Phytochemical Analysis and Effect of Methanolic Root/Leaf Extracts of *Thaumatococcus danielli* on some Biochemical Parameters in Wistar Rat

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Abstract: This study was to determine the phytochemical composition of dried leaves and roots of *Thaumatococcus danielli* and their effect on some biochemical parameters in Wistar rats. Phytochemical screening revealed that flavonoid, saponin and tannin were present with trace amounts of alkaloid and cardiac glycosides. Phytochemical quantification using GC-FID revealed that kaempferol was higher in leaf (18.75ug/g) than root (9.55ug/g). Tannin was higher in root (10.39ug/g) than leaf (8.93ug/g), while phenol was higher in leaf (6.66ug/g) than root (3.96ug/g). Saponin, rutin, phytate and catechin contents were within same range in both leaf and root. A total of 226 albino rats of weight between 180 to 220g were used for toxicity testing for 4 weeks. The LD_{50} for leaf and root was 330mg/kg body weight (bw) and 250mg/kg bw respectively, upon intraperitoneal administration. Biochemical analysis of the serum showed significant increase ($P<0.05$) in ALT level in week 2 treatment for both extracts across all groups when compared to the control. AST level was significantly increased ($P<0.05$) in albino rat groups treated with16.5mg/kg of leaf extract; 6.25mg/kg and 12.5mg/kg of root extract in week 2, while ALP activity was significantly decreased across all groups treated with both extract only in weeks 2 and 3. Albumin was significantly ($P<0.05$) increased in week 2 treatment with leaf extract at doses of 16.5mg/kg and 33mg/kg and in week 3 at all doses of the root extract administered. Thus, continuous administration of leaf and root extracts of *Thaumatococcus danielli* especially at medium and high doses may cause mild injury to the liver.
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1. Introduction

The use of plants as medicine dates back to antiquity, before written history of civilization. Medicinal plants are the source of treatment for many diseases and ailments throughout the developing world because they contain various bioactive principles which have the potential to cause beneficial or detrimental effects (Adewunmi and Ojewole, 2004; Rao *et al*., 2004).

People often think that medicinal herbs being natural are safe and free from undesirable effects, but fail to understand that herbs are composed of bioactive chemicals some of which may be toxic. Although there is increased acceptance and use of herbal remedies worldwide, care must be taken not to consume harmful plants or high doses of plant extracts that could have deleterious effects on vital body organs either in short term or long term.

Toxic effects due to herbal medicine may manifest in a number of organs such as kidney, liver, stomach, nervous system and blood. The liver is a vital organ for maintaining of metabolic functions and detoxification of exogenous and endogenous substances such as foreign compounds, drugs and viral infections. When the liver is exposed to such substances, its protective capacities are overpowered, cellular necrosis and increase in serum levels of biochemical parameters like Alanine aminotransferase (ALP) and Aspartate aminotransferase (AST) are usually observed. The administration of herbal preparation without any standard dosage coupled with inadequate scientific studies of their safety has raised concerns on their toxicity.

Toxicity studies in animals help to assess the potential health risk in humans caused by adverse effects of chemical compounds present in plant extracts. The adverse effects may manifest in form of alterations in levels of biomolecules such as enzymes and metabolic products, normal functioning and histomorphology of the organs (Ashafa *et al*., 2009).

1.1 Profile of *Thaumatococcus danielli*

Thaumatococcus danielli is a tropical plant found in the rain forest of West Africa particularly Nigeria, Ghana and Cote d'iviore (Yeboah *et al.,* 2003; Arowosoge and popola, 2005; Ojekale *et al*., 2007). It

is the source of the natural sweetener, Thaumatin, regarded as a sweet protein (Chinedu *et al*., 2014).

In Nigeria, this plant is used among different ethnic groups for wrapping and boiling food to add to its savour and also to preserve and extend the shelf life of the food. With the widespread uses of the leaves of this plant in food packaging, researches have been made in to its sterility, it was reported that *Thaumatococcus danielli* leaves extracts had no significant antimicrobial activity, but has been shown to possesses hypoglycaemic properties, thus, it can be used in the treatment of diabetes mellitus (Emudainohwo *et al.,* 2015). All the plants part is rich in minerals and crude fibre. The fruit is also a good source of Calcium, Magnesium and Phosphorus (Shalom *et al.*, 2014). The high fibre content can be helpful in preventing intestinal and digestive disorders such as constipation, flatulent, pile, colon and rectum cancers (Showemimo and Olarewaju, 2004). *Thaumatococcus danielli* has versatile uses in folk medicine and nutrition.

