment there was no change in grooming behavior across groups on day 1 however, following 7-day treatments with BB there an increase in grooming behavior when compared to untreated group.

Conclusion

The result from this study indicates that the stem bark methanol extract of *Bombax buonopense* possess anxiolytic effect in streptozotocin - induced diabetic rats. The stem bark methanol extract of *Bombax buonopense* also possess antidepressant effect and stress relieving effect.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

The experimental protocol for the use of animal in this study was approved by the Ethics Committee of the Faculty of Pharmacy (EC/FP/022/08), University of Benin, Nigeria.

Contribution of Authors

We declare that this work was carried out by the authors named in this article all liabilities pertaining to claims relating to the content of this article will be borne by the Authors. AMA designed the study, the experiments, data collection and analysis was done by AMA and SO.

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