

Full Length Research Paper

Evaluation of the effect of ethanolic extract of *Croton zambesicus* on the testes of Swiss albino mice

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The possible effect of *Croton zambesicus* administration on vital organs has been less investigated despite its extensive traditional use in tropical Africa. We therefore aim at elucidating the effect of ethanolic extract on the testes. The aqueous fraction of ethanolic leaf extract of *C. zambesicus* (5 and 10 mg/Kg body weight) was administered to verify its effect on sperm concentration, sperm motility, sperm progressivity, malondialdehyde and catalase activities for a period of five consecutive days. The result showed that there is a significant increase in sperm production, sperm motility and sperm progressivity in the treated group when compared with the control; while there was a reduction in malondialdehyde and catalase activity in all the treated groups. The slight increase in the weight of the measured parameters also indicated the positive effect of the extract in the normal metabolic activities in the treated groups. This investigation has shown that the leaf extract possesses promising profertility property which can be exploited in fertility therapy.

Key words: Testes, *Croton zambesicus*, sperm production, sperm motility, sperm progressivity, malondialdehyde.

INTRODUCTION

Croton zambesicus, a component of tiger bush, is a medicinal plant grown in villages and towns in Nigeria (Okokon et al., 2005). Some of its medicinal properties and components are reflected in the works of Block et al. (2002), Abo et al. (1999), Menut et al. (1995), Mekkawi (1985), Odetola and Bassir (1980), and Ryley and Peter (1970). For instance Abo et al. (1999) investigated the antimicrobial potential of *C. zambesicus*. The leaf decoction is used in Benin as anti-hypertensive, anti-microbial (urinary infections) and to treat fever associated with malaria (Adjanohaun et al., 1989; Watt and Breyer-Brandwijk, 1962).

The genus *Croton* is well known for its diterpenoid content and a lot of different types of diterpenes (phorbol esters, clerodane, labdane, kaurane, trachylobane, pimarane, etc.) have been isolated from this genus (Block et al., 2004). Ngadjui et al. (2002) revealed that compounds isolated from *C. zambesicus* which are used in traditional medicine for the treatment of a number of diseases including malaria, were characterized and screened for anti-plasmodial activity using inhibition of growth of *Plasmodium berghei* in mice. The compounds include abiatane diterpenoids, quinines, triterpenoids and flavonoid. It was clear from the results that flavonoid exhibited strong anti-malarial activity against the multidrug resistant K1 strain of *Plasmodium falciparum*. Labdane, clerodane and trachylobane diterpenes have been identified in the stem bark of *C. zambesicus* (Ngadjui et al., 2002). Recently Block et al. (2002) identi-

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fied a new cytotoxic trachylobane diterpene from the leaves of *C. zambesicus*. In order to continue their investigations on the composition of the cytotoxic dichloromethane extract of the leaves, Block et al. (2004) have isolated and characterised two new trachylobane and one isopimarane diterpenes together with trans-phytol, α -amyrin and sterols. Okokon et al. (2005) concluded that the alkanoid fractions of the leaf and stem of *C. zambesicus* is active against microorganism and that the essential oil found in the leaves also contain P-cymene, linalool and beta-caryophyllene.

Despite these huge achievements on the successful isolation of some important phytochemicals, there is very little literature concerning the study of the effect of *C. zambesicus* on vital organs such as the testes. We therefore set to elucidate the possible effects of ethanolic extract of *C. zambesicus* on the function of the testes. This will in a way guide the usage and dosage of this important plant.

MATERIALS AND METHODS

Plant materials

The leaves of *C. zambesicus* were procured from a local market in Ile-Ife, Osun-State, Nigeria. It was identified by the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, were a voucher was deposited at the Herbarium. The leaves were oven dried at 40°C for 6 days and then grounded to a fine powder.

Preparation of extract

The powdered material (100 g) was percolated with ethanol. The extract obtained yield (26.27%) was partitioned between dichloromethane and water. The aqueous fraction was concentrated in vacuum at 20°C (yield 3.98%). The fraction was dissolved in normal saline and administered orally at a dose of 5 and 10 mg/kg as the plant extract for a period of five consecutive days.

Animal treatment

Fifteen Swiss male albino mice (27 – 30 g) were used for the experiment. They were maintained under standard laboratory conditions in the Animal Holdings of Igbinedion University, Okada, Nigeria. They were fed with standard pelleted diet and water *ad-libitum*. The animals were randomly assigned into groups A, B and C (n = 5). Groups B and C were administered with 5 and 10 mg/kg doses of the extract respectively. An equivalent volume of normal saline was given to group A (control group) for five consecutive days. The care and handling of the animals is in line with the rules and guidelines of animal right committee of the Igbinedion University, Okada, Nigeria.