The fruit aril contains the sweetener called Thaumatin which is used in food and confectionery industries as taste and flavour enhancer (Ojekale *et al*, 2007). Thaumatin being a sweet protein and not carbohydrate (Arowosoge and Labode, 2006), has been shown to be very ideal for diabetic as it is non-caloric (Lim, 2012). In folk medicine, the leaf sap is used as antidote against venoms, stings and bites. Leaf and root sap are used as sedative and for treating insanity (shalom *et al.*, 2014).

The fruit is used as laxative (Adeyemi *et al*., 2014). The seed is used as an emetic and for pulmonary problems (shalom *et al*., 2014). It has been shown by research that the leaf extract of this plant contains Tannin, Saponins, anthraquinones, Terpenoids and Steroids (Adeyemi *et al*., 2014; Shalom *et al.,* 2014). These secondary metabolites could be associated with sedative properties of this plant extract (Edewor-Kuponiyi, 2013). Plate 1 shows a photograph of *Thaumatococcus danielli* root while Plate 2 is the photograph of its leaves.

1.2 Statement of the Problem

The tradition of collecting, processing and using plants and plant-based medication have been handed down from generation to generation. Resurgence interest in the use of medicinal plants and products especially in developing nations is attributed to the fact that herbal medicine is relatively cheap, readily available, easier method of preparation and administration and the assumption that they are relatively less toxic.

Interestingly, there have been some scientific reports on the potential of herbal medicine to cause injury to vital organs such as liver and kidney (Chikezie *et al*., 2015). However, some plants that were earlier reported to be safe are now shown to be hepatotoxic, haematotoxic having mild to severe adverse side effect on the skin, biologic and metabolic functions (Ekpenyong *et al.*, 2012).

Plate 1: *Thaumatococcus danielli roots*

Plate 2: *Thaumatococcus danielli leaves*

Another serious challenge with the use of herbal plants is lack of standardization and only few have been investigated to the level of biochemical, toxicological and patho-histological characterization. Therefore, this research is undertaken to ascertain the level of safety of leaf and root extracts of *Thaumatococcus danielli* given it's used as sedative.

1.3 Objectives of Study

To determine the phytochemical composition of root and leaf extracts of *Thaumatococcus danielli* and to assess the effect of root and leaf extract of *Thaumatococcus danielli* on some biochemical parameters (liver enzymes).

2. Materials and Methods

2.1 Sample Collection and Extraction

Fresh leaves and roots of *Thaumatococcus danielli* were collected from the bush at Rukpokwu

community of Rivers State, identified in Plant Science and Biotechnology Department, University of Port-Harcourt. The roots and leaves (samples) were shade-dried at room temperature to a constant weight, and thereafter ground to powder, packed into dark polythene bags and stored in a desiccator for subsequent uses.

About 200g of each powdered sample (leaves and root) was dissolved in 1 litre of methanol at room temperature for 72 hours. This was then filtered using Wahtman No. 1 filter paper and the filtrate was transferred into a rotary evaporator at 40°C - 42°C. Each residue obtained was further dried in a water bath at 37°C- 40°C. The crude extracts were stored in a refrigerator at 4°C.

2.2 Preparation of Solutions

The vehicle used for the reconstitution of the extract was 5% of sodium carbonate (Na_2Co_3), it was prepared by dissolving 5g of Na_2Co_3 in 100ml of distilled water. On the basis of solubility of the extract in the 5% Na_2Co_3 solution, 40mg/ml and 50mg/ml were prepared as follows: 40mg/ml, prepared by dissolving 1g (1000mg) of extracts (roots and leaves) in 25ml of 5% Na_2Co_3, 50mg/ml: this was prepared by dissolving 1g (1000mg) of each extract in 20ml of 5% Na_2Co_3.

2.3 Animals and Treatment

A total of 226 Wistar rats of weighing between 180g-220g were bought from the animal house of the department of human Physiology, University of Nigeria, Enugu State and acclimatized for one week in the animal house of Biochemistry Department, University of Port Harcourt. During acclimatization, the animals were fed with rat pellets and water ad libitum. The leaf and root extracts of *Thaumatococcus danielli* were administered based on the experimental design.

2.4 Collection of Blood for Biochemical Analysis

Three animals (rats) were sacrificed weekly after day 7, 14, 21 and on day 29 from each group by putting in a desiccators containing cotton wool soaked in chloroform to anaesthetize the rats. The blood samples were obtained by cutting the jugular vein of the rat on the neck by means of surgical blade.

The blood for biochemical assay was collected in sterile plain tubes without anti-coagulant and left to clot. Thereafter, it was centrifuged at 5000rpm for 10 min, and serum was separated. Serum biochemical indices estimated for liver function test were AST, ALP, ALT, total protein, albumin, total and conjugate bilirubin levels.

