

The effect of aqueous ethanolic extract of *Stachytarpheta cayennensis* on the histology of the liver and fasting blood sugar of non-diabetic and diabetic wistar rats

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Citation

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Abstract

Thirty-two presumably healthy rats of both sexes weighing between 160g and 200g were used in this study. The rats were randomly distributed into four groups viz A, B, C and D each having eight rats. Type 1 Diabetes mellitus was induced in groups B and D by a single intraperitoneal dose of 65mg/kgbw of streptozotocin. Group A served as the normal control while Group B served as the diabetic control and both received daily oral doses of 0.4ml of normal saline. Groups B and D received daily oral doses of 250mg/kgbw of *Stachytarpheta cayennensis* aqueous ethanolic leaf extract. This study aimed at validating the traditional claims of hypoglycaemic effect of the herb lasted for 21 days and thereafter the remaining rats were sacrificed and the liver dissected out for histological studies. Results showed that the plant material is capable of causing hypoglycaemia both in normal and diabetic rats. Liver histology showed congestion of the tissue in the diabetic control group and non-diabetic group treated with plant material. This is seen as sinusoidal engorgement and poorly defined outline of hepatocytes. Also observed in the study were proptosis, cervical lymphadenopathy, epistaxis, general malaise and passage of the loose stools in the groups treated with plant material. High mortality rate of over 50% were recorded mainly in the third week of study. It is therefore necessary to note that chronic administration of *S. cayennensis* may be deleterious to the body, its hypoglycaemic and antihyperglycaemic effects notwithstanding.

INTRODUCTION

Stachytarpheta cayennensis commonly called Ogwu ugwa, aran umon and panle by the Ibo, Efik and Yoruba tribes of Nigeria respectively belongs to the Verbanaceae family. It has a long history of use traditionally in the management of several health challenges including diabetes mellitus. Diabetes mellitus which is defined as a state in which homeostasis of carbohydrates and lipid metabolism is improperly regulated by insulin (Tiwari and Rao, 2002) has a common denominator hyperglycaemia. Chronic hyperglycaemia causes glucose toxicity whose detrimental effect is mediated and complicated through oxidative stress (Tiwari and Rao, 2002).

Diabetic complications are minimized by controlling the chronic hyperglycaemia. It is because of this that management of diabetes focuses on bringing down blood glucose levels thereby abolishing glucose toxicity.

The liver plays a central role in metabolism in the body including carbohydrate metabolism (Levinthal and Tavill,

1999). Levinthal and Tavill (1999) recorded a study demonstrating that a total hepatectomy in a dog results within a few hours from hypoglycaemic shock underscoring the important role the liver plays in maintaining normoglycaemia. In the presence of hepatic disease, the metabolic homeostasis of glucose is impaired (Picardi et al., 2006). This study also seeks to elicit any possible adverse effect on the histology of the liver that may hinder the regulatory mechanisms of the liver.

MATERIALS AND METHODS

PLANT MATERIAL

Matured leaves of *Stachytarpheta cayennensis* were harvested from an abandoned, uncompleted building in Calabar, Nigeria in the month of February 2010. They were authenticated by comparison with the herbarium section of Botany Department (University of Calabar, Calabar) file voucher specimen. The leaves were air-dried in the laboratory at room temperature and then powdered. The powdered plant material (2kg) was homogenized with an

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electric blender in 4.2 litres of ethanol and water (4:1, v/v) at room temperature. The extract obtained was concentrated to dryness under reduced pressure with a yield of 46.99g (2.35%)

ANALYSIS OF AQUEOUS ETHANOLIC EXTRACT OF LEAVES

Freshly prepared extract was used to quantitatively analyse the bioactive constituents following standard methods described by Harbone (1993) and Trease and Evans (1983)

ANIMALS

Thirty-two presumably healthy wistar rats of both sexes weighing between 160g and 200g were used in this study after approval by the Ethics Committee of the University of Calabar, Calabar-Nigeria. The animals were housed in well-ventilated cages in the animal house of the Department of Anatomy, University of Calabar, Calabar-Nigeria. They were fed with normal rat chow and given water freely using plastic containers with well fitted stainless steel nozzles. After a period of two weeks for acclimatization, they were randomly distributed into four groups A, B, C and D of eight rats each.

INDUCTION OF EXPERIMENTAL DIABETES

Streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) was reconstituted in normal saline (Atangwho et al., 2010). After an overnight fast, a single intraperitoneal injection of 65mg/kg body weight of STZ was given to twenty rats. After 72 hours of induction of diabetes fasting blood glucose was measured using One Touch Ultra Mini Glucometer (Lifescan Inc., USA) and only rats with blood glucose levels 12.7mmol/l (230mg/dl) and above were considered to be diabetic (Cetto et al., 2000). From the diabetic rats, eight rats each were randomly assigned to groups B and D

EXPERIMENTAL DESIGN

S. cayennensis extract was reconstituted using normal saline solution.

Group A: Normal control was given 0.4ml of normal saline solution once daily.