Testes weight, volume and density estimation

On the day six, the mice were decapitated and the testes collected and weighed using a Metler (153) balance. The volume was also measured by water displacement method. The density was calculated arithmetically by dividing the mass by the volume.

Sperm characteristics

The testes from each mouse were carefully exposed and removed. They were trimmed free of the epididymis and adjoining tissues. From each separated epididymis, the caudal part was removed and placed in a beaker containing 1 mL physiological saline solution. Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Sperm motility, concentration and progressive motility were determined as earlier described (Monica, 2000). Semen drops were placed on the slide and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined under the microscope using X40 objective for sperm motility. Sperm count was done under the microscope using improved Neubauer haemocytometer.

Assay for Malondialdehyde (MDA)

The testes were removed and blotted dried on a filter paper and weighed. A 10% homogenates of each testis in chilled phosphate buffer was immediately prepared with a Potter-Elvehjem homogenizer. The homogenates were centrifuged (3000 rpm for 10 min) and the supernatant immediately stored in the freezer (-20°C) and assayed within 48 h. MDA an index of lipid peroxidation was determined using the method of Bueger and Aust (1978). 1.0 ml of the supernatant was added to 2 ml of the tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent (TCA-TBA-HCl) boiled at 100°C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA-complex of $1.56 \times 10^5 \text{ M}^{-1}\text{CM}^{-1}$.

Determination of catalase activity

This was determined adopting the methods of Aksenov and Njaa (1981). Hydrogen peroxide was prepared with phosphate buffer; 0.2 ml of sample was added to 1.8 ml of 30 mM of hydrogen peroxide (H_2O_2) substrate in a 2 ml curvette. The phosphate buffers were used as a blank. The absorbance for the test sample, blank and standard was read against a blank at 240 nm at 30s interval for 1 min. The enzyme activity was calculated using the molar extinction coefficient of 40.00 per M per CM expressed as unit per ml.

Determination of total protein

This was determined using Biuret method (Gonall et al., 1949). 5.0 ml of blank Biuret reagent prepared by dissolving $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystal in 500 ml of distilled water was added to sample blank. These were mixed well and allowed to stand for 20 min at room temperature 25 - 27°C. Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using: optical density for standard \times concentration of standard.

Statistical analysis

Data were expressed as mean \pm SEM. The data were analysed by student's t-test using statistical software STATISTICA VERSION 5. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Close examination of the experimental animals showed

Table 1. Weights of the animals before and after treatment (mean \pm SEM).

| Parameters | Group A Control | Group B 5 mg/kg | Group C 10 mg/kg |
|-------------------|--------------------|--------------------|---------------------|
| Initial weight | 27.1 \pm 4.16 | 29.2 \pm 5.10 | 30.0 \pm 4.80 |
| Final weight | 28.0 \pm 4.23 | 30.2 \pm 5.90 | 31.2 \pm 5.20 |
| Weight difference | 0.9 \pm 0.07 | 1.0 \pm 0.80 | 1.2 \pm 0.40 |

n = 5, P > 0.05 (not significantly different).

Table 2. Weight, volume and density of the testes of treated and control animals (mean \pm SEM).

| Parameter | Group A Control | Group B 5 mg/kg | Group C 10 mg/kg |
|-------------------------------|--------------------|--------------------|---------------------|
| Weight (g) | 0.28 \pm 0.0063 | 0.29 \pm 0.0052 | 0.31 \pm 0.0057 |
| Volume (ml) | 0.27 \pm 0.0062 | 0.27 \pm 0.0050 | 0.28 \pm 0.0055 |
| Density (g/ml ⁻³) | 1.03 \pm 1.01 | 1.07 \pm 1.04 | 1.11 \pm 1.03 |

n = 5; P > 0.05 (not significantly different)

Table 3. Malondialdehyde (MDA) concentration in the testes (mean \pm SEM).

| Parameters | Group A Control | Group B 5 mg/kg | Group C 10 mg/kg |
|----------------------------------|--------------------|--------------------|---------------------|
| MDA (mol/mg protein) | 0.76 \pm 0.00 | 0.45 \pm 2.56* | 0.40 \pm 2.19* |
| Catalase activity (U/mg protein) | 109 \pm 0.00 | 90 \pm 5.06* | 82 \pm 5.62* |

*P < 0.05 (significantly different) vs. control n = 5.

Table 4. Characteristic of sperm in control and experimental animal (mean \pm SEM)

| Parameters | Group A Control | Group B 5 mg/kg | Group C 10 mg/kg |
|--|--------------------|--------------------|---------------------|
| Sperm concentration ($\times 10^6$ /mL) | 56.71 \pm 6.19 | 68.24 \pm 7.35* | 79 \pm 7.61* ** |
| Sperm motility (%) | 69.56 \pm 3.14 | 76.51 \pm 2.45* | 89 \pm 2.79* ** |
| Progressivity | b1 | a1 | a1 |

*P < 0.05 (significantly different) vs. control.