2.5 Qualitative and Quantitative Phytochemical Analyses

Qualitative analyses were carried out using the methods of Trease and Evans (1989) and Harborne (1998) to identify the different photochemical, while phytochemical Quantification was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector.

2.6 Biochemical Assay

The following liver function test were conducted to investigate derangement in the liver of the animals used for the study: aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined by the colorimetric method of Reitman and Frankel (1957) using a commercial assay kit from Randox Laboratories Ltd, UK; Alkaline phosphatase (ALP) was estimated by the colorimetric method using assay kits from Randox Laboratories Ltd.

Serum protein and serum albumin were estimated by Biuret method and Bromocresol Green (BCG) binding method respectively using a commercial assay kit from Randox Laboratories Ltd. Total and conjugated bilirubin was determined using commercial kits from Randox Laboratories Ltd following colorimetric method described by Jendrassik and Grof (1938).

2.7 LD_{50} Determination

From the results of toxicity range test, 25 albino rats were obtained for each extract (5 animals per group), making a total of 50 animals for both extracts (leaf and root), with doses stated below. Clinical signs and symptoms of toxic effects and mortality within 24hrs were observed.

Doses (mg/kg):

500mg/kg = 2.5ml of 40mg/ml
400mg/kg = 2ml of 40mg/ml
300mg/kg = 1.5ml of 40mg/ml
200mg/kg = 1ml of 40mg/ml
100mg/kg = 0.5ml of 40mg/ml

For the LD_{50} determination, Karber's method was used.

$$\mathbf{LD_{50}} = LD_{100} - \Sigma \frac{\text{(sum of dose difference x mean death)}}{\text{Number of animal per group}}$$

2.8 Experimental Design for Sub-Acute Toxicity Testing

A total of one hundred and forty (140) Wistar rats weighing between 180 to 220g were grouped into 7 of 20 rats in each, while group 1 served as normal control for both extract. The treatment was for a period of 4 weeks. For the roots: Group 1: Normal control treated with 1ml of distilled water daily. Group 2: Treated with 6.25mg/kg/day (Low dose). Group 3: Treated with 12.5mg/kg/day (Medium dose). Group 4: Treated with 25mg/kg/day (High dose). For the leaves: Group 2: Treated with 8.25mg/kg/day (Low dose). Group 3: Treated with 16.5mg/kg/day (Medium dose) Group 4: Treated with 33mg/kg/day (High dose).

2.9 Statistical Analysis

Results were analyzed using SPSS (IBM-SPSS). Data obtained were expressed using descriptive statistic. Significant difference between the treatment

groups and the control was determined using one way analysis of variance (ANOVA).

3. Results And Discussion

Results of phytochemical analyses are presented in Tables 1 and 2 while Tables 3 and 4 gave results of LD_{50} of leaf and root extract respectively. Phytochemical analyses indicated that flavonoids were significantly present in both leaves and roots.

The flavonoids in the leaves were Kaempferol-(18.57ug/g), Rutin (11.25ug/g), Catechin (5.51ug/g), Anthocyanin (2.49ug/g) and Epicatechin (1.80ug/g), while the root showed 9.55ug/g, 11.35ug/g, 5.67ug/g, 1.37ug/g and 1.87ug/g respectively. Saponin concentration was between 14.00ug/g to 14.33ug/g in roots and leaves respectively.

Phytate content of both leaves and roots was low (0.23ug/g – 0.29ug/g). Phenol concentration was higher in the leaves (6.66ug/g) than in the root (3.96ug/g), while tannin was more in the roots (10.39ug/g) than the leaves (8.93ug/g). Lunamarine and Ribalinidine (Alkaloids) were higher in the roots (2.08ug/g and 4.76ug/g) than the leaves (1.98ug/g and 3.09ug/g) respectively.

This report agrees with findings of Ojekale *et al*. (2007) and Chinedu *et al*. (2014) that the leaf extract of *Thaumatococcus danielli* contains alkaloids, saponins, flavonoids, tannins, but cardiac glycoside, phenol were absent.

Flavonoids are important group of polyphenols widely distributed among the plant flora. Kaempferol, a member of flavonoids is a potent promoter of apoptosis (Ramos, 2007). It also modifies a host of cellular signalling pathways and has numerous anticancer properties.

Flavonoid (Anthocyanin) have many health promoting effects which include anti-oxidant, anti-inflammatory, anticancer, enzyme inhibition, anti-allergic and anti-viral effects (Chikezie *et al*., 2015). Several studies have shown that certain flavonoids can protect low density lipoprotein from being oxidized (Donald and Cristobal, 2006). The oxidation of low density lipoproteins have been recognized to play an important role in atherosclerosis, hypertension and excessive cholesterol in the blood (Middleton and Kandaswami, 1993; Ojekale *et al*., 2007; Ramawat *et al*., 2009). The leaves and roots extracts of *Thaumatococcus danielli* contain appreciable concentrations of Lunamarine (1.98ug/g in the leaves and 2.08ug/g in the roots), Ribalinidine in the leaves (3.09ug/g) and Roots (4.76ug/g) and negligible concentration of spartein (0.0002ug/g) in both leaves and roots.

