

Effect of Aqueous Leaf Extract of *Dissotis Roundifolia* on Plasma Glucose and Creatinine Concentration in Normal Albino Rats

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Abstract

Dissotis rotundifolia is a broad spectrum medicinal plant that has received worldwide recognition. This study was designed to investigate the effect of aqueous leaf extract of *Dissotis rotundifolia* on plasma glucose and creatinine level in normal albino rats. A total of 6 albino wistar rats each weighing 150g were assembled and randomly divided into 2 groups (control and tests) consisting of 3 rats each. The control group received pelleted growers mash and distilled water all through the period of experiment while the test group received 2ml/kg body weight of extract twice daily. Blood samples was collected into lithium heparin bottle for creatinine and sodium oxalate tube for glucose determination. The data obtained were subjected to statistical analysis using SPSS version 20. Results showed a significant increase in the mean plasma glucose and creatinine levels in rats treated with *Dissotis rotundifolia* compared with normal control group. This suggests a deleterious effect of *Dissotis rotundifolia* at the investigated dosage and thus, may not be safe for oral consumption. Hence, there is need for further studies in this regard in order to ascertain the safety of *Dissotis rotundifolia*.

Keywords: *Dissotis rotundifolia*; Glucose; Creatinine; medicinal plants; Albino Wistar rat

INTRODUCTION

Medicinal plants are commonly used in treating and preventing specific ailments and diseases, and are generally considered to play a beneficial role in healthcare (Aja et al., 2015). Makanjuola et al., (2014), reported that approximately 80% of the developing world's population meets their Primary Healthcare needs through traditional medicine. The demand and dependency on medicinal plants as remedy for different ailment is steadily increasing not only in developed countries but also in industrialized nations.

Dissotis rotundifolia (sm) Triana belongs to the family Melastomataceae. These plants exhibit a wide range of biological and pharmacological activities such as anti-inflammatory, anti-anemic, anti-malaria, anti-microbial, anti-diabetic and so many properties (Ayevbomwan et al., 2017). It is wide spread in Nigeria and other West African countries. *Dissotis rotundifolia* is a creeping herb with purple or sometimes pinkish flowers. Its medicinal uses include treatment of coughs, tooth ache, migraines, jaundices, blennorrhoea, bronchitis, fever, pneumonia, asthma, tubercular conditions, yellow fever, and diarrhoea (Balogun and Owoseni, 2013). The antitrypanosomal potential of the crude

extract at different doses has been established in rats treated orally or intraperitoneally. The plant also has been found to be rich in ascorbic acid (Vitamin C) when compared with some common garden fruits and vegetables (Balogun and Owoseni, 2013). It is used in East Africa for the treatment of bilharzias and in Cameroun, the leaves are used for dysentery (Aja et al., 2015). In tropical Africa, the whole plant is used as a remedy for rheumatism and yaws and as an antihelmintic and in Liberia for diarrhea (Abere et al., 2010). Hot water extract of *Dissotisrotundifolia* given orally is used for hookworm infestations (Ansah et al., 2016).

Creatinine is a by-product of muscle metabolism in which creatine in the muscle is converted nonenzymatically to creatinine (Bhattacharjya and Goswami, 2016). Because the total body content of creatine is fairly constant, there is a continual production of creatinine and a continual excretion of it in the urine (Allen, 2012). The typical 70 kg adult man produces about 2 g of creatinine per day (Bhattacharjya and Goswami, 2016). Creatinine is a more specific indicator of renal glomerular filtration rate than blood urea nitrogen (BUN), which is more easily affected by nonrenal factors (Allen, 2012).

Plasma glucose level is the concentration of glucose present in the blood of humans and other animals (Cox and Nelson, 2013). Elevated glucose levels (hyperglycemia) may also occur with pancreatic neoplasm, hyperthyroidism, and adrenal cortical hyperfunction as well as other disorders (Lehninger et al., 2017). Decreased glucose levels (hypoglycemia) may result from excessive insulin therapy or various liver diseases.

This research aims to determine the effect of aqueous leaf extract of *dissotisrotundifolia* on plasma glucose and creatinine level in normal albino rats.

MATERIALS AND METHOD

Chemicals/Reagents

The chemicals and reagents used in this study include distilled water, methylated spirits, glucose kit, Creatinine kit (product of Randox Laboratory Limited). All other reagents used were of standard analytical grade.

