

Full Length Research Paper

Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats

Toyin M. Salman*, Luqman A. Olayaki and Wahab A. Oyeyemi

Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.

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The effects of the aqueous extract of the leaves of *Telfairia Occidentalis* (fluted pumpkin) were studied on some haematological indices, sperm parameters and blood glucose in male albino rats. The experiment was divided into two parts. In the first part, twelve (12) male rats were divided into two groups of six (6) rats each. Group 1 rats served as control and were given 10 ml/kg of normal saline while the group 2 rats were treated with 200 mg/kg of the aqueous extract for two weeks. At the end of the treatment period, haematological indices (packed cell volume, haemoglobin concentration, red and white blood cell counts), sperm parameters (sperm motility, viability and counts) and blood glucose were determined. In the second part of the experiment, twenty four (24) male rats were divided into four (4) groups of six (6) rats each. Group 1 rats served as control while groups 2, 3 and 4 rats were given 200 mg/kg of the extract for 5, 7 and 14 days, respectively. Haematological indices were determined in these rats at the end of the treatment. The results showed that *T. occidentalis* significantly increased all the haematological indices and sperm parameters. In contrast, however, the extract significantly reduced the blood glucose levels. The results also showed that the increase in the haematological indices started after the seventh day of treatment. The results suggest that *T. occidentalis* could increase haematological indices within seven days. It could also improve sperm quality and could be a potent hypoglycemic agent.

Key words: *Telfairia occidentalis*, Haematological indices, blood glucose, sperm quality, rats.

INTRODUCTION

Telfairia Occidentalis (family cucurbitacea) is a tropical vine grown in West Africa and highly reputed in traditional medicine (Badifu et al., 1995). It is particularly cultivated from Sierra Leone to southern Nigeria among other areas (Burkett et al., 1968). It is commonly called "Ugwu" in Nigeria. The fruits of the plant are large and inedible but the seeds contain up to 30% protein and can be boiled and eaten, or ground into powder for soup. The seeds of the plant can also be fermented for several days and eaten as a slurry (Badifu and Ogunsua, 1991). The roots and leaves have been shown to contain highly toxic alkaloids and saponins (Akube, 1980). The Leaves are also rich in essential and non-essential amino acids, vita-

mins and minerals (Tindal, 1968; Fasuyi, 2006).

The herbal preparation of the plant has been employed in the treatment of sudden attack of convulsion, malaria and anaemia (Gbile, 1986). Based on its use as a haematinic, its effects on haematological indices had been scientifically investigated and reported. The diet preparation of the air-dried leaves of the plant significantly increased red blood cell count, white blood cell count, packed cell volume and haemoglobin concentration in rats (Alada, 2000) while the dietary preparation made with the sun-dried leaves had no significant effect on haematological parameters in birds (Fasuyi and Nonyerem, 2007); indicating that the potency of the plant depends on the method of preparation of the plant for consumption. However, it is not known whether or not the aqueous extract of the leaves will have any significant effects on haematological indices. A thorough search of the literature also reveals that there is little or no informa-

*Corresponding author. E-mail: drsalman111@yahoo.com. Tel: +2348032337422.

tion on the effects of this vegetable plant on reproductive functions and blood glucose.

The present study was therefore designed to investigate the effects of the aqueous extract of the leaves of *T. occidentalis* on some haematological and reproductive indices and blood glucose using rat as a model.

MATERIALS AND METHODS

Animal model

Wister strain albino rats (150 -170g) obtained from the Central Animal House, College of Medicine, University of Ibadan, were used for the study. The rats were housed in wire mesh cages under standard conditions (Temperature, 25-29°C, 12 h light and 12 h darkness cycles) and fed with standard rat pelleted diet and water. The study was generally conducted in accordance with recommendations from the declaration of Helsinki on guiding principles in the care and use of animals.