Table 1: Result of Phytochemical Screening

Parameters	Leaves	Root
Alkaloids	+	+
Flavonoids	++	++
Steroids	-	-
Tannins	++	++
Resins	-	-
Proteins	+	+
Saponins	++	++
Cardiac glycosides	+	+
Terpenoids	-	-

Key: (+) trace amount, (++) significantly present, (-) Absence

Table 2: Result of Phytochemical Analysis using GC-FID

Phytochemicals	Leave ug/g	Root ug/g
Phytate	0.23	0.29
Epidcatechin	1.80	1.87
Oxalate	1.20	0.84
Anthocyanin	2.49	1.37
Tannin	8.93	10.39
Phenol	6.66	3.96
Lunamarine	1.98	2.08
Ribalinidine	3.09	4.76
Catechin	5.51	5.67
Rutin	11.25	11.35
Kaempferol	18.57	9.55
Saponin	14.33	14.00
Spartein	0.0002	0.0002

Table 3: Result of LD_{50} of Leaf Extract

Doses (mg/kg)	No of Death	Mean Death	Dose Diff.	Mean Death x Dose Diff.
100	0	0	0	0
200	1	0.5	100	50
300	2	1.5	100	150
400	3	2.5	100	250
500	5	4	100	400
Total				**850**

Now by applying Karber's method for the determination of the LD_{50},

$$LD_{50} = LD_{100} - \frac{\sum \text{sum of Dose Diff x Mean Death}}{\text{No of animals per group}}$$

$LD_{50} = 500 – (850/5) = 500 – 170 = 330$

$\therefore LD_{50} = 330mg/kg$

Table 4: Result of LD_{50} of Root Extract

Dose (mg/kg)	No of Death	Mean Death	Dose Diff.	Mean Death x Dose Diff.
100	0	0	0	0
200	2	1	100	100
300	4	3	100	300
400	4	4	100	400
500	5	4.5	100	450
Total				**1250**

$LD_{50} = LD_{100} - \frac{\sum \text{Sum of Dose Diff x Mean Death}}{\text{No of animals per group}}$

$LD_{50} = 500 – (1250/5) = 500 – 250 = 250$

:. LD_{50} = 250mg/kg.

Alkaloids are potential bioactive compounds which have been used as CNS stimulant, topical anaesthetic in ophthalmology, powerful pain relievers and are known to exert antipuretic action (Ramawat *et al*., 2009). According to Okwu (2004), the basis for use of Alkaloids as therapeutic agents is due to their analgesic, anti-spasmodic and bactericidal effects.

Saponins have been reported to possess both beneficial (lowering of blood cholesterol) and deleterious effects (cytotoxic, haemolysis and permeabilization of the intestine (Wink, 2010). Saponin content in both leaves (14.33uglg) and Roots (14.00ug/g) may be suggested to exert beneficial effect.

Saponins also have antimicrobial and anti-inflammatory activities but high concentration of saponins in the body can reduce uptake of certain nutrients including glucose and cholesterol, leading to hypercholesterolemia effect (Price *et al*., 1987; Sparg *et al*., 2004). The knowledge of phytate levels in plants and food is necessary because high concentration of phytate can cause indigestion of food and flatulence (Nwoko and Bragg, 1977). Phytic acid intake of 4.00 – 9.00mg/100g reduces iron absorption by 4-5 folds in human (Grases *et al*., 2001). But phytate in moderate levels has an anti-oxidant effect and can also prevent colon cancers by reducing stress in the lumen of intestinal tracts. Phenol was significantly present in both samples: leaves (6.66ug/g) and roots (3.96ug/g).

Phenolic compounds are secondary metabolites with a wide range of biological activities including increase bile secretion, reduction of blood lipid and cholesterol level, antioxidant and antimicrobial activity against bacterial strains staphylococcus (Wink, 2010).

The concentration of oxalate in the leaves (1.20ug/g) and roots (0.84ug/g) was quite low compared to values reported for okra (61.5mg/100g), sweet potato (29.1mg/100g), Tomato (6.5mg/100g), orange (2.2mg/100g), onion (2.9mg/100g), carrot (7.7mg/100g). Oxalate is often referred to as anti-nutrient.