Preparation of *Dissotisrotundifolia* Leaf Extract

The plant *Dissotisrotundifolia* was obtained from the Niger Delta University premises. After collecting the plants, they were thoroughly washed and air dried. 100g of the fresh leaves was homogenised using laboratory mortar and pestle and mixed with 1liter of distilled water at 1g/10ml distilled water. The active ingredient of the homogenised sample was extracted using clean cheese cloth. The filtrate was stored in a clean bottle and refrigerated under 3°C. 2ml/kg body weight of the filtrate was administered to the rat twice daily (12 hourly).

Administration of Extract

The plant extracts were administered to the test rats according to their weight. The extract was administered twice daily (morning and evening) at the dose of 2ml/kg body weight.

Experimental Animals

Normal female albino rats between the ages of 6-7 weeks were used for this experiment. The rats were obtained from the animal house, in the Faculty of Basic Medical Science, College of Health Science, Niger Delta University, Wilberforce Island Amassoma Bayelsa State. The rats were moved from the animal house to the Department of Biochemistry where they were maintained and allowed free access to pelleted growers mash and clean water for a period of two weeks to acclimatize to the new environment.

Feed

The rats were fed with pelleted growers mash weighing 150g twice daily.

Experimental Design

After acclimatization for two weeks and basal test was carried out on the rat, they were starved overnight (fasting) and then grouped randomly into two groups containing three rats each.

Groups 1

Control-This group was given pelleted growers mash and distilled water all through the period of experiment.

Group 2

Test-Required amount of extract was administered orally at 2ml/kg body weight using gavage, twice daily for 15 days. Pelleted growers mash and distilled water were also given to them throughout the experiment.

Collection of Blood

Little Vaseline was applied on the tail of the rat and then massaged gently towards the tip for some time until the top showed evidence of blood accumulation. Thereafter, the tip of the tail was incised and blood was collected immediately with lithium heparin bottle for creatinine and sodium oxalate tube for glucose determination.

Assays of Biochemical Parameters

The Glucose and Creatinine assays were performed according to the procedure described by Randox Laboratory Ltd.

Histological Assessment of Tissue

The rats were sacrificed and dissected to obtain the heart, liver and kidney for histological

studies. The tissue samples were immediately preserved by immersion into 10% formalin. The fixed tissue sample were cleared in xylene and embedded in paraffin wax and sections were cut using 5-micron in a rotatory microtome. The sections were then examined using the light microscope after staining with hematoxylin and eosin dye and interpreted by an expert histologist.

Statistical Analysis

All measurement will be expressed as Mean $\pm$ Standard deviation. The statistical significance will be evaluated using SPSS version 20, (One-way Analysis of Variance (ANOVA). Values are considered statistically significant when $P < 0.05$.

RESULTS

Table 1 and Figure 1 showed that the plasma glucose concentration increased progressively from Day 0 to Day 15. When compared to the test group a statistical ($P < 0.05$) increase was observed in from Day 0 to Day 15.

Table 1: Mean plasma glucose concentration (mg/dl) of control and test rats administered aqueous extract of *Dissostisrotundifolia* for 15 days.

	DAY 0	DAY 1	DAY 5	DAY 10	DAY 15
CONTROL	52.21 $\pm$ 14.81 ^a	44.00 $\pm$ 17.40 ^a	41.09 $\pm$ 15.12 ^a	38.05 $\pm$ 16.23 ^a	33.85 $\pm$ 22.77 ^a
TEST	58.18 $\pm$ 7.82 ^b	67.34 $\pm$ 18.23 ^b	70.08 $\pm$ 13.99 ^b	88.91 $\pm$ 29.45 ^c	95.40 $\pm$ 11.82 ^d

Values are reported as mean $\pm$ SD of triplicate determinations. Values are statistically different from control at $P < 0.05$ * One Way Analysis of Variance (ANOVA). Values with the same superscript are not statistically different.

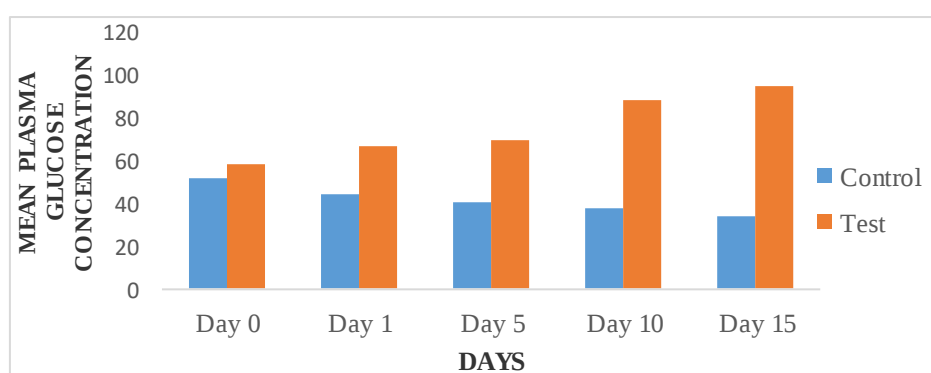


Figure 1: Mean plasma glucose concentration (mg/dl) of control and test rats administered aqueous extract of *Dissostis rotundifolia* for 15 days.

