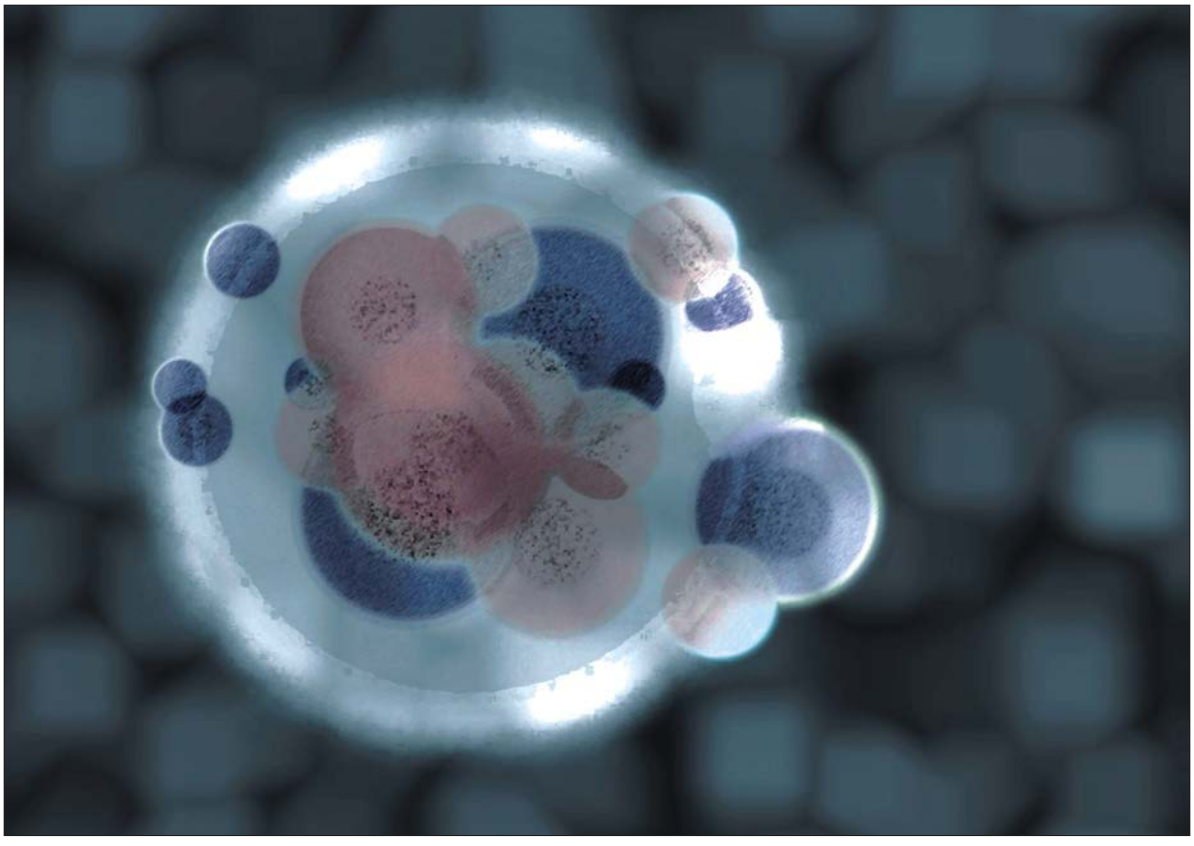

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2017.381.387

Research Article
Antioxidant and Biochemical Evaluation of *Thaumatococcus daniellii* Seeds in Rat

Shalom Nwodo Chinedu, Franklyn Nonso Iheagwam, Chisom Juliet Anichebem, Gbemisola Beatrice Ogunnaike and Opeyemi Christianah Emiloju

Department of Biochemistry, College of Science and Technology, Covenant University, PMB 1023, Canaanland, Ota, Ogun State, Nigeria

