Asian Journal of Scientific Research

ISSN 1992-1454

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Asian Journal of Scientific Research 8 (3): 367-373, 2015
ISSN 1992-1454 / DOI: 10.3923/ajsr.2015.367.373

Phytochemistry and Reproductive Activities of Male Albino Rats Treated with Crude Leaf Extract of Great Bougainvillea (*Bougainvillea spectabilis*)

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ABSTRACT

This study was aimed at assessing the reproductive activities of male albino rats treated with crude leaf extract of *Bougainvillea spectabilis*. The total of thirty sexually matured male albino rats of about eleven weeks, weighing between 120-180 g were divided into five groups (A, B, C, D and E) with 6 rats in each group. Rats in group A served as the control and were fed with normal commercial feed only; groups B, C, D and E received 150, 300, 450 and 600 mg kg^{-1} Body Weight (BW) of the test substance, respectively. The results of the phytochemistry showed high content of phytates (49.27%) and other bioactive compounds in the leaf extracts. Results on the sperm parameters revealed significant reduction ($p<0.05$) in the sperm count, viability and motility. Sperm head abnormalities were also significant in the different groups with the highest recorded at 600 mg kg^{-1} BW (8.75%). Although, there were no significant difference in the epididymides weight of rats in the different groups, the testes weight was significantly reduced ($p<0.05$). From the present results, there is a dire need to always apply caution on the use of *B. spectabilis* in combating diseases considering the possible adverse effects that it could pose on spermatogenic pathways.

Key words: *Bougainvillea spectabilis,* phytochemistry, sperm count

INTRODUCTION

Since the beginning of time, humans have relied directly or indirectly on plant products for food, shelter, clothing, furniture and therapeutic purposes. At that time, the therapeutic properties of plants were not fully exploited due to little or no information on their bioactive components, but overtime, with the discovery of medicinal plants and the efficacy of their inherent bioactive compounds, the exploration and exploitation of plant products for the treatment of ailments became more apparent. The myriad of synthetic drugs in developing countries notwithstanding, current estimates suggest that a large portion of the population still rely greatly on medicinal plants and their products to meet primary health care needs (Saikia and Lama, 2011). This is occasioned by the belief that phytomedicine are safer, readily available and affordable compared to the synthetic products (Calixto, 2000; Zhang, 2005; Ikpeme *et al*., 2013).

Bougainvillea spectabilis commonly referred to as 'Great Bougainvillea' is one of such plants that has been explored and exploited in herbal medicine for the treatment and prevention of various ailments. This has been attributed to the phytoconstituents such as oxalates, flavonoids, glycosides, alkaloids, phenolic compounds, which was reported as the basis of its efficacious

therapeutic properties (Mishra *et al*., 2009). For instance, D-pinitol present in the leaves of *Bougainvillea* has been found to possess hypoglycemic effect for the treatment of diabetes (Narayanan *et al*., 1987; Bates *et al*., 2000; Saikia and Lama, 2011). Studies have also shown that Great Bougainvillea possesses antiviral, antibacterial and antifertility potentials (Narwal *et al*., 2001; Umamaheswari *et al*., 2008; Mishra *et al*., 2009). The leaves are also a good source of antioxidant for combating the deteriorating effects of free radicals in the body (Chaires-Martinez *et al*., 2009).

Although Great *Bougianvillea* has high medicinal values, recent researches suggest that there could be some adverse effects in the body upon its consumption. In a study conducted by Mishra *et al*. (2009), it was revealed that the leaf extract of *B. spectabilis* drastically reduced testosterone and estrogen levels of mice. Similarly, Adebayo *et al*. (2005), reported that the extract of *B. spectabilis* had a beneficial effect by reducing the serum and cholesterol level in rat and also reduced hematological indices of the rats. In line with these reports, it becomes quite pertinent to x-ray its biochemical components as well as their effects on sperm parameters in male albino rats. Thus, this study was aimed at assessing the reproductive performance of male albino rats treated with crude leaf extract of *B. spectabilis*.