Table 2 and Figure 2 revealed that a significant increase in creatinine was observed in control from Day 0 to day 15. When compared to control, a statistical ($p < 0.05$) increase was observed in the test group from Day 0 to day 15.

Table 2: Mean plasma creatinine concentration (mg/dl) of control and test rats administered aqueous extract of *Dissostis rotundifolia* for 15 days.

	DAY 0	DAY 1	DAY 5	DAY 10	DAY 15
CONTROL	29.85±18.91 ^a	27.47±24.38 ^a	23.80±10.10 ^a	15.66±6.90 ^a	52.24±31.32 ^a
TEST	37.83±5.76 ^b	44.38±20.67 ^b	47.19±13.02 ^b	52.96±26.91 ^b	54.47±33.79 ^b

Values are reported as mean±SD of triplicate determinations. Values are statistically different from control at $P < 0.05$ * One Way Analysis of Variance (ANOVA). Values with the same superscript are not statistically different.

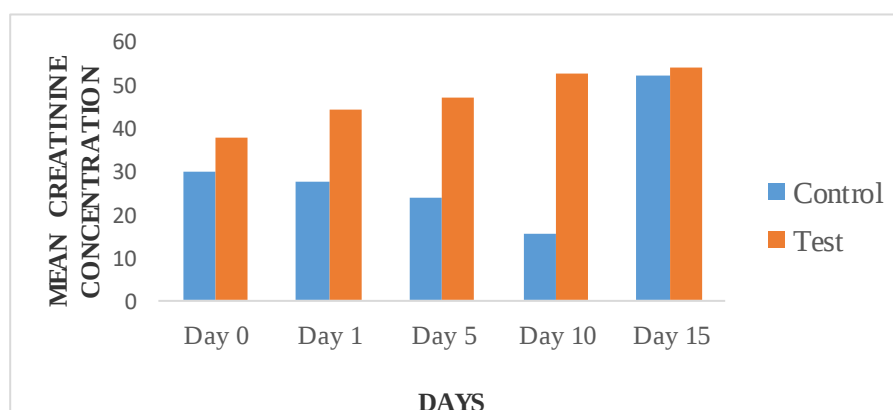


Figure 2: Mean plasma creatinine concentration (mg/dl) of control and test rats administered aqueous extract of *Dissostis rotundifolia* for 15 days.

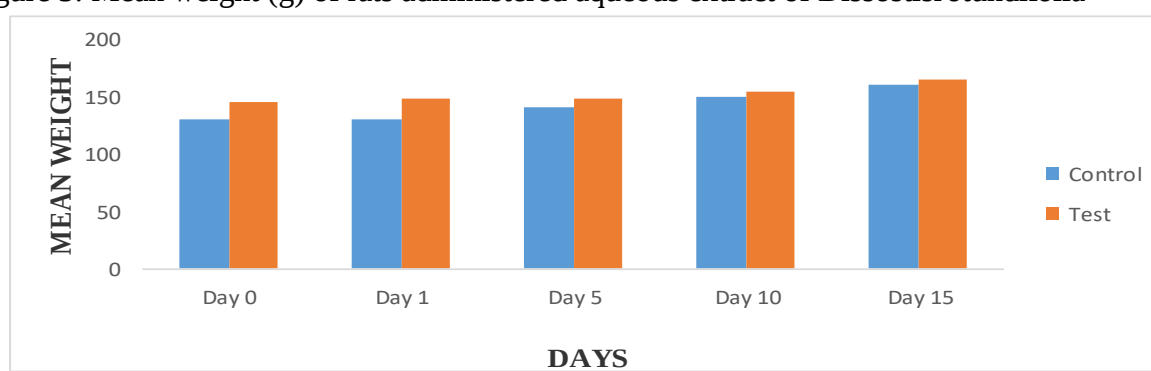
From the result table 3 showed that the rats in the control progressively increased in weight from day 0 to 15. It was also observed that the rats in the test group increased in weight from day 0 to day 15.