Abstract

Background and Objective: Despite numerous reports of medicinal uses of *Thaumatococcus daniellii* (*T. daniellii*) plant, there remains a dearth of information on the *in vivo* effect of the seed. In this study, the antioxidant and biochemical effects of *T. daniellii* seeds in the liver and kidney of male wister rats were assessed. **Materials and Methods:** Seeds were macerated with ethanol and filtrate was concentrated to yield an ethanolic crude extract. Rats were orally dosed with vitamin C, 500, 1000 and 1500 mg kg^{-1} of the extracts for 14 days. Antioxidant and biochemical parameters were evaluated. Liver histology was examined. Data were subjected to one-way analysis of variance and expressed as Mean ± SEM. **Results:** It was observed that *T. daniellii* seed reduced the amount of total body weight gain but did not have any effect on organ weight ($p<0.05$). Treatment also resulted in significant ($p<0.05$) increase in hepatic SOD and GSH. It was only at the highest dose that renal GSH increased significantly ($p<0.05$). These antioxidant effects were associated with maintaining bilirubin concentration and reducing AST activity in the liver ($p<0.05$). Histopathological observations were in correlation with the biochemical results showing that there was no pathologic abnormality. **Conclusion:** The results of this study indicate that *T. daniellii* seed is a source of natural antioxidant and may be exploited for the treatment of kidney and liver diseases.

Key words: *Thaumatococcus daniellii*, *in-vivo* effect, biochemical parameters, antioxidant, liver histology, liver diseases

Citation: Shalom Nwodo Chinedu, Franklyn Nonso Iheagwam, Chisom Juliet Anichebem, Gbemisola Beatrice Ogunnaike and Opeyemi Christianah Emiloju, 2017. Antioxidant and biochemical evaluation of *Thaumatococcus daniellii* seeds in rat. J. Biol. Sci., 17: 381-387.

Corresponding Author: Shalom Nwodo Chinedu, Department of Biochemistry, College of Science and Technology, Covenant University, PMB 1023, Canaanland, Ota, Ogun State, Nigeria Tel: +2348065940573

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Thaumatococcus daniellii (Benn.) Benth is a monocotyledonous herb found in rain forests and coastal areas of West and Central Africa[1-3]. This plant is a large rhizomatous flowering herb which grows to about 3-4 m in height with large papery leaves[4]. It bears pale purple flowers and a soft crimson coloured fruits containing a few shiny black seeds[4]. It is commonly referred to as miraculous fruit, miraculous berry, serendipity berry and katamfe/katempfe. In Nigeria, it is popular referred to as soft cane[5]. It was one of the underutilized and neglected plant species in Nigeria, which grows wildly mainly in cocoa-growing areas of Southwest Nigeria[4,6]. It is categorized as a non-woody fibre (NWF) plant used to supplement to wood fibre in paper manufacturing[7]. The plant, commonly known as "ewe eran" in Southwest Nigeria[8], is popular for being the natural source of thaumatin, a globally traded protein sweetener[9-11]. This low-calorie sweetner is about 2000 times sweeter than sucrose and suitable for diabetic patients[12]. Thaumatin is found in the arils which are located at the top of the seeds[9,13]. The seeds are black, hard and impervious, wrapped around by a transparent sticky gel-like sac[14]. The seed was used by locals in ethnomedicine as an emetic and in treating pulmonary problems[15]. The jelly on the seed can be used as a substitute for agar[16]. Nonetheless, it has little to no antimicrobial effect[17]. More so, while on a field trip to the South Western part of Nigeria in a group of four researchers, it was discovered that the seed of the plant when grinded and added to food could have a positive effect on sight and total wellness of an individual though there is presently no scientific basis to support these claims. Several *in vitro* studies have been done on *T. daniellii* seed, but little was known on the *in vivo* effect of the seed[14,16]. This present study evaluated the biochemical, antioxidant and histopathological effects of *T. daniellii* seed in experimental rats.

MATERIALS AND METHODS

Chemicals and reagents: Tris-HCl, ethylenediaminetetraacetic acid (EDTA), pyrogallol, Ellman's Reagent (DTNB) were obtained from Sigma–Aldrich, Germany. Sodium nitrate, thiobarbituric acid (TBA), trichloroacetic acid (TCA) were obtained from Fischer Scientific, UK. All other chemicals and reagents were of analytical grades.