Group B: Diabetic control was given 0.4ml of normal saline solution once daily.

Group C: Non-diabetic, received 250mg/kgbw (0.4ml) of *S. cayennensis* extract once daily.

Group D: Diabetic received 250mg/kgbw (0.4ml) of *S. cayennensis* extract once daily

Experiment lasted for 21 days; thereafter the wistar rats after an overnight fast were anaesthetized using chloroform inhalation. The peritoneum was stripped open and the liver dissected out.

HISTOPATHOLOGICAL EVALUATION

Tissue samples from the liver were fixed in 10% buffered neutral formalin, dehydrated in ascending grades of alcohol, cleared with xylene and embedded in paraffin wax. Thin paraffin sections at 5µm were stained using haematoxylin-eosin staining technique (Luna, 1968)

STATISTICS

Significance of differences was established using the unpaired Student's t-test. Values at $p < 0.05$ were considered significant

RESULTS

PHYSICAL OBSERVATION

Proptosis was observed in the second week of study in groups C and D that received plant extract. Passage of loose stools and increased urination were observed from the first week of study. Apparent general malaise and high mortality of over 50% especially in the third week of study was observed in all the groups except the normal control (Group A). The study had to be shortened to 21 days because of this Cervical lymphadenopathy and epistaxis were observed in some rats in groups (C and D) that received *S. cayennensis* leaf extract especially later in the second week of study.

EFFECT OF ON BLOOD GLUCOSE LEVEL.

The results of blood glucose levels in all the groups were shown in Table 1

Figure 1

Table 1: The effect of on wistar rat fasting blood glucose levels

	Group A Normal Control	Group B Diabetic control	Group C Non-diabetic 250mg/kgbw	Group D Diabetic 250mg/kgbw
Day 0	4.72±0.06	*14.57±2.95	4.01±0.27	*22.45±0.07
Day 7	4.38±0.67	21.90±0.63	2.85±0.12	3.80±1.67
Day 14	4.67±0.05	22.73±0.76	3.66±0.45	5.75±2.76
Day 21	4.88±0.10	22.93±6.79	2.78±0.46	*3.90±1.13

Data represent mean ± SEM n=3 $p < 0.05$

At the beginning of treatment, blood glucose levels in Groups B (Diabetic control) and D (diabetic, received

The effect of aqueous ethanolic extract of *Stachytarpheta cayennensis* on the histology of the liver and fasting blood sugar of non-diabetic and diabetic wistar rats

250mg/kgbw. of *S. cayennensis* leaf extract) were significantly higher ($p < 0.05$) than the blood glucose of the normal control. Blood glucose levels of Group A (normal control) remained within the same range throughout the study. In group B (Diabetic control) blood glucose continued to rise till the end of the experiment. In group C (Non-diabetic, received 250mg/kgbw of *S. cayennensis* leaf extract) blood glucose level continued to decrease till the termination of treatment but this decrease was not statistically different from the normal control and from the value at the beginning of the experiment. In group D (Diabetic, received 250mg/kgbw of *S. cayennensis* leaf extract), there was a significant decrease in blood glucose level from the onset of treatment to the end of treatment. Though the blood glucose here was lower than the normal control, this variation is not significantly different from it.

Quantitative Phytochemical analysis, proximate and micronutrient composition of *S. cayennensis* aqueous ethanolic leaf extract.

Tables 2-5 show the percentage phytochemical and proximate composition and vitamin and mineral composition of *S. cayennensis*

Figure 2

Table 2 – Percentage phytochemical composition of

Alkaloids (%)	Flavonoids (%)	Saponins (%)	Tannins (%)	Phenols (%)
0.83±0.01	0.72±0.01	0.61±0.02	0.43±0.01	0.22±0.01

Data represent mean ± SEM n=3

Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols in *S. cayennensis* aqueous ethanolic leaf extract

Figure 3

Table 3 – Percentage proximate composition of aqueous ethanolic leaf extract

Carbohydrate (%)	Protein (%)	Fat (%)	Fibre (%)
21.58±0.80	9.28±0.18	3.66±0.02	4.10±0.12

Data represent mean ± SEM n=3

Result revealed that *S. cayennensis* aqueous ethanolic extract is composed of carbohydrate (21.58±0.80%), protein (9.28±0.18%), dietary fibre (4.10±0.12%) and fat (3.66±0.02%)

