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# Effects of *Dissostis rotundofolia* Stem Extract Consumption on some Biochemical Parameters and Hepato-Renal Organs in *Rattus norvegicus* Rats

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#### Abstract

Dossostis rotundofolia is a plant with many ethno-medicinal uses in tropical Africa. The aim of this study was to assess the effects of the consumption of stem extract of this plant on some biochemical parameters as well as the histology and morphology of hepato-renal organs in Rattus norvegicus rats. Five millilitres blood specimens were withdrawn into lithium heparin anti-coagulated bottles from each of the ten rats (control group) and another ten rats administered with two millilitres of stem extract of the plant twice daily for twenty-eight days (experimental group) followed by the excision of hepato-renal organs for histology/morphological study. Thereafter, the following biochemical parameters: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, creatinine and triglyceride were quantitatively measured using a spectrophotometer. The results showed no statistically significant differences (p>0.05) in the mean values of all the measured biochemical parameters (experimental group) as compared with that of the control group which revealed conformity with the histo/ morphological study of the hepato-renal organs. However, the mean value of triglyceride being exception was significantly decreased (p < 0.05). In conclusion, Dissostis rotundofolia stem extract may have no adverse effects on biochemistry and histo/morphology on hepatorenal organs. The use of this plant stem extract for herbal remedy of triglyceridaemia is therefore recommended.

*Keywords:* Dissostis rotundofolia, Stem extract consumption, Biochemical parameters, Hepato--renal organs, Rattus norvegicus rats

## Introduction

*Dissostis rotundofolia* which belongs to the kingdom plantae and family melastomataceae is a common plant that has several ethno-medicinal uses in tropical Africa. It is a perennial and slender creeping herb having prostate or ascending stems up to 40 cm high while its leaves which are 1.5-7.0 cm long and 0.8-4.0 cm wide, are ovate to ovate-lanceolate or suborbicular (Mshana *et al.*, 2000; Dokosi, 1998).

In West Africa, this plant is mainly used for treating rheumatism and painful swellings while its leaves decoction is used to relieve stomach ache, dysentery, diarrhoea, stop abortion, conjunctivitis, cough, circulatory problems and venereal diseases. However, in East Africa it is used for treating bilharzias (Odugbemi, 2008). The stem of this plant can grow up to 40 cm long is ascending and rooting at the nodes. In cross-section it is rectangular and lightly covered with soft hairs hence it is described as pilose (Abere *et al.*, 2009).

This plant is used widely in traditional herbal medicine systems in Nigeria (Odugbemi, 2008), Tanzania and Cameroun (Noumi, 2010), Liberia and Ghana (Watt and Breyer-Brandwijk, 1962). In recent years many studies have been channelled on indigenous plants that are cultivated as subsistence crops with such plants reported to contain chemical bioactive compounds that are known to contribute to their medicinal value. These plants are eaten as component of supporting food, as main dishes or as dessert. Implicitly, there are evidences that the plant is used in the traditional setting for the management of several illnesses including gastrointestinal disorders (Odugbemi, 2008).

Regardless of the fact that many drugs are used for disease management, research has shown that most of them have serious adverse effects on humans such as erectile dysfunction, diarrhoea, and hypergastrinemia (Sheen and Triadafilopoulos, 2011). However, researchers have reported pharmacological effects of *Dissostis rotundofolia* in various test models among which include anti-microbial, antidiarrhoea and anti-trypanosomiasis effects (Abere *et al.*, 2010).

The aim of this research is to investigate the effects of Dissostis *rotundofolia* stem extract on some biochemical parameters and hepato-renal organs of *Rattus norvegicus* rats.

## Materials and Methods Study area

This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

# Animal used

Healthy female *Rattus norvegicus* rats weighing 180- 200g (nine weeks old) were used for this study. These rats were obtained from the animal house in the Department of Pharmacology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria and kept in a standard animal house in the Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria and allowed free access to food (pelleted growers mash) and distilled water for a period of two weeks to acclimatize to the new environment prior to the commencement of the study.

## **Ethical clearance**

This study got ethical approval from the ethical committee of Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria and all the procedures carried were in conformity to the National Guidelines for Animal usage in research.