Collection of plant samples and extracts preparations: Fresh fruits of *T. daniellii* were obtained from local farmers in Ekiti, Ekiti State, Nigeria and authenticated by Dr. J.O. Popoola in the Department of Biological Sciences, Covenant University, Ota, Nigeria. The seeds were removed, air-dried at room temperature (25°C) and pulverized. The powder was macerated in 80% ethanol (1:10 w/v) for 72 h. The resulting mixture was filtered using Whatman (No.1) filter paper and the filtrate was concentrated to dryness at 60°C on a rotary evaporator to give the crude ethanol extract.

Experimental animals and procedure: Thirty male wister rats, 4-6 weeks old, weighing between 100 and 150 g were purchased from the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria. The animals were maintained under standard laboratory conditions in the animal house of Covenant University in compliance with Covenant University Ethics Committee guide for care and use of laboratory animals. Animal feed and water were given *ad libitum*. The rats were acclimatized for 2 weeks after which they were randomly distributed into five (5) groups. Group 1 served as the control, Group 2 was dosed with vitamin C (10 mg kg^{-1} bwt) daily as drug standard, Group 3, Group 4 and Group 5 was daily administered 500, 1000 and 1500 mg kg^{-1} b.wt., dose of *T. daniellii* seed respectively for 14 days. At the end of 14 days, the animals were fasted overnight and sacrificed under light ether anaesthesia. Brain, heart, kidney, liver, testes and spleen was excised. Kidney and liver were biochemically and histologically examined.

Preparation of tissue homogenates: Tissue homogenates were prepared using the procedure of Amin *et al.*[18] with slight modification. 0.2 g of hepatic and renal tissue were weighed in 1.8 mL phosphate buffer saline then minced by a homogenizer (Raider Professional, Hamburg, Germany) for 15 min followed by centrifugation for 10 min at 3000 rpm. The formed supernatants were collected and stored at -20°C for antioxidant and biochemical analysis.

Biochemical assay

Estimation of antioxidant parameters: Superoxide dismutase (SOD): 1 mL each of 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol were added to 50 µL of tissue homogenate. Change in absorbance at 420 nm for 3 min in a spectrophotometer was noted. One unit of enzyme activity was equivalent to 50% inhibition of the rate of autooxidation of pyrogallol as determined by a change in absorbance min^{-1} at 420 nm[19].

Reduced glutathione (GSH): Three hundred microliter of 10% TCA was added to 300 µL of tissue homogenate. It was centrifuged at 5000 rpm for 10 min before 500 µL of the supernatant was transferred to a separate tube. 250 µL of

Ellman' s reagent (19.8 mg of DTNB in 100 mL of 0.1% sodium nitrate) and 1500 µL of phosphate buffer (0.2M, pH 8.0) were added and absorbance was read at 412 nm[20].

Malondialdehyde (MDA): One mL of tissue homogenate was added to 2 mL of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA). The mixture was boiled at 100°C for 15 min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank[21].

Estimation of liver function parameters: Commercial test kits obtained from Randox laboratories, England, UK were used for aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL) and direct bilirubin (DBIL) analysis according to manufacturer's instruction.

Histopathology: Liver tissues were separated from two animals from each group and stored in 10% formalin. They were later sectioned using a microtome, dehydrated in graded alcohol, embedded in paraffin section and stained with hematoxylin and eosin (H&E). Specimens were evaluated with a light microscope. Histopathological changes were evaluated by a pathologist[22].

Statistical analysis: Statistical analysis was performed using IBM Statistical Package for the Social Sciences statistical package V 23.0. Data were expressed as means of six replicates±SEM and were subjected to one-way analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT). They were considered statistically significant at $p<0.05$.