MATERIALS AND METHODS

Collection and preparation of plant materials: The leaves of *B. spectabilis* were obtained from Calabar, Cross River Nigeria and was properly identified and authenticated in the herbarium unit of the Department of Botany, University of Calabar. The leaves were oven dried at 35°C using an electric oven (Sheldon Manufacturing Inc. USA, Model: 1510E-2) and blended using an electric blender (Christison 37 BLIB, Model: 204C BC). The aqueous extract was prepared by filtering and dissolving the powdered extract in distilled water.

Experimental animals and administration of extracts: Thirty sexually matured male albino rats weighing between 120-180 g were purchased from Animal House Unit of the Department of Genetics and Biotechnology, University of Calabar, Calabar. They were allowed to acclimatize for a period of 2 weeks under standard environmental conditions at room temperature, 12:12 h light/dark cycle with water and feed *ad libitum,* before commencement of treatment. Ethical care and handling of experimental animals was observed at all times and the study was approved by the University of Calabar Ethical Committee. The rats were divided into 5 groups (A, B, C, D and E) to include 6 rats per group in a Completely Randomized Design (CRD). Rats in group A served as the control and were fed with normal commercial feed, while groups B, C, D and E received 150, 300, 450 and 600 mg kg^{-1} body weight of the test substance via oral gavage for a period of 65 day. At the end of the experiment, the rats were sacrificed using diethyl ether. The testes and epididymides were surgically removed and weighed while the semen was obtained for sperm analysis.

Phytochemical screening

Test for tannins: The 0.5 g of the sample was weighed into a plastic bottle and 50 cm^3 of water was added and shaken for 1 h in a shaker. It was then filtered and 5 cm^3 of the extract was measured into a test tube, mixed with 3 cm^3 of 0.1 MHCl and 3 drops of ferrocyanide. It was allowed to stand for 10 min before measuring in the UV-visible spectrometer at 605 nm. Blank was also determined (Trease and Evans, 2000).

Test for saponins (frothing test): Froth emulsion test was used. Two milliliters of the aqueous extract was mixed with 6 mL of distilled water in a test tube. The mixture was shaken and observed for the presence of stable froth. Froth (foam) showed the presence of saponins in the dry stem sample. To further confirm saponin presence, drops of olive oil was added to the frothing mixture and shaken. The formation of stable emulsion confirmed the presence of saponin (Harbone, 2000).

Test for glycosides: Five grams of the sample was weighed into a 500 mL round bottom flask. Two hundred milliliters of distilled water was added and allowed to stand for 2 h before it was distilled into a 2.5% sodium hydroxide. One hundred milliliter of the distillate was collected and 25 mL aliquot was taken with additional 8 mL of 6 M NH_4OH and 2 mL of 5% Kl and titrated with 0.02 M $AgNO_3$ solution until a permanent turbidity indicates the end point (Harbone, 2000).

$$\text{Glycosides (mg/100g)} = \frac{\text{Titre values (mL)} \times \text{Extract vol. (mL)} \times 100}{\text{Aliquot volume (mL)} \times \text{Sample weight (g)}}$$

Test for flavonoids: Five grams of the sample was weighed into a beaker and extracted with 50 cm^3 of 80% methanol at room temperature for 1 h. The solution was filtered using filter paper. The filtrate was evaporated to dryness over water bath and oven. The weight of the dried extract was taken and the result recorded (Trease and Evans, 2000).

Test for alkaloids: Alcohol extract was obtained by dispensing 2 g of sample in 10 mL of absolute ethanol and after shaking by hand for 30 min, it was filtered using Watman filter paper and the filtrate was used as the extract. Two milliliters of the extract from each sample was mixed with drops of Mayer's reagent in the test tube. The formation of orange brown precipitate indicated the presence of alkaloids in the sample (Harbone, 2000).

Test for phytate: Two grams of the sample was weighed into 25 cm^3 portion of 0.5 M HCl and was shaken for 30 min. Two milliliters of ferric chloride solution was added to the extract and ferric phytate precipitate was formed. This precipitate was converted to sodium phytate by the addition of 3 cm^3 sodium hydroxide solution. The precipitate was digested with acid mixture of equal portions of concentrated sulphuric acid and perchloric acid in a digestion set. The liberated phosphorus was quantified colorimetrically at 620 nm after colour development with molybdate reagent (Harbone, 2000).