## Source of Dissostis rotundofolia stem sample

The *Dissostis rotundofolia* was collected from the environment of College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

## **Preparation of sample**

*Dissostis rotundofolia* stem were cut into pieces with the aid of a knife and sun-dried. When properly dried, the sample was ground into powder with the aid of electric blender. Two hundred and forty grams (240g) of this powdery sample was weighed with the aid of a standard weighing balance and macerated in 2400ml of distilled water (1g/10ml) and stirred periodically for three days, filtered with sieve to eliminate particles followed by the collection of the filtrates which were subsequently concentrated on a water bath to yield thick brown viscous pastes.

## Administration of aqueous stem extract

Aqueous stem extract of *Dissostis rotundofolia* was administered to the experimental rats orally twice a day (morning and evening) throughout the duration of the experiment (28 days) with the aid of gavage, at the dose of 2ml/180g body weight.

# Apparatus and equipment used

The following apparatus and equipment: centrifuge (Biochemical Engineering Inc. USA), thermostat water bath (HH-4 Techmel and Techmel, USA), weighing balance, spectrophotometer, lithium heparin bottles, gavage, blender, volumetric flask, conical flask, test tubes, test tubes racks, distilled water, dissecting set, methylated spirit, cotton wool, cages, micro pipettes, gloves and cuvettes were used for this study.

## **Chemical reagents**

All reagents used were obtained from a standard commercial supplier, Anams Medical Laboratory and Chemical supply, Idumota, Lagos State, Nigeria and were of analytical grade.

## Experimental design Pilot study

A pilot study was carried out to ascertain the minimum dose of *Dissostis rotundofolia* that can cause 50 percent death  $(LD_{50})$  in the experimental *Rattus norvegicus* rats

A total of sixteen rats having approximately a weight range between 180-200g were used. These rats which were grouped into three with four rats/group were designated as A, B and C respectively with each rat in group A administered with five millilitres of Dissostis rotundofolia stem extract/180g body weight as a single dose for one day, group B administered with ten millilitres of Dissostis rotundofolia stem extract//180g body weight as a single dose for one day and group C administered with fifteen millilitres of Dissostis rotundofolia stem extract//180g body weight as a single dose for one day and thereafter monitored for 24 hours for any signs and symptoms of Dissostis rotundofolia toxicity including death. The rats were considered dead when they failed to respond to agitation. No adverse signs, symptoms and death were observed in all the rats. After this experiment, twenty Rattus norvegicus rats were now grouped into two with ten rats per group. The sample size for the rats was determined utilizing the Resource Equation method in accordance with (Wan and Wan, 2017).

**Group 1 (control group):** This group of rats were fed with pelleted grower mash and distilled water throughout the experiment (28 days)

**Group 2 (experimental group):** Each of the rats in this group was administered with two millilitres of aqueous stem extract of *Dissostis rotundofolia* orally (twice daily)/ 180g body weight. These rats were fed with pelleted grower mash and distilled water throughout the experiment (28 days)

## Collection of blood specimens and hepatorenal organs

The tail of the restrained rat was cleansed with a ball of cotton wool soaked in methylated spirit. A little vaseline was then smeared on the tail to reduce friction while massaging to redness. Gentle massage towards the tip of the tail continued until the tip became red; sign of blood accumulation. The red tip of the tail was then slightly and carefully incised with a new and sterilized blade and further massaged gently as the blood trickled into lithium heparin anticoagulated bottles, mixed gently and spun for 10 minutes using a macro-centrifuge. Cotton wool soaked in methylated spirit was again used to cleanse the incised area of the tail. After the 10 minutes spinning the obtained plasma was decanted into universal sample tubes kept on the side bench under room temperature (28°C) and used for all the biochemical analyses carried out immediately. After which the rats were anaesthetized using chloroform technique with the liver and kidney organs excised

## Assays of biochemical parameters

The following biochemical parameters as shown below were assayed.

- i. Alanine aminotransferase in accordance with the colorimetric method as described by Egoro *et al.* (2020) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.
- ii. Aspartate aminotransferase in accordance with the colorimetric method as described by Egoro *et al.* (2020) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.
- iii. Alkaline phosphatase in accordance with the colorimetric method as described by Egoro *et al.* (2019) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.
- iv. Urea in accordance with Urease Berthelot method as described by Egoro *et al.* (2020) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.
- v. Creatinine in accordance with Jaffe reaction method as described by Egoro *et al.* (2020) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.
- vi. Triglycerides in accordance with the colorimetric method as described by Ezeh *et al. (2020)* using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.

#### Statistical analysis

The results obtained from the control and experimental groups were expressed as mean and standard deviation while the differences between the groups were compared using the student's "t" tests. A p-value of  $p \le 0.05$  was considered statistically significant.