RESULTS

Table 1 shows the amount of total body weight gain in rats treated with *T. daniellii* seed was significantly lower than the control ($p<0.05$). There was no difference in relative organ weight between all groups. Only the group dosed with 1000 mg kg^{-1} had their heart weight significantly ($p<0.05$) lower than the control.

Table 2 indicate groups treated with *T. daniellii* seed exhibited an increase in hepatic SOD activity and GSH concentration ($p<0.05$). There was no significant effect on renal SOD activity and GSH concentration. However, a significant ($p<0.05$) increase in renal GSH concentration was observed at the highest concentration. *T. daniellii* seed had no effect on hepatic TBARS level. Nevertheless, a significant increase was observed in renal TBARS level.

The effect of *T. daniellii* seed on liver function parameters were shown in Table 3. Though there was no effect of *T. daniellii* seed on TBIL and DBIL, ALT and AST were significantly increased and decreased respectively when compared with the control group ($p<0.05$).

Figure 1 shows the representative histological sections of liver tissue treated with 500-1500 mg kg^{-1} of *T. daniellii* seed where mild periportal infiltration by inflammatory cells was observed as a non-toxic sign of by the extracts concentration.

Table 1: Effect of *T. daniellii* seed on rat and relative organ weights

Group (g)	Control	Vit. C	500 mg kg^{-1}	1000 mg kg^{-1}	1500 mg kg^{-1}
TBWG	27.33±2.03^{b}	22.25±3.97^{ab}	14.00±2.04^{a}	21.67±6.49^{ab}	16.33±3.33^{ab}
Liver	4.47±0.29^{a}	3.88±0.16^{a}	4.02±0.15^{a}	4.03±0.57^{a}	4.23±0.16^{a}
Kidney	0.81±0.05^{a}	0.78±0.05^{a}	0.80±0.05^{a}	0.75±0.09^{a}	0.80±0.04^{a}
Spleen	0.55±0.05^{a}	0.54±0.03^{a}	0.57±0.08^{a}	0.61±0.08^{a}	0.46±0.04^{a}
Heart	0.51±0.04^{b}	0.51±0.03^{b}	0.48±0.04^{b}	0.38±0.03^{a}	0.50±0.03^{b}
Brain	1.89±0.12^{a}	1.57±0.08^{a}	1.68±0.13^{a}	1.51±0.16^{a}	1.72±0.16^{a}
Testes	0.91±0.22^{a}	0.84±0.16^{a}	1.09±0.17^{a}	1.25±0.24^{a}	1.26±0.16^{a}

Values are expressed as Mean±SEM of 6 replicates. Values with different superscripts in a row are significantly different at $p<0.05$ compared to the control, TBWG = Total body weight gain

Table 2: Effect of *T. daniellii* seed on antioxidant defence system in rat liver and kidney

Groups	Control	Vit. C	500 mg kg^{-1}	1000 mg kg^{-1}	1500 mg kg^{-1}
SOD (U mg^{-1} protein)					
Liver	7.76±0.43^{a}	7.94±0.76^{a}	18.07±4.04^{b}	16.53±2.01^{b}	28.17±2.08^{c}
Kidney	5.76±0.81^{a}	2.88±0.09^{a}	4.24±0.51^{a}	3.86±0.59^{a}	5.18±1.74^{a}
GSH (mM mg^{-1} protein)					
Liver	13.70±1.34^{a}	12.52±1.18^{a}	20.32±2.86^{b}	18.81±2.35^{b}	14.03±1.08^{a}
Kidney	18.40±1.01^{a}	21.16±1.34^{b}	16.96±1.62^{a}	18.31±2.86^{a}	22.61±1.36^{b}
TBARS (mM mg^{-1} protein)					
Liver	2.73±0.54^{a}	2.03±0.10^{a}	3.65±0.29^{a}	3.49±0.20^{a}	3.03±0.22^{a}
Kidney	1.72±0.14^{a}	2.35±0.20^{a}	4.45±0.19^{b}	5.94±0.42^{b}	4.15±0.25^{b}