Test for oxalate: Two grams of sample was weighed and extracted with dilute HCl. The oxalate in the extracted sample was precipitated with calcium chloride as calcium salts. The precipitated extract was washed with 50 cm^3 of 25% H_2SO_4 and dissolved in hot water. It was then titrated with 0.05 M $KMno_4$ (Harbone, 2000).

Sperm quality analysis

Determination of epididymides and testes weight: The epididymides and testes were dissected out at the end of the experiment and excess blood damped with cotton wool and placed in a clean weighing balance (Grand G model: JJ 500 ld = 0.01 g) to record the weight.

Estimation of sperm count: This was carried out according to the method of Ekaluo *et al*. (2009). The epididymal content was obtained by macerating with fine scissors known weights of the caput

and cauda epididymides in a glass petridish, containing warmed buffered physiological saline in the ratio of 1:10 w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80 μm stainless mesh. A tissue-free aliquot was loaded into the Neubauer haemocytometer (Deep1/10, Labart, Germany). Five different counts were done for each sample, and the means were taken as the mean count for each male rat in millions of sperm cells mL^{-1}.

Estimation of sperm viability: This was estimated using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin-nigrosin stain and air dried smears were prepared on glass slides for each sample according to Bjorndahl *et al*. (2003). The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin-nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 min and multiplied by 100.

Estimation of sperm motility: The sperm suspension was diluted in 2 mL of warmed buffered physiological saline and dropped on glass slides. This was viewed under light microscope to determine the motile and non-motile sperm cells by their movement (WHO., 1992).

Sperm head abnormalities: A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al*. (2009).

Statistical analysis: All data collected on sperm parameters were subjected to Analysis of Variance (ANOVA) using Predictive Analytics Software (PASW), version 18.0. Significant means were separated using the least significant difference at 5% probability level.

RESULTS

Phytochemistry: The phytochemical constituents of *B. spectabilis* leaf extract as shown in Fig. 1 revealed that tannins content was 27.64%, while, saponins was 14.08%. Glycosides was 11.49%, flavonoids (10.05%), alkaloids (4.10%), phytate (49.27%) and oxalate content was 27.65%.

Effect of *B. spectabilis* leaf extract on sperm profile: The results revealed that *B. spectabilis* had no significant effect ($p>0.05$) on the epididymides weight of the rats (Table 1). On the contrary, the testicular weight showed significant differences among the different groups, which reduced with increasing dose. On sperm count, viability and motility, there were significant differences ($p<0.05$) between rats in the control group and the treatment groups, which reduced as dose of the extract increased although there was no significant difference in the sperm count of rats in the control group (9.380×10^6 mL^{-1}), 150 mg kg^{-1} BW (8.610×10^6 mL^{-1}) and 300 mg kg^{-1} BW (8.523×10^6 mL^{-1}), respectively. Sperm head abnormalities increased as the concentration increased from the control (2.75%) to rats treated with 600 mg kg^{-1} BW (8.75%).

DISCUSSION

Medicinal plants are reported to be more accessible and safe (Naik *et al*., 2003; Ikpeme *et al*., 2013) and are thus exploited in the treatment of different human diseases such as bacterial and viral infections, treatment of diabetes as well as in the improvement of fertility. Although,

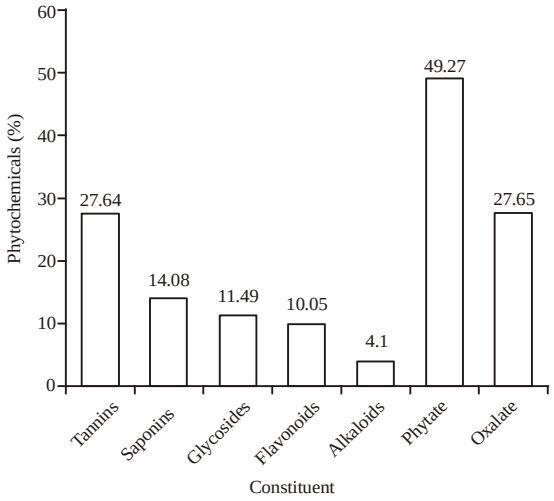

Fig. 1: Phytochemical constituents present in the crud leaf extract of *B. spectabilis*