Plant material

The fresh leaves of *T. occidentalis* (fluted pumpkin) were purchased from a market in Ilorin, Kwara state, Nigeria. They were authenticated by a staff in the herbarium of the Department of Botany, University of Ilorin, Ilorin, Kwara state, Nigeria. The leaves were shade-dried and reduced to a powdery form by grinding. 500 g of powdered sample was soaked in distilled water for 48 h, after which it was sieved with a white cloth. The filtrate was then evaporated in water bath to dryness to obtain a solid extract. The extract was then dissolved in normal saline and used for the study.

Experimental design

The experiment was divided into two parts. In the first part of the experiment, twelve (12) male rats were divided into two groups of six (6) animals per group. Group 1 consists of rats which received 10 ml/kg normal saline and served as the control. Group 2 rats received 200 mg/kg of *T. occidentalis* for 2 weeks. Administration was by oral route. At the end of the treatment, haematological indices, blood glucose and sperm parameters were determined. In the second part of the experiment aimed at determining when new blood cells start to appear in the circulation after treatment with *T. occidentalis*, twenty four (24) male rats were divided into four (4) groups of six (6) rats each. Group 1 rats served as the control while groups 2, 3 and 4 rats were treated with 200 mg/kg of the extract for 5, 7 and 14 days, respectively. Haematological indices were then determined after the treatment.

Haematological indices and blood glucose

After treatment, blood samples were obtained from the tails of the rats for the determination of the blood parameters under investigation: packed cell volume, haemoglobin concentration, red blood cell count and white blood cell count. Packed cell volume was measured by spinning the blood samples with the microhaematocrit centrifuge for 5 min before reading with the haematocrit reader. Heparinized capillary tubes were supplied by British Drug House (BDH). Haemoglobin levels were measured by the cyanomethaemoglobin method. The red and white blood cell counts were done using the haemocytometer. Blood glucose was also determined by the glucose oxidase method using a glucometer (Life Scan data file,

2000). Values obtained using the glucometer have been shown to correlate excellently with those from the use of standard biochemical methods (Ajala et al., 2003; Devreese and Leroux-Roels, 1993).

Sperm motility, viability and counts

The rats were anaesthetized using ether 24 h after the last administration of the extract and the caudal epididymis was immediately dissected. An incision (about 1 mm) was then made in the caudal epididymis. A drop of sperm fluid was squeezed on the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was then assessed by calculating motile spermatozoa per unit area and was expressed in percentage. Epididymal sperm counts were done using the haemocytometer and were expressed in million/ml of suspension as earlier described (Adeeko and Dada, 1998). The sperm viability was also determined using Eosin/Nigrosin stain as earlier described (Raji et al., 2005a).

Statistical analysis

Data were expressed as mean \pm SEM. Statistical significance was determined using the student's t-test. $P < 0.05$ was considered significant.

RESULTS

The results of the effects of the aqueous extract of *T. occidentalis* on packed cell volume, haemoglobin concentration, white blood cell count, red blood cell count and glucose levels are shown in Table 1. Oral administration of the aqueous extract for two weeks resulted in significant increases in PCV, Hb concentration, RBC and WBC counts. The increases were 13, 39, 72 and 23%, respectively. In contrast, the extract also caused about 46% reduction in blood glucose levels. The results also showed that *T. occidentalis* significantly increased sperm motility ($P < 0.05$). The sperm motility of 77.7 ± 3.8 percent observed in the *T. occidentalis*-treated rats was significantly higher than 58.6 ± 1.7 percent observed in the control rats (Table 2). This represents 32.4% increase in sperm motility when compared with the control. In addition, there was a significant increase ($P < 0.05$) in sperm viability and sperm counts from 57.5 ± 3.6 percent and 39.5 ± 3.0 million/ml in the control rats to 75.2 ± 1.5 percent and 47.8 ± 2.5 million/ml respectively in the treated rats; representing about 30.7 and 21.1% increases in sperm motility and counts, respectively.