Values are expressed as Mean±SEM of 6 replicates. Values with different superscripts in a row are significantly different at $p<0.05$ compared to the control

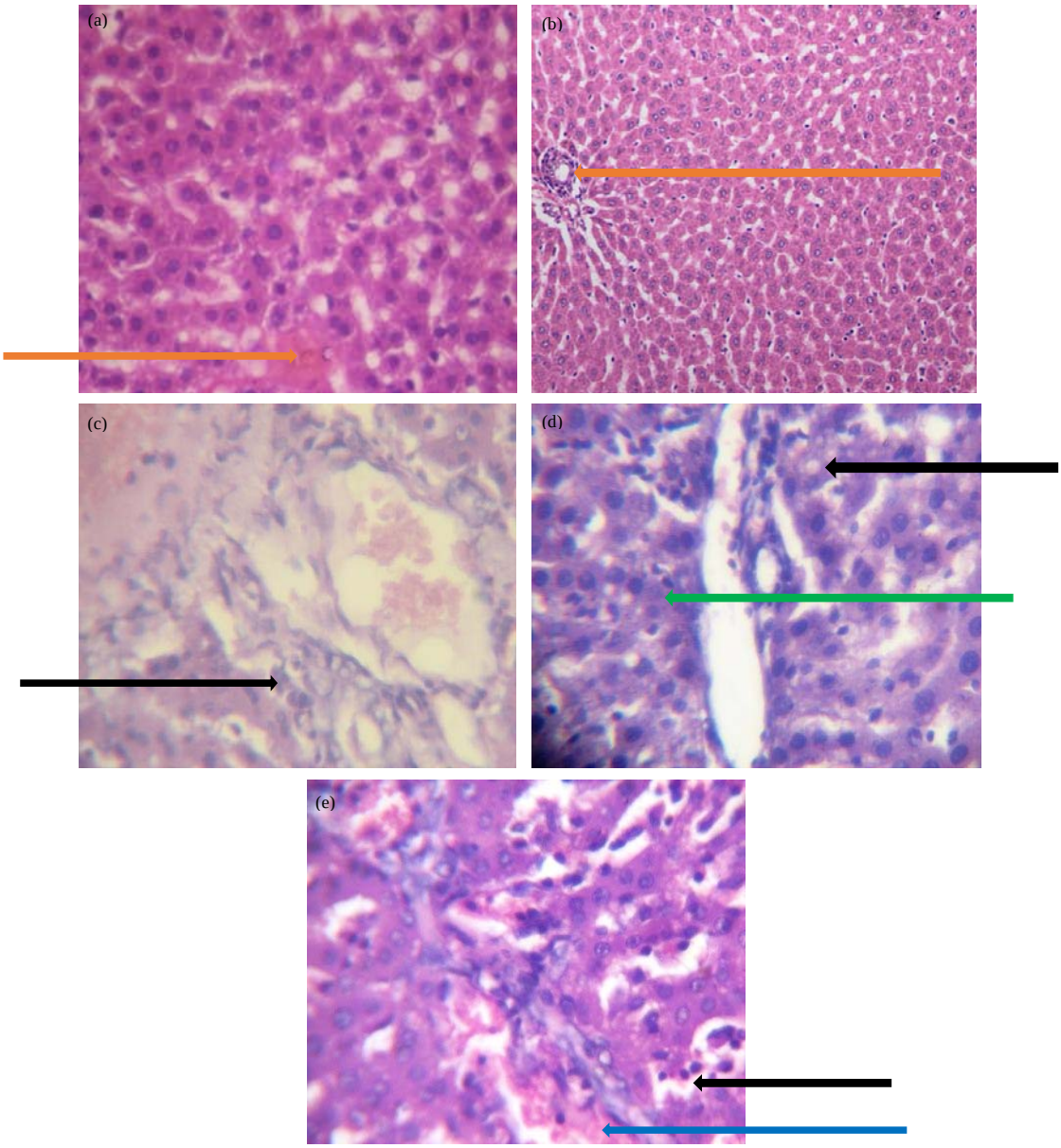

Fig. 1(a-e): Hematoxylin and eosin-stained cross-sectional liver views. (a) Normal histology of control group (b) group administered with Vit. C, (c) Histology of group orally administered 500 mg kg^{-1} show very mild periportal infiltration by inflammatory cells (black arrow), (d) Histology of group orally administered 1000 mg kg^{-1} show very mild periportal infiltration by inflammatory cells (black arrow) and disseminated steatosis (green arrow) and (e) Histology of group orally administered 1500 mg kg^{-1} show mild periportal infiltration by inflammatory cells (black arrow) and congestion of vessels (blue arrow) (Magnification 400X)

Table 3: Effect of *T. daniellii* seed on liver function parameters in rat liver

Groups	Control	Vit. C	500 mg kg^{-1}	1000 mg kg^{-1}	1500 mg kg^{-1}
ALT (U L^{-1})	29.31±4.4^{a}	36.55±1.24^{a}	76.56±4.20^{b}	75.18±4.02^{b}	72.14±1.92^{b}
AST (U L^{-1})	301.30±17.39^{b}	315.33±4.67^{b}	180.11±11.74^{a}	199.46±1.70^{a}	176.30±12.17^{a}
DBIL (µmol L^{-1})	31.52±7.97^{a}	36.93±6.58^{a}	48.34±11.12^{a}	33.21±10.32^{a}	26.47±2.04^{a}
TBIL (µmol L^{-1})	23.31±6.86^{a}	20.97±1.54^{a}	16.07±3.26^{a}	16.30±0.84^{a}	14.73±2.92^{a}

Values are expressed as Mean±SEM of 6 replicates. Values with different superscripts in a row are significantly different at $p<0.05$ compared to the control

DISCUSSION

From the result, there was no decrease beyond 10% of the animal organs weight indicating that *T. daniellii* seed may be nontoxic to the organs.The observed difference in weight gain may be as a result of the high crude fibre, low fat content and fat yield potential of the seed which has been reported in previous studies[15,16]. It may also be as a result of the crude fibre present in the seed binding to cholesterol in the gut and delaying gastric emptying and cholesterol synthesis thus reducing body weight. This is in line with the study conducted by Iheagwam *et al.*[23] and Elemo *et al.