Table 3: Mean weight (g) of rats administered with aqueous extract of *Dissostisrotundifolia*.

	DAY 0	DAY 1	DAY 5	DAY 10	DAY 15
Control	131 ^a	131 ^a	142 ^a	151 ^a	162 ^a
Test	146.2 ^a	149.2 ^a	150.0 ^a	156.6 ^a	166.7 ^a

Values are reported as mean±SD of triplicate determinations. Values are statistically different from control at $P < 0.05$ * One Way Analysis of Variance (ANOVA). Values with the same superscript are not statistically different.

Figure 3: Mean weight (g) of rats administered aqueous extract of *Dissostisrotundifolia*



Liver: Histopathological sections of the liver of the control animal showed normal plates of hepatocytes separated by sinusoids () unlike those of test animals that received the extract. Photomicrograph of the liver tissue showing central vein, hepatocytes and sinusoids consistent with normal histology of the liver. The **TEST** group shows infiltration of inflammatory cells around the central vein. Also seen are prominent kupffer cells. Substance administered is hepatotoxic

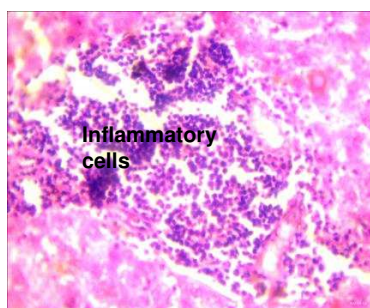
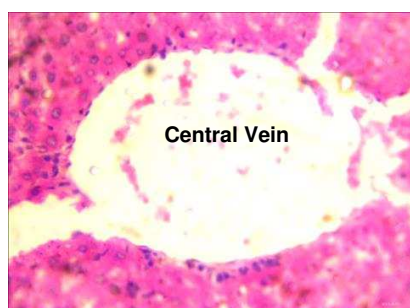


PLATE 1: Haematoxylin and Eosin stain liver Tissue, X 400 Magnification

Kidney:

Photomicrograph of the kidney tissue stained with haematoxylin and eosin. The CONTROL kidney slides shows normal glomerulus showing Bowmans space and messengial cells, the tubules display normal epithelium consistent with normal histology of the kidney. TEST slide membranous defeat of the glomerulus with cells leaking into the stroma. Substance administered is toxic to the kidney.

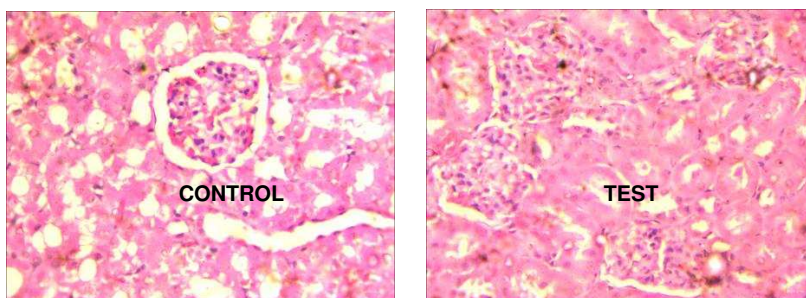


PLATE 2: Haematoxylin and Eosin stain kidney Tissue, X 400 Magnification

Heart: Photomicrograph of the heart tissue showing normal histology. Both Test and control are consistent with normal histology of the heart. Substance administered is non-toxic to the tissue.

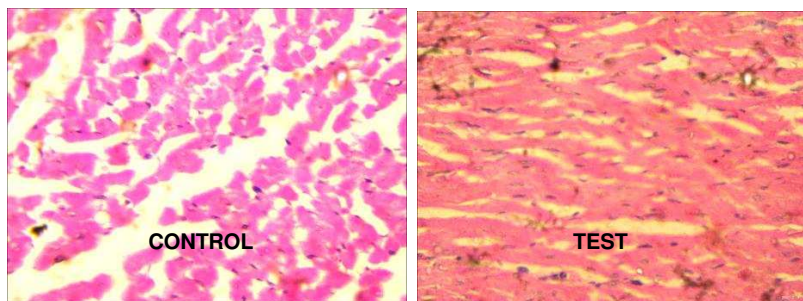


PLATE 3: Haematoxylin and Eosin stain heart Tissue, X 400 Magnification

DISCUSSION

Medicinal plants are of great importance to the health of individuals and communities and their medicinal values lie in some chemical substances that produce definite physiological actions on the human body (Ahn, 2017). Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals in the example of salicylic acid, as a hormone in plant defense. Numerous phytochemicals with potential and established biological activity have been identified. The

phytochemical content and pharmacological actions of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety (Ahn, 2017).