Figure 4

Table 4 – Vitamin composition of aqueous ethanolic leaf extract

Vitamin A mg/100g	Vitamin C mg/100g	Thiamine (B ₁) mg/100g	Riboflavin (B ₂) mg/100g	Niacin (B ₃) mg/100g
4.14±0.03	56.91±1.03	0.05±0.01	0.17±0.01	0.41±0.02

Data represent mean ± SEM n=3

Results revealed the presence of Vitamin A (4.14±0.03mg/100g), Vitamin C (56.91±1.03mg/100g), Thiamine (0.05±0.01mg/100g), Riboflavin (0.17±0.01mg/100g) and Niacin (0.41±0.02mg/100g)

Figure 5

Table 5 – Mineral composition of aqueous ethanolic leaf extract

Magnesium (mg/100g)	Calcium (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)	Phosphorus (mg/100g)	Iron (mg/100g)	Copper (mg/100g)	Selenium (mg/100g)
60.00±2.40	90.85±2.31	225.47±1.18	11.07±0.23	68.03±1.00	0.65±0.01	0.02±0.01	0.01±0.00

Data represent mean ± SEM n=3

Results revealed the presence of Magnesium (60.00±2.40mg/100g), Calcium (90.85±2.31mg/100g), Potassium (225.47±1.18mg/100g), Sodium (11.07±0.23mg/100g)

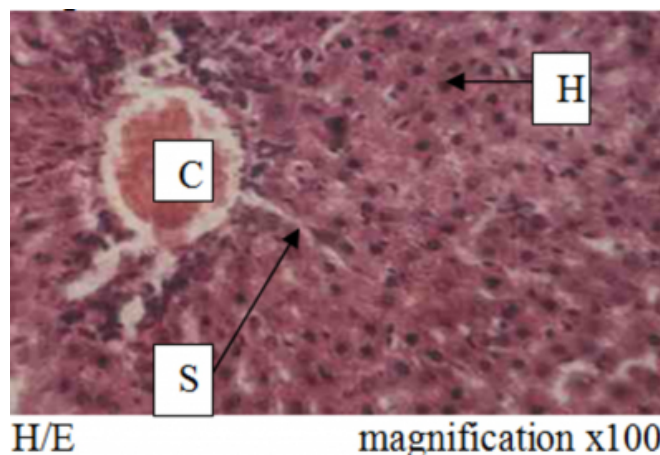
Phosphorus (68.03± 1.00mg/100g), Iron (0.65±0.01mg/100g), Copper (0.02±0.01mg/100g) and Selenium (0.01±0.00mg/100g)

Histopathological examination of liver

Histological examination of liver sections are shown in figures 1-4

Figure 6

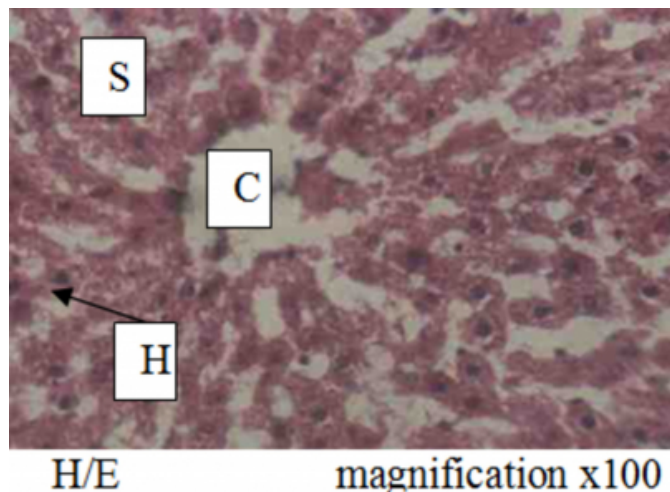
Figure 1 – showing liver section of normal control (Group A)



Section reveals central vein (C), hepatocytes (H) arranged in cords radiating from the central vein. Also present are sinusoids (S).

Figure 7

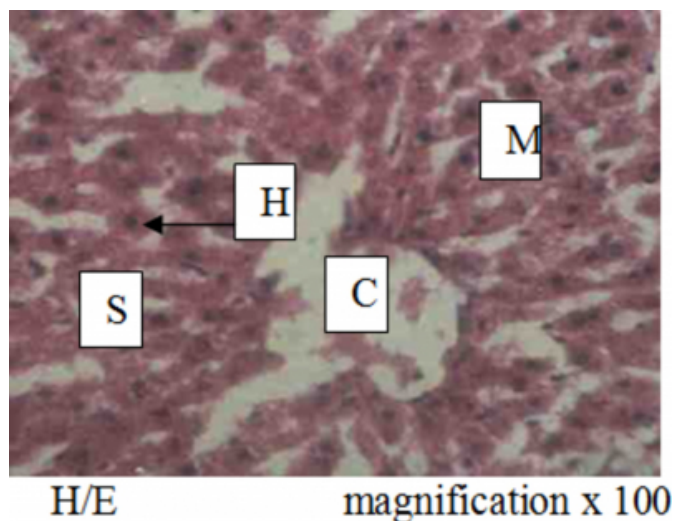
Figure 2 – showing liver section of diabetic control (Group B)