*[24]. Due to the metabolic processes that are simultaneously carried out in cells, reactive oxygen species (ROS) are produced[25]. Endogenous and exogenous antioxidants (enzymatic and non-enzymatic) protect cells from ROS attack[26]. Plants are made up of secondary metabolites and phytoconstituents with excellent antioxidant properties, which help in prevention and management of diseases[27]. SOD, an endogenous enzymatic antioxidant exerts its function by scavenging radicals while GSH (an endogenous non enzymatic antioxidant) conjugates these radicals preventing cellular injury through lipid peroxidation of cell membranes[28]. MDA is the consequent product of lipid peroxidation and an index of measuring the metabolic level of oxidative stress[29].The observable increase in SOD and GSH after *T. daniellii* seed administration may be as a result of synergistic stimulatory effects of various active components present in the seed on the synthesis of these enzumes. Flavonoids possess antioxidant properties[28] while these elements are cofactors responsible for the normal functioning of SOD's four isozymes[30]. Chinedu *et al.*[14] and Abiodun *et al.*[16] have previously reported the presence of flavonoids and micronutrients (copper, nickel, manganese and iron) respectively in the seeds. Conversely, high oxalate content in the seed might be responsible for the increase in renal MDA as they are known to aid kidney stone formation[31]. Liver function bio markers are used to assess hepatotoxicity and hepatocyte integrity[32]. The significant decrease of AST and insignificant effect on DBIL and TBIL despite the high concentration of *T. daniellii* seed dosage suggests a hepatoprotective ability of the seed since liver membrane integrity was not affected. The antioxidant activity of this seed may consequently be responsible for the hepatoprotective effect of *T. daniellii* seed. This relationship has been previously reported by Kamisan *et al.*[33]. Slight to mild periportal infiltration by inflammatory cells was observed in the liver histology. This observation has been reported as an indicator of positive immune response[34]. The result obtained in this study was in conformity with *in vivo* studies carried out on other parts of *T. danielli* particularly the leaves[28,35].