If consumed in large amounts may be harmful to health (Noonan and Savage, 1999) and can increase risk of kidney stone development because of increased concentration in urine. At moderate intake levels, about 40% of the total oxalate is excreted in the urine (Kohlmeier, 2003) as minute crystals. In the body oxalic acids combine with divalent metal cations such as Ca^{2+} and iron (II) fe^{2+} to form crystals and has been reported to interfere with calcium absorption (Kohlmeier, 2003).

Acute toxicity study of the leaf extract of *Thaumatococcus danielli* revealed that the LD_{50} value is 330mg/kg and the root 250mg/kg when administered intraperitoneally (Tables 4 and 5). Emudainohwo *et al*. (2015) reported LD_{50} value of above 6000mg/kg for leaf extract of *Thaumatococcus danielli* administered orally which is far higher than reported in this study.

Table 5: AST level of Albino rats treated with Methanol leaf extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	20.00 ± 5.57^{ns}	23.67 ± 8.65^{ns}	14.33 ± 0.88^{ns}	12.67 ± 3.48^{ns}
Medium Dose	9.00 ± 2.00^{ns}	89.00±0.00*	33.33 ± 15.01^{ns}	15.00 ± 8.00^{ns}
High Dose	9.00 ± 2.00^{ns}	18.67 ± 7.36^{ns}	12.33 ± 2.73^{ns}	19.00 ± 0.00^{ns}
Normal	16.33±4.81	16.33±4.81	16.33±4.81	16.33±4.81

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant ($p<0.05$) compared to the control, ns: not significant as compared to the control.

According to Osibemhe *et al*. (2016), a test drug administered orally is considered extremely toxic at $LD_{50} \leq 1$mg/kg, highly toxic at 1-50mg/kg, moderately toxic at 50-500mg/kg, slightly toxic at 500-5000mg/kg, practically non-toxic at ≥ 5000-15,000mg/kg. Abnormality in liver enzyme activities is widely used as an index of toxicity effect on the liver, a major organ of detoxification processe.

Table 5 shows that methanolic leaf extract of *Thaumatococcus danielli* in Low and high doses (8.25mg/kg and 33mg/kg) administered for 4 weeks has no significant ($p<0.05$) effect on AST level of the albino rats compared to the control group, but was significantly ($p<0.05$) increased in the medium dose treated rats at week 2.

In Table 6, the methanolic Leaf extract of *Thaumatococcus* danielli administered for 4 weeks significantly ($p<0.05$) increased ALT level in week 2 for all the treated groups compared to the control group. Although elevated ALT level were observed at week 1 and 4 in the medium dose (16.5mg/kg) treated rats, week 1 and 3 in high dose (33mg/kg) treated group, those changes in the enzymes activity were insignificant at $p<0.05$.

Several biochemical parameters in the blood are measured to determine the functional intergrity of the liver. Significant changes in some of these parameters singly or in combination with others may suggest toxicity sign. Findings from this study shows that methanolic leaf extract of *Thaumatococcus danielli* in low and high doses (8.25mg/kg and 33mg/kg) administered for 4 weeks has no significant effect on AST level of the albino rats compared to the control group, but AST level was significantly ($p<0.05$) increased in the medium dose treated rats at week 2.

The ALT level significantly ($p<0.05$) increased in week 2 for all the treated groups compared to the control group. Elevated AST activity in week 4 of the medium dose treated group was statistically insignificant at $p<0.05$. ALT levels showed no significant ($p<0.05$) difference in weeks 1, 3 and 4 in all the studied groups for 4 weeks, but in week 2, there were significant ($p<0.05$) increases in low, medium and high dose (6.25mg/kg, 12.5mg/kg and 25mg/kg) groups.

Elevated ALT level in week 4 of the medium dose (12.5mg/kg) treated group was statistically insignificant at $p<0.05$. There is no clinical significance to the occurrence of low level of biochemical markers but elevated activities of liver enzymes indicate several mechanisms which may be due to liver damage, mitochondrion toxicity, hypersensitivity reaction and immune response (Pentilla *et al*., 1975).

Some observable changes in AST and ALT levels after some weeks of treatment with the leaf and root extracts of this plant may not be due to hepatoxicity, this is because these enzymes (AST and ALT) are also found in other cells of the body. AST is found in liver cells and brain, kidneys, pancreas, cardiac and skeletal muscles (Kasper *et al*., 2005).

On the other hand ALT is present in high concentrations in the liver and at low concentrations in variety of tissues such as heart, spleen, kidney, lungs and skeletal muscle, thus, ALT is considered more specific for hepatic injuries (Emeka and Obidia, 2009). Clinically, circulatory shock, trauma, muscles disease, myocardial infarction and haemolytic disease are associated with elevated ALT levels (Allston, 1993). In acute viral hepatitis, ALT level is always higher than AST, but most patients with chronic hepatitis C may show lower or normal ALT level (Emeka and Obidia, 2009).