Section reveals engorgement of the sinusoids (S), fewer hepatocytes

Figure 8

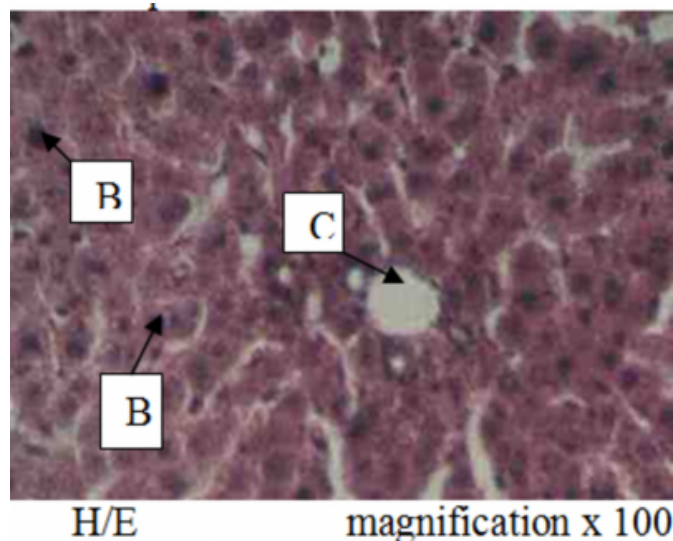
Figure 3 – showing liver section of non-diabetic wistar rats treated with 250mg/kgbw of aqueous ethanolic leaf extract of (Group C)



Section reveals mild sinusoidal engorgement and an apparently higher population of hepatocytes when compared with the diabetic control.

Figure 9

Figure 4 – showing liver section of diabetic wistar rats that received 250mg/kg body weight of aqueous ethanolic leaf extract



Section reveals a central vein, well-outlined hepatocytes and sinusoids. It resembles the section of the normal control.

DISCUSSION

Streptozotocin which was used to induce experimental diabetes in this study acts by generation of reactive oxygen species. It also causes accumulation of thiobarbituric acid reactive substances (TBARS) which causes hepatocyte degeneration (Ohkuwa et al., 1995; Kakkar et al., 1995) so the marked distortion seen in the untreated diabetic group is expected. This will further worsen the hyperglycaemia. The liver plays a crucial role in the regulation of carbohydrate metabolism (Levinthal and Tavill, 1999) in the body and in detoxifying harmful substances. It regulates blood glucose levels by altering the glucogen stores in the liver (Guo et al., 2009) by glucogenogenesis and glyconogenesis (Holstein et al., 2002; Picardi et al., 2006). In the presence of hepatic disease, the metabolic homeostasis of glucose is impaired (Picardi et al., 2006; Nielsen et al., 2005)

Hypoglycaemic effect seen in this study with *S. cayennensis* has also been obscured by Adebayo et al (2007) and Abuh et al., (1990). Medicinal plants with hypoglycaemic activity are known to increase circulating insulin levels in normoglycaemic rats (Abuh et al., 1990). The mechanism of action of the reduction of blood glucose could be by increasing circulating levels of insulin (Adebayo et al., 2007)

Present in the herb are several potent antioxidants and these

include some minerals e.g. Selenium, some vitamins e.g. B vitamins, vitamin C and some phytochemicals like alkaloids and flavonoids. Flavonoids stimulate glucose uptake in peripheral tissues, regulate the activity of the rate-limiting enzymes in the carbohydrate mechanism and probably insulinomimetic (Cazarolli et al., 2008). Although each of these substances is in small proportions, it is possible that they work in concert to bring down blood glucose and restore normal hepatic functions. *S. cayennensis* leaf extract seem to elicit an immune reaction as evidenced by cervical lymphadenopathy. This may also explain the mild signs of toxicity seen in non-diabetic treated group (Group C) liver section.

Frequent passage of loose stool may be responsible for the general weakness observed.

Cases of exophthalmos seen in the treated groups may suggest an increase in intraocular pressure. The cause of this is not understood.

Epistaxis, though usually have no obvious underlying cause could result from hypertension, clotting disorder or tumors. Congestion of the liver may also be a result of increase in blood pressure. It is not clear what is responsible for these features of an increase in blood pressure but it may be possible that some of the components of the plant material may have a salt and water retaining property thereby expanding the intravascular space giving rise to increase in blood pressure.

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