CONCLUSION

This research has shown that *Thaumatococcus daniellii* seed is a natural source of antioxidant principles. It also showed that the *in vivo* antioxidant properties were favourable when compared to vitamin C. This seed can be exploited for antioxidant bioactive components for the amelioration of liver and kidney diseases.

SIGNIFICANCE STATEMENTS

This study indicates the possible antioxidant properties of *Thaumatococcus daniellii* seed on rats' kidney and liver, which is highly comparable with vitamin C (a known antioxidant). The study could also lead to the discovery of a novel antioxidant lead compound, in the seed, for amelioration of liver and kidney diseases.

ACKNOWLEDGMENT

This study was supported by Covenant University Institutional grant (CUCRID RG 007.11.14/FS).

REFERENCES

1. Onwueme, I.C., B.E. Onochie and E.A. Sofowora, 1979. Cultivation of *Thaumatococcus danielli*-the sweetener. World Crops, 31: 321-335.
2. Nwonuma, C.O., E.O. Irokanulo, C.E. Iji, O.O. Alejolowo and C.O. Adetunji, 2016. Effect of *Thaumatococcus daniellii* leaf rat-feed on potassium bromate induced testicular toxicity. Asian Pac. J. Reprod., 5: 500-505.
3. Adeogun, O., A. Adekunle and A. Ashafa, 2016. Chemical composition, lethality and antifungal activities of the extracts of leaf of *Thaumatococcus daniellii* against foodborne fungi. Beni-Suef Univ. J. Basic Applied Sci., 5: 356-368.
4. Adedosu, O.T., J.A. Badmus, G.E. Adeleke and G.O. Olalere, 2017. *Thaumatococcus daniellii* extract modulates glibenclamide activity and ameliorates heamatological disorders, oxidative stress and dyslipidemia associated with diabetes mellitus in rats. Br. J. Pharm. Res., 16: 1-12.
5. Segun, A.A., F.O. Samuel and A.T. Aminat, 2015. Assessment of antibacterial activity of essential oil extracted from leaves of *Thaumatococcus danielli* (Benn.) Benth. in light of its inhibitory impact on extracellular protease of *Shigella dysenteriae*. Int. J. Biochem. Res. Rev., 5: 9-19.
6. Oluwatayo, I.B. and A.O. Ojo, 2014. Socioeconomic contributions of neglected and underutilized species to livelihood security in rural southwest Nigeria: *Thaumatococcus danielli* as a test case. Mediterr. J. Social Sci., 5: 311-317.