The preliminary analysis of the plasma glucose and creatinine of the normal albino rats showed glucose concentration ranging from 38-52mg/dl and creatinine ranged from 15-52mg/dl. Continual administration of the extract brought about a significant increase in the mean plasma glucose which ranged from 58-95mg/dl and creatinine level ranged from 38-54mg/dl. The increase in plasma glucose and creatinine concentration may be as a result of the plant extract. Glucose is a simple sugar that circulates in the blood of animals as blood sugar. Glucose serves as a primary fuel for energy production, especially for the brain, muscles and several other body organs and tissues. Glucose also serves as a building block for larger structural molecules of the body, such as glycoproteins and glycolipids (Clarke and Foster, 2012). Elevated plasma glucose concentration (hyperglycemia) a condition in which an excessive amount of glucose circulates in the blood plasma. Symptoms include frequent hunger, frequent thirst, increased volume of urination, blurred vision, fatigue, restlessness, weight loss, poor wound healing, dry mouth, and dry or itchy skin (Geijselaers et al., 2017). Reduced plasma glucose concentration (hypoglycemia) is when blood sugar decreases to below normal levels. This may result in a variety of symptoms including clumsiness, trouble talking, confusion, loss of consciousness, seizures, or death (Yanai et al., 2015).

Result from this study showed that the aqueous extract of *Dissotis rotundifolia* caused a significant increase in plasma glucose concentrations in days 1, 5, 10, and 15, when compared with the normal control (Table 1). This decrease could have been due to the plant inducing the production of insulin by mobilizing glucose into the cell. Early report on the phytochemical and antimicrobial effect of *Dissotis rotundifolia* by Balogun and Owoseni (2013) showed the presence of saponin, tannin, flavonoid, anthraquinone, steroid, and cardiac glycosides. This result is not in agreement with those reported by Mohamed et al., (2019) which showed a significant decrease in plasma glucose level in STZ-induced diabetic albino rats following oral administration of ethanol and aqueous-ethanol extracts of *R. communis* leaves.

This finding suggests that extracts exhibited a potent hyperglycemic activity which may be due to the presence of alkaloids, flavonoids and saponins which could act synergistically and independently as a stimulant for the inhibition of insulin resulting to the significant increase in glucose level.

Creatinine is a waste product formed in muscle by creatine metabolism. Creatine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle (Ezeugwunne et al., 2017). Its retention in the blood is evidence of kidney impairment. Serum creatinine is an important indicator of renal health because it is an

easily-measured by-product of muscle metabolism. Creatinine itself is an important biomolecule because it is a major by-product of energy usage in muscle via a biological system involving creatine, phosphocreatine and adenosine triphosphate (Ezeugwunne et al., 2017). Creatinine is chiefly filtered out of the blood by the kidneys. There is little or no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise (Jhee et al., 2018). Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance, which reflects the glomerular filtration rate (Allen, 2014).

Result from this study showed that the aqueous extract of *Dissotis rotundifolia* caused a significant increase in creatinine concentrations in day 1, 5, 10, and 15, when compared with the normal control (Table 2). Similar report by Ogbodo, et al., (2018) revealed a significant increase ($p=0.000$) of mean serum creatinine level in rats following treatment with *Phyllanthus amarus* when compared with the control subjects (129.20 ± 1.93 ; 83.90 ± 10.14). Eweka and Adaze, (2011) reported a distortion in renal structures after oral administration of *P. amarus* leaf extract on the kidneys of adult wistar rats. The observed increase in creatinine level suggest deficiency in glomerular filtration and tubular secretion. Furthermore, the aqueous extract of *Dissotis rotundifolia* caused a significant increase in the mean weight of the test rats when compared with normal control.

CONCLUSION

In conclusion, the administration of aqueous extract of *Dissotis rotundifolia* showed a significant increase in the mean plasma glucose and creatinine level with a corresponding increase in the mean weight of rats when compared to the normal control. This suggests a deleterious effect of *Dissotis rotundifolia* at the investigated dosage and thus, may not be safe for oral consumption. Hence, there is need for further studies in this regard in order to ascertain the safety of *Dissotis rotundifolia*. Furthermore, biochemical and histopathological investigations are needed to explore the possible use of different doses of this extract and their constituents.

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