**P < 0.05 (significant different) 5 mg/kg vs. 10 mg/kg.

a1- Rapid linear progressive motility.

b1- Slow linear progressive motility.

n = 5.

no pathological symptoms throughout the experimental period. The consumption of food was also appreciably okay. This explains the reason for a slight increase observed in the weight difference even though it was not significantly different (P > 0.05) when the weight of the treated animals were compared with the control (Table 1). The results showed nonsignificant increase P > 0.05 when the volume, weight and density of the testes in the treated group were compared with the control (Table 2). Assay for MDA (an index of lipid peroxidation) and catalase activity showed a significant reduction (P < 0.05)

when the treated group was compared with the control group (Table 3). There was, however, a significant increase (P < 0.05) in the sperm concentration, sperm motility, and progressivity in the treated group in contrast to the control. This significant increase was observed to be dose dependent (Table 4).

This present study demonstrated that *C. zambesicus*, a very important ornamental plant in Africa, is a good booster of testicular functions in Swiss mice. The increment (P > 0.05) in the (body) weight difference, when the control group was compared with the treated (Table 1)

implies an equal continuous progressivity of the body cells through the cell cycle to accommodate tissue growth or cell turn over (Heath et al., 1999). The increment in the weight, volume and density of the testes, as compared with the control group, might be related to the proliferative and differentiating changes in the surface epithelium of the seminiferous tubules which enhance the active role of the extract in spermatogenesis (Heath et al., 1999; Senger, 1999). The increment observed in the measured parameters (Tables 1 and 2), clearly demonstrates the non interference of the extract in the normal metabolic activities (Ezeasor, 1985) which was reflected in the slight increase in the weight of the treated group even though it was not statistically significant.

The leaf extract of *C. zambesicus* demonstrates an antioxidant activity in the testes as it inhibits lipid peroxidation which was measured by the concentration of MDA (Table 3). The significant reduction in the concentration of MDA in the treated group implies its role in scavenging natural free radicals by donating electron to it (Mira et al., 2000). Akpantah et al. (2006) in a related work with *Garcinia kola* seed concluded that the flavonoid in *G. kola* has antioxidant activity which acts in synergy with natural antioxidant. Flavonoids have been associated with antimicrobial effects in various studies using plant extracts (Nweze et al., 2004; Abo et al., 1999; Corthout et al., 1991). However, more research is required to determine other roles of flavonoids in *C. zambesicus*. The flavonoid isolated in *C. zambesicus* (Ngadjui et al., 2002; Okokon et al., 2005) may have possessed the antioxidant activity which is responsible for the reduction in MDA (an index of lipid peroxidation) even at high dosage (Table 4). The activity of this antioxidant must have acted in synergy with the catalase which is also an antioxidant. This suggests that the extract prevent lipid peroxidation. The little literature concerning the phytochemical study of *C. zambesicus*, made Block et al. (2002) to identify a new cytotoxic trachylobane diterpene from the leaves of *C. zambesicus* and just very recently, Block et al. (2004) isolated and characterised two new trachylobane and one isopimarane diterpenes together with trans-phytol, amyryn and sterols.

This study which revealed the antioxidant properties of *C. zambesicus* in testes, further confirm its non toxic effect and its importance in African traditional medicine. The observed increase in sperm concentration in the treated group was dose dependent (68.24 ± 7.35 and $79 \pm 7.61 \times 10^6$ mL for 5 and 10 mg/kg respectively). This is a significant improvement over the control ($56.71 \pm 6.19 \times 10^6$ mL). This is a confirmation of the importance of *C. zambesicus* as a potent antioxidant and free radical scavenger (Ngadjui et al., 2002; Okokon et al., 2005) which ameliorated the increased free radicals generated by the natural and experimental stress. Although the mechanism of action of the extract for the increased sperm concentration is yet to be elucidated, it may however be connected with the induction of the germinal

epithelial cells called spermatogonia to continuously proliferate to replenish themselves and to differentiate through definite changes of development (Guyton and Hall, 2000). Data for sperm motility and progressivity (Table 4) showed a significant increase ($P < 0.05$) when the treated group was compared with the control group. This could be due to the influence of the extract on the mitochondria in the body of the tail of the spermatozoon to synthesize energy in the form of adenosine triphosphate (Guyton and Hall, 2000). In conclusion, this plant has been demonstrated to have a promising profertility property which can be exploited in fertility therapy.

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