Table 1: Effect of *B. spectabilis* leaf extract on sperm profile of male albino rats

	Concentration of extract (mg kg^{-1})				
Parameters	Control	150	300	450	600
Sperm count (×10^6 mL^{-1})	9.38±0.36^{c}	8.61±0.36^{bc}	8.52±0.26^{bc}	7.70±0.29^{ab}	6.76±0.35^{a}
Sperm viability (%)	86.55±1.43^{d}	78.97±1.46^{c}	72.44±2.23^{bc}	67.38±3.69^{ab}	63.91±0.89^{a}
Sperm motility (%)	65.75±2.17^{c}	61.00±2.04^{c}	52.25±2.95^{b}	50.50±2.63^{b}	42.75±1.25^{a}
Sperm head abnormality (%)	2.75±0.48^{a}	4.75±0.25^{b}	6.75±0.48^{c}	7.25±0.48^{c}	8.75±0.25^{d}
Epididymal weight (g)	0.66±0.08^{a}	0.63±0.08^{a}	1.24±0.09^{a}	1.23±0.03^{a}	1.10±0.20^{a}
Testicular weight (g)	1.38±0.05^{b}	1.28±0.01^{b}	1.24±0.09^{ab}	1.23±0.03^{a}	1.10±0.02^{a}

$^{[abc]}$Means followed with different superscript along the same horizontal array indicate a significant difference at ($p<0.05$)

medicinal plant products are used in controlling these diseases, there may also be some side effects on sex parameters upon their consumption (Ikegami *et al*., 2003; Izzo, 2004), especially on people who directly consume these plants with little or no idea on the useable dosage. The quantitative phytochemical analysis of the leaf extract in the current study revealed the presence of variant bioactive compounds, which may singly or synergistically worked to exert the changes on the sperm parameters of the rat model in the different treatment groups.

Our results revealed drastic decrease in the testicular weight, sperm count, viability and motility of the rats in a dose-dependent manner. This reduction may be directly linked to the various phytoconstituents in the extract. There are reports that some phytochemicals such as saponins and alkaloids exert toxic effects on reproductive hormones (Dewick, 2002; Francis *et al*., 2002; Robert and Wink, 1998). The aforementioned phytochemicals may be the underlying factors for reduction in the above reproductive parameters at the highest dosage of 600 mg kg^{-1} BW, which may contain more quantities of these compounds. Although, there is much interest on the use of plant products to control disease conditions such as diabetes, infertility, cancer, inflammation, etc., Ikpeme *et al*. (2014), Ravi *et al*. (2012) and Nagavani *et al*. (2010), it should be noted that it may also generate adverse pharmacological effects on sperm profile of the organism as evidenced in our results. It is reported that some medicinal plants disrupt spermatogenic pathways, which might eventually lead to decreased sperm count, viability, motility (Ikpeme *et al*., 2007, 2010, 2014; Ekaluo *et al*., 2009). This suggests that the administration of extract to the rats may have resulted in the alteration of spermatogenesis resulting in reduction of the sperm count from 9.380×10^6 of the control to 6.755×10^6 for the highest dose (600 mg kg^{-1}).

Similarly, the disruption in the spermatogenic pathway of the rats probably caused by administration of the test substance may have resulted in the sperm head abnormalities such as pin heads, double heads and round heads. This concomitantly reduced the viability of the sperm cells dose-dependently. The morphological disruption of the sperm cells by the test substance may as well result in the weakening of the cells (Ikpeme *et al*., 2007), thus leading to decreased sperm motility. It is important to mention that decreases in sperm count, motility and viability are of great concern, knowing the doom it could spell on reproductive efficiency. This implies that great caution should be exercised in the utilization of *B. spectabilis,* the reported pharmacological and therapeutic capacities not with standing.

CONCLUSION

The results of the present study revealed the reproductive activities of *Bougainvillea spectabilis* extract. Though there are reports on the use of this plant in the treatment of ailments, considering the mitigation in sperm count, viability, motility and increased sperm head abnormalities of the rats caused by administration of the crude extract, the recommendation of its intake should be done with great care.

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