## **Results and Discussion**

The results of the biochemical parameters measured in the *Rattus norvegicus* rats (control group) and *Rattus norvegicus* rats administered with aqueous stem extract of *Dissostis rotundofolia* for 28 days (experimental group) are presented in Table 1.

Dissostis rotundofolia is a common plant that has been reported to have various ethno-medicinal uses in tropical Africa (Mshana et al., 2000) and is regarded as one of the most widely used medicinal plants in Africa for the management of several illnesses including gastrointestinal disorders. In this study comparison was made between the mean values of plasma biochemical parameters such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatise, urea, creatinine and triglycerides in Rattus norvegicus rats that were not administered with the stem extract of *Dissostis* rotundofolia (control group) and those that were administered with 2ml of the stem extract of Dissostis rotundofolia for 28 days (experimental group) as shown in Table 1. The liver and kidneys of these Rattus norvegicus rats in both the control and experimental groups were also examined histo/morphologically for comparison purpose.

As shown in Table 1 the mean values of liver enzymes biomarkers (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) and renal biomarkers (urea and creatinine) revealed no statistically significant differences (p > 0.05) in the plasma of *Rattus* norvegicus rats administered with 2 ml of the stem extract of Dissostis rotundofolia for 28 days (experimental group) as compared with that of Rattus norvegicus rats that were not administered with stem extract of Dissostis rotundofolia (control group). The biochemical findings as established in this study are in conformity with the histo/morphological study which revealed that the extract was not toxic to the liver and kidneys after the administration of 2 ml of the Dissostis rotundofolia twice daily on each of the experimental rats for a period of 28 days as there were no changes in these organs when compared with that of the control group as shown in figure 1 and figure 2 respectively.

However, the result of this study went further as shown in Table 1 to reveal a statistically significant decrease (p<0.05) in the mean value of plasma triglycerides of the *Rattus norvegicus* rats following the administration of 2ml aqueous stem extract of *Dissostis rotundofolia*/180g body weight for 28days (experimental group) as compared to that of the *Rattus norvegicus* rats that were not administered with aqueous stem extract of *Dissostis rotundofolia* (control group). This finding is as established in this study

## Conclusion

The present study has demonstrated that administration of two millilitres aqueous stem extract of *Dissostis rotundofolia*/180g body weight of *Rattus norvegicus* twice daily for a period of 28 days may cause a significant decrease in plasma triglycerides while the hepato-renal biomarkers are not adversely affected as confirmed by the histo/ morphological study of these organs.

Table 1: Biochemical Parameters in Plasma of *Rattus norvegicus* Rats following the Administration of Aqueous Stem Extract of *Dissostis rotundofolia* for 28 days (experimental group) as compared with that of the Control *Rattus norvegicus* Rats

Parameters	Control group (n=10)	Experimental group (n=10)	p-values	Remarks
AST (U/L)	$4.10 \pm 1.06$	$4.12 \pm 1.08$	p>0.05	NS
ALT (U/L)	$4.90 \pm 1.83$	$4.92 \pm 1.84$	p > 0.05	NS
ALP(IU/L)	$11.82 \pm 3.49$	$11.85 \pm 3.51$	p > 0.05	NS
Urea (mmol/L)	$3.98 \pm 1.46$	$4.00 \pm 1.48$	p > 0.05	NS
Creatinine	$49.80 \pm 4.29$	$50.00 \pm 4.32$	p > 0.05	NS
Triglyceride (mmol/L)	$1.44 \pm 0.70$	$0.58 \pm 0.03$	p<0.05	S

Values are expressed as mean  $\pm S.D$ .

Keys AST(U/L) = aspartate aminotransferase ALT(U/L) = alanine aminotransferase ALP(IU/L) = alkaline phosphatase n = number of rats S = statistically significantNS = not statistically significant



Figure 1: Shows the photomicrograph of the liver stained with Haematoxylin and Eosin ×100

Slide A (control group) shows normal hepatic stroma with hepatocytes as well as sinusoid and central vein. Slide B (experimental group) shows no significant changes as compared with the control group.



Figure 2: Shows the photomicrograph of the kidney stained with Haematoxylin and Eosin ×100

Slide C (control group) shows normal kidney stroma with Bowman's capsule and glomerulus (renal corpuscle) as well as renal tubules. Slide D (experimental group) shows no significant changes as compared with the control group.

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