The results from the second part of the experiment also showed that although, there was an increase in the haematological parameters of rats that were treated for five days, this was not statistically significant. However, there was statistically significant difference in haematological parameters in the rats that were treated for seven and fourteen days when compared with the control (Table 3).

Table 1. Effects of *Telfairia occidentalis* on RBC counts, HB concentration, WBC counts, PCV and blood glucose concentration.

| Group | RBC ($10^{12}/L$) | Hb (g/dl) | WBC ($10^9/L$) | PCV (%) | Glucose (mg/dl) |
|---------|---------------------|-------------|------------------|-------------|-----------------|
| Control | 2.1 ± 0.1 | 10.1 ± 0.6 | 4.9 ± 0.8 | 39.6 ± 0.9 | 88.3 ± 2.0 |
| Treated | 3.6 ± 0.2* | 14.1 ± 0.3* | 6.0 ± 0.5* | 44.8 ± 1.4* | 80.3 ± 3.9* |

Values in asterisk are significantly different from the control at $P < 0.05$.

Table 2. Effects of aqueous extract of *Telfairia occidentalis* on sperm motility, viability and counts in rats.

| Groups | Motility (%) | Viability (%) | Counts (million/ml) |
|---|--------------|---------------|---------------------|
| Control | 58.7 ± 1.6 | 57.5 ± 3.6 | 39.5 ± 3.0 |
| <i>T. occidentalis</i> leaf extract (200 mg/kg) | 77.7 ± 3.7* | 75.2 ± 1.5* | 47.8 ± 2.5* |

*Values in asterisk are significantly different from the control at $P < 0.05$.

Table 3. Effects of aqueous extract of *Telfairia occidentalis* on RBC counts, Hb concentration, WBC counts and PCV in the control and rats treated for 5, 7, and 14 days.

| Group | RBC ($10^{12}/L$) | Hb (g/dl) | WBC ($10^9/L$) | PCV (%) |
|---------|---------------------|-------------|------------------|-------------|
| Control | 6.8 ± 0.3 | 12.2 ± 0.8 | 6.2 ± 0.4 | 35.7 ± 2.4 |
| 5 Days | 7.2 ± 0.5 | 13.8 ± 0.8 | 8.4 ± 1.1 | 43.0 ± 2.5 |
| 7 Days | 7.5 ± 0.2* | 14.8 ± 0.4* | 10.1 ± 1.4* | 45.2 ± 1.5* |
| 14 Days | 8.3 ± 0.2* | 15.3 ± 0.4* | 11.0 ± 1.5* | 50.8 ± 1.2* |

*Values in asterisk are significantly different from the control at $P < 0.05$.

DISCUSSION

The present study has shown that the aqueous extract of *T. occidentalis* caused significant increases in packed cell volume, haemoglobin concentration, red blood cell count and white blood cell count in addition to a significant decrease in blood glucose. The increase in the haematological indices observed in this study is consistent with the observations made when rats were fed with the diet preparation of the air-dried leaves of *T. occidentalis* for four weeks (Alada, 2000). The present study has also shown for the first time that new blood cells would have started appearing in the circulation after the fifth day of treatment with *T. occidentalis* and the increase would become significant after the seventh day of treatment and beyond.

The increases in the haematological indices observed following treatment with *T. occidentalis* extract might not be unconnected with the chemical composition of the leaves of *T. occidentalis*. The chemical composition had been shown to include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamide, vitamin C and minerals such as zinc, iron, calcium and magnesium. The amino acid profile of *T. occidentalis* had also been shown to be very rich and includes alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cystine, leucine,

arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine (Tindal, 1968; Fasuyi, 2006). Some of these constituents are well-established haemopoietic factors that have direct influence on the production of blood in the bone marrow. For instance, iron is a well-known haemopoietic factor (Ganong, 2005). Moreover, the amino acids derived from *T. occidentalis* could also be used for the synthesis of the globin chains of the haemoglobin and this could also contribute to the increase in haemoglobin concentration. The significant increase observed in this study is however inconsistent with the insignificant change in haematological parameters observed when birds were fed with the dietary preparation of the sun-dried leaves of the plant (Fasuyi and Nonyerem, 2007). The insignificant change observed with the sun-dried leaves might be due to the denaturing of the active ingredients especially proteins in the leaves during exposure to sunlight. In addition, the inconsistency may be an indication of a species variation in the responses to the effects of the plant.