Thus, an absence of changes in ALT and AST levels does not exclude some degree of dysfunction in clinical examination.

Table 6: ALT level of Albino rats treated with Methanol leaf extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	9.33 ± 2.67^{ns}	38.00±5.51*	16.00 ± 4.04^{ns}	8.00 ± 0.00^{ns}
Medium Dose	15.00 ± 7.37^{ns}	42.00±6.81*	6.33 ± 1.20^{ns}	19.00 ± 2.00^{ns}
High Dose	21.00 ± 6.66^{ns}	44.33±13.28*	18.00 ± 3.00^{ns}	6.00 ± 2.00^{ns}
Normal	11.00±3.79	11.00±3.79	11.00±3.79	11.00±3.79

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant ($p<0.05$) compared to the control, ns: not significant as compared to the control.

Table 7 shows that administration of methanolic Leaf extract of *Thaumatococcus danielli* for 4 weeks significantly ($p<0.05$) increased ALP level at week 1 in the low dose (8.25mg/kg) treatment group when compared to the control. In week 2 and 3, all the treated groups showed significant ($p<0.05$) decreases but were markedly increased in week 4 of low and high dose treated groups.

Table 8 shows that methanolic root extract of *Thaumatococcus danielli* has no significant ($p<0.05$) effect on AST level in weeks 1, 3 and 4 at different doses (6.25mg/kg, 12.5mg/kg and 25mg/kg)

administered for 4 weeks compared with normal control, but increased (P<0.05) in week 2 of low and medium (12.5mg/kg and 25mg/kg respectively) dose treated groups. Elevated AST activity in week 4 of the medium dose treated group was statistically insignificant at p<0.05.

In Table 9, the ALT levels showed no significant (p<0.05) difference in weeks 1, 3 and 4 in all the studied groups for 4 weeks, but in week 2, there were significant (p<0.05) increases in low, medium and high dose (6.25mg/kg, 12.5mg/kg and 25mg/kg) groups. Elevated ALT level in week 4 of the medium dose (12.5mg/kg) treated group was statistically insignificant at p<0.05.

Table 7: ALP level of Albino rats treated with Methanol leaf extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	459.98±9.36*	12.12±0.24*	65.86±4.03*	556.22±71.27*
Medium Dose	122.29±1.39ns	15.98±0.19*	63.90±8.76*	329.76±6.40ns
High Dose	170.19±36.91ns	14.15±0.30*	71.44±8.61*	439.15±11.84*
Normal	164.50±18.99	164.50±18.99	164.50±18.98	164.50±18.99

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant (p<0.05) compared to the control, ns: not significant as compared to the control.

Table 8: AST level of Albino rats treated with Methanol Root extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	20.33±5.46ns	57.00±19.00*	17.00±5.03ns	13.00±0.00ns
Medium Dose	16.33±4.81ns	70.33±2.85*	21.67±1.33ns	48.00±4.10ns
High Dose	25.00±6.00ns	11.67±2.60ns	9.67±1.45ns	15.00±8.00ns
Normal	16.33±4.81	16.33±4.81	16.33±4.81	16.33±4.81

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant (p<0.05) compared to the control, ns: not significant as compared to the control.

Table 9: ALT level of Albino rats treated with Methanol Root extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	19.67±11.89ns	29.67±4.67*	14.67±1.45ns	6.67±1.33ns
Medium Dose	48.00±23.00ns	37.00±4.16*	18.00±3.00ns	26.50±18.50ns
High Dose	8.33±4.33ns	27.33±1.20*	15.00±3.00ns	8.00±4.00ns
Normal	11.00±3.79	11.00±3.79	11.00±3.79	11.00±3.79

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant (p<0.05) compared to the control, ns: not significant as compared to the control.

Table 10 shows that administration of the methanolic root extract of *Thaumatococcus* danielli for 4 weeks had no significant (p> 0.05) difference in ALP activity in week 1 and week 4 at all doses (6.25mg/kg, 12.5mg/kg and 25mg/kg), but in weeks 2 and 3, all treated groups showed significant (p<0.05) decrease when compared to the control group. It was also observed that all the treated groups in week 4 showed elevated ALP activity which was insignificant at p<0.05.

Table 10: ALP level of Albino rats treated with Methanol Root extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	158.10±67.31ns	44.46±18.20*	70.64±6.64*	290.48±9.51ns
Medium Dose	125.93±35.78ns	48.41±8.41*	79.60±10.73*	327.89±27.22ns
High Dose	296.05±10.91ns	10.45±0.73*	69.69±5.23*	494.29±11.62ns
Normal	164.50±18.99	164.50±18.99	164.50±18.98	164.50±18.99

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant (p<0.05) compared to the control, ns: not significant as compared to the control.