The significant reduction in blood glucose suggests that the vegetable plant also possesses hypoglycemic property. This is consistent with the reports that a number of antimalarial agents including quinine, chloroquine and *Morinda lucida* extract possess hypoglycemic activities (Abu-sharka, 1994; Olajide et al, 1999). *T. occidentalis*

has also been employed in the treatment of malaria. Hypoglycemic property therefore appears to be common to the above-mentioned anti-malarial agents.

The significant increases in the sperm motility, viability and counts could also be attributed to the actions of some of its active ingredients which have well-documented spermatogenic activities. The administration of *T. occidentalis* extract could therefore be construed as a steady supply of additional nutrients to the treated rats over the control rats. Carbohydrate-rich *T. occidentalis* for instance, could have increased sperm motility and viability by increasing glucose metabolism leading to the production of pyruvate and energy. Pyruvate is known to be the preferred substrate essential for the activity and survival of sperm cells (Egbunike et al., 1986). Moreover, the amino acid arginine is a biochemical precursor in the synthesis of putrescine, spermidine and spermine which are essential for sperm motility (Steven, 2000). Zinc also promotes growth, sexual maturation and reproduction. In fact, studies have shown that serum and semen zinc levels were lower in infertile males than the fertile males (Mohan et al., 1997). Vitamin C had also been reported to improve sperm motility and fertility in smokers (Dawson et al., 1992) and boar (Ivos et al., 1971), respectively.

Although, serum testosterone was not estimated in this study, the increase in sperm counts may be due to an increase in serum testosterone concentration. Testosterone is needed for growth and development of male reproductive organs (Mooradian et al., 1987) and in association with follicular stimulating hormone, it acts on the seminiferous tubules to initiate and maintain spermatogenesis.

The ability of *T. occidentalis* to increase sperm motility, viability and counts, as observed in this study, is of great interest because these parameters are determinants of the fertilizing capacity of sperm cells. In fact, studies have shown that agents which reduce the fertilizing capacity of sperm cells do so by reducing these parameters. For instance, the reduction in the fertility of male rats treated with chloroquine and the extracts of *Morinda lucida* and *Alstonia boonei* had been attributed to significant reduction in sperm motility, viability and counts induced by these agents (Adeeko and Dada, 1998; Raji et al., 2005a; Raji et al., 2005b). Immotile or sluggishly motile spermatozoa would not penetrate the cervical mucus and thus could fail to fertilize the ova. Fewer number of viable sperm cells could also reduce the chances of fertilization. The increase in the sperm parameters induced by *T. occidentalis* could therefore increase the fertilizing capacity of spermatozoa.

Current evidence suggests that a multi-faceted therapeutic approach to improving male fertility involves identifying harmful environmental and occupational risk factors, while correcting underlying nutritional imbalances to encourage optimal sperm production and function (Steven, 2000). In this respect, studies have shown that nutritional therapies with zinc (Tikkiwal et al., 1987), vitamin C (Dawson et al., 1987), vitamin E (Vezina et al., 1996)

and arginine (Scibona et al., 1994) proved beneficial in treating male infertility. Since *T. occidentalis* contains most of these substances, it could therefore be a natural product that may be very useful in the treatment and management of infertility especially that associated with reduction in sperm performance.

This study suggests that aqueous extract of *T. occidentalis* could improve haematological parameters and sperm quality and it could be a potent hypoglycemic agent. Further studies aimed at elucidating the mechanism of hypoglycemic effect of this vegetable plant and its effects on male reproductive hormones and fertility are required.

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