In Table 11, the albumin concentration of Albino rats treated with Methanol leaf extract of *Thaumatococcus danielli* for 4 weeks showed no significant ($p<0.05$) difference in all the doses (8.25mg/kg, 16.5mg/kg and 33mg/kg) administered in weeks 1, 3 and 4, but significantly ($p<0.05$) increased in week 2 at medium and high doses of treatment when compared to normal control group. Table 12 indicates that the albumin level of albino rats after treatment with Methanol root extract of *Thaumatococcus danielli* for 4 weeks showed no significant ($p<0.05$) difference in all the doses administered in week 1 and 4, but significantly ($p<0.05$) increased in week 3 at all doses of treatment. A significant ($p<0.05$) increase was noticed in high dose treated group in week 2 when compared to normal control group.

Table 11: Albumin level of Albino rats treated with Methanol leaf extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	4.11 ± 0.67^{ns}	4.20 ± 0.35^{ns}	3.79 ± 0.51^{ns}	4.07 ± 0.11^{ns}
Medium Dose	2.65 ± 0.42^{ns}	4.94±0.15*	4.31 ± 0.28^{ns}	3.73 ± 0.56^{ns}
High Dose	3.71 ± 0.37^{ns}	5.08±0.10*	3.79 ± 0.26^{ns}	4.03 ± 0.61^{ns}
Normal	3.06±0.70	3.06±0.70	3.06±0.70	3.06±0.70

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant ($p<0.05$) compared to the control, ns: not significant as compared to the control.

Table 12: Albumin level of Albino rats treated with Methanol Root extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	3.30 ± 1.06^{ns}	4.01 ± 0.05^{ns}	4.86±0.01*	3.20 ± 0.37^{ns}
Medium Dose	3.19 ± 0.32^{ns}	3.84 ± 0.15^{ns}	4.52±0.104*	3.87 ± 0.42^{ns}
High Dose	4.17 ± 0.07^{ns}	4.80±0.24*	4.57±0.20*	3.54 ± 0.20^{ns}
Normal	3.06±0.70	3.06±0.70	3.06±0.70	3.06±0.70

Values are mean of five replicate ± Standard error of the mean (SEM). *Values are significant ($p<0.05$) compared to the control, ns: not significant as compared to the control.

Table 13 shows that the total protein concentration of albino rats treated with Methanol leaf extract *of Thaumatococcus danielli* for 4 weeks was significantly ($p<0.05$) higher in week 1 for high dose (33mg/kg) treated rats, while low (8.25mg/kg) and medium dose (16.5mg/kg) of treatment showed no significant ($p<0.05$) difference with normal control group.

In week 2, medium and high dose treated rats showed significant increases. Table 14 shows that the total protein concentration of Albino rats treated with Methanol leaf extract *of Thaumatococcus danielli* for 4 weeks was not significantly ($p<0.05$) different from the control in week 1. A significant ($p<0.05$) increase was observed in low (12.5mg/kg) and high dose (25mg/kg) treated groups in week 2. All the doses tested showed significant ($p<0.05$) increase in week 3, but in week 4, a significant ($p<0.05$) increase was only recorded in high dose treated rats.

Table 14 shows that total bilirubin concentration of albino rats after treatment with methanolic leaf extract for 4 weeks was significantly ($p<0.05$) decreased in all treated groups at weeks 2,3 and 4 when compared to normal control, however, no significant changes were observed during the first week (week 1).

Table 13: Total Protein level of Albino rats treated with Methanol leaf extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	6.38 ± 1.02^{ns}	6.54 ± 0.003^{ns}	9.19±0.08*	6.66 ± 0.91^{ns}
Medium Dose	4.87 ± 0.84^{ns}	7.58±0.58*	13.09±3.03*	5.53 ± 1.07^{ns}
High Dose	7.87±0.95*	7.53±0.17*	8.99±0.22*	9.01 ± 4.33^{ns}
Normal	4.07±1.42	4.07±1.42	4.07±1.42	4.07±1.42

Table 14: Total Protein level of Albino rats treated with Methanol Root extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	3.81±0.14^{ns}	9.32±0.34*	10.93±0.30*	5.11±0.30^{ns}
Medium Dose	9.80±4.99^{ns}	6.48±0.40^{ns}	12.68±1.23*	5.28±0.09^{ns}
High Dose	6.72±0.24^{ns}	8.90±0.44*	14.99±1.56*	7.36±0.36*
Normal	4.07±1.42	4.07±1.42	4.07±1.42	4.07±1.42

Table 14: Total Bilirubin level of Albino rats treated with Methanol leaf extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	0.63±0.25^{ns}	0.06±0.02*	0.01±0.005*	0.04±0.01*
Medium Dose	0.89±0.01^{ns}	0.004±0.002*	0.06±0.012*	0.18±0.14*
High Dose	0.93±0.54^{ns}	0.08±0.03*	0.06±0.02*	0.12±0.07*
Normal	0.80±0.06	0.80±0.06	0.80±0.06	0.80±0.06

Values are mean of 5 replicate ± Standard error of the mean (SEM). *Values are significant ($p<0.05$) compared to the control, ns: not significant as compared to the control.

Table 15 shows that the concentration of conjugate bilirubin of albino rats treated with methanol leaf extract of *Thaumatococcus danielli* was not significantly ($p<0.05$) affected at all doses administered for 4 weeks. Furthermore, Table 16 shows that conjugate bilirubin level of albino rats treated with Methanol root extract of *Thaumatococcus danielli* for 4 weeks showed significant ($p<0.05$) increase in medium dose treated group in week 1, but no observable differences in weeks 2, 3 and 4 at all doses (6.25mg/kg, 12.5mg/kg and 25mg/kg) of treatment when compared to normal control group.

Table 15: Conjugate Bilirubin level of Albino rats treated with Methanol Leaf extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	0.33±0.23^{ns}	0.13±0.03^{ns}	0.01±0.004^{ns}	0.42±0.17^{ns}
Medium Dose	0.11±0.2^{ns}	0.12±0.04^{ns}	0.02±0.01^{ns}	0.16±0.16^{ns}
High Dose	0.78±0.67^{ns}	0.21±0.06^{ns}	0.015±0.01^{ns}	0.74±0.08^{ns}
Normal	0.59±0.49	0.59±0.49	0.59±0.49	0.59±0.49

Table 16: Conjugate Bilirubin level of Albino rats treated with Methanol Root extract of *Thaumatococcus danielli* for four weeks

Group	Week 1	Week 2	Week 3	Week 4
Low Dose	0.11±0.11^{ns}	0.12±0.01^{ns}	0.004±0.00^{ns}	0.74±0.05^{ns}
Medium Dose	1.59±0.18*	0.16±0.03^{ns}	0.04±0.01^{ns}	0.52±0.02^{ns}
High Dose	0.09±0.03^{ns}	0.04±0.001^{ns}	0.004±0.00^{ns}	0.80±0.004^{ns}
Normal	0.59±0.49	0.59±0.49	0.59±0.49	0.59±0.49

Values are mean of 5 replicate ± Standard error of the mean (SEM). *Values are significant ($p<0.05$) compared to the control, ns: not significant as compared to the control.

Both total and conjugate bilirubin measurement can be used to determine the excretory function of the liver and assessment of haemolytic anaemia (Kpomah *et al*., 2012). The observed non- significant changes in conjugate bilirubin suggest intact functional liver.

The administration of methanolic Leaf extract of *Thaumatococcus danielli* for 4 weeks significantly ($p<0.05$) increased ALP level at week 1 in the low dose (8.25mg/kg) treatment group when compared to the normal control.

Elevated ALP level often indicate obstruction of biliary system (hepatobiliary diseases) and bone diseases (Reust and Hall, 2011). In this study significant increases observed may not point to hepatobiliary diseases but could come from extrahepatic sources (Hasan and Owyed, 2003; Giannini *et al*., 2005). Albumin plays a major role in maintaining plasma osmotic pressure as well as transportation of lipids and hormone (Saidu *et al*., 2007).

Almost all blood proteins in human blood are synthesized in the liver, thus impairment of synthetic function of the liver is an indication of liver damage (Whitby *et al*., 1989). Significant ($p<0.05$) increase in

total protein and albumin levels after 2 weeks treatment with both extracts could further suggest non-toxic effect of the extract on the liver.

The observed high levels of total protein in medium and high dose treated rats could be due to high protein content in the extracts. Ekpeyong *et al.* (2012) had earlier stated that high protein diet is associated with high hepatic synthesis and hence high serum protein.

4. Conclusion and Recommendations

Phytochemical analyses showed significant concentration of flavonoids which is a powerful antioxidant with other phytochemicals which are of health benefit.

But, sub-acute administration of leaf and root extracts of this plant caused some alterations in biochemical parameters.

Thus, caution should be applied when used therapeutically at medium and high dose concentrations.

It is recommended that chronic toxicity studies for duration of at least 60 days be designed to explicitly define some observed alterations in biochemical parameters. Testing for the presence of heavy metals should also be carried out.

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