7. Oluwadare, A.O., 2016. Pulpsheet properties of soda pulp of miraculous berry (*Thaumatococcus daniellii* Benth.) stalk as a global fibrous raw material for papermaking. Egerton J. Sci. Technol., 15: 190-203.
8. Ekpo, I.A., R.B. Agbor, E.C. Okpako, A.N. Osuagwu, B.E. Ekanem and P.A. Otu, 2012. Effect of crude oil and simulated acid rain on the growth and physiology of *Thaumatococcus daniellii*. J. Biodivers. Environ. Sci., 2: 21-25.
9. Boadi, S., M. Baah-Acheamfour, F. Ulzen-Appiah and G.M. Jamro, 2014. Nontimber forest product yield and income from *Thaumatococcus daniellii* under a mixed tree plantation system in Ghana. Int. J. For. Res., Vol. 2014. 10.1155/2014/524863.
10. Oluwadare, A.O., A.F. Gilbert and O.A. Sotannde, 2014. A comparison of soda and soda-ethanol pulps of *Thaumatococcus daniellii* Benth (Miraculous Berry) stalks. Br. J. Applied Sci. Technol., 4: 2181-2193.
11. Arowosoge, O.G.E. and L. Popoola, 2006. Economic analysis of *Thaumatococcus danielli* (Benn.) Benth. (Miraculous berry) in Ekiti State, Nigeria. Nig. J. Food Agric. Environ., 41: 264-269.
12. Olabanji, S.O., G.A. Osinkolu, D.A. Pelemo, E.I. Obiajunwa and A.T. Oladele, 2014. PIXE analysis of *Thaumatococcus danielli* in Osun State of Nigeria. Nucl. Instruments Methods Phys. Res. Sect. B: Beam Interact. Mater. Atoms, 318: 182-186.
13. Yeboah, S.O., T.H. Hilger and J. Kroschel, 2003. *Thaumatococcus daniellii* (Benn.) Benth.-A natural sweetener from the rain forest zone in West Africa with potential for income generation in small scale farming. J. Applied Sci., 6: 854-859.
14. Chinedu, S.N., A.Y. Oluwadamisi, S.T. Popoola, B.J. David and T. Epelle, 2014. Analyses of the leaf, fruit and seed of *Thaumatococcus daniellii* (Benth.): Exploring potential uses. Pak. J. Biol. Sci., 17: 849-854.
15. Lim, T.K., 2012. Edible Medicinal and Non-Medicinal Plants, Volume 6 Fruits. Springer, New York, ISBN: 978-94-007-5627-4, pp: 259-264.
16. Abiodun, O.A., R. Akinoso, O.O. Olosunde, J.A. Adegbite and O.A. Omolola, 2014. Nutritional quality and essential oil compositions of *Thaumatococcus danielli* (Benn.) tissue and seed. Food Chem., 160: 286-291.
17. Ojekale, A.B., S.C.O. Makinde and O. Osileye, 2007. Phytochemistry and anti-microbial evaluation of *Thaumatococcus danielli*, Benn. (Benth.) leaves. Nig. Food J., 25: 176-183.
18. Amin, K.A., K.S. Hashem, F.S. Alshehri, S.T. Awad and M.S. Hassan, 2017. Antioxidant and hepatoprotective efficiency of selenium nanoparticles against acetaminophen-induced hepatic damage. Biol. Trace. Elem. Res., 175: 136-145.
19. Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.
20. Sedlak, J. and R.H. Lindsay, 1968. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., 25: 192-205.
21. Buege, J.A. and S.D. Aust, 1978. Microsomal lipid peroxidation. Methods Enzymol., 52: 302-310.
22. Akharaiyi, F.C., B. Boboye and V.O. Akpambang, 2015. Antibacterial and biochemical effects of ethanol leaf extract of *Senna hirsuta* mill using animal model-mice. J. Microbiol. Biotechnol. Food Sci., 4: 292-296.
23. Iheagwam, F.N., S.N. Chinedu, O.C. Emiloju, C.J. Anichebem and O.K. Okolie, 2017. *Thaumatococcus daniellii* seed improves lipid profile in male wistar rats. FASEB J., 31: 973-976.
24. Elemo, B.O., O.B. Adu, O.O. Ogunrinola, T.O. Efuwape, K.O. Olaleye, A.A. Kareem 2011. Biological evaluation of *Thaumatococcus danielli* waste protein. Pak. J. Nutr., 10: 1048-1052.
25. Moradi-Sardareh, H., N. Mohammadi, F. Oubari, M.R. Nikbakht and R.H. Kia *et al*., 2014. Biochemical factors, MDA levels and antioxidant activity in opium addicted hamsters. Int. Res. J. Biol. Sci., 3: 21-24.
26. Sumanth, M. and A.C. Rana, 2006. *In vivo* antioxidant activity of hydroalcoholic extract of *Taraxacum officinale* roots in rats. Indian J. Pharmacol., 38: 54-55.
27. Ganesan, P., P. Arulselvan and D.K. Choi, 2017. Phytobioactive compound-based nanodelivery systems for the treatment of type 2 diabetes mellitus-current status. Int. J. Nanomed., 12: 1097-1111.
28. Xu, G.K., X.Y. Qin, G.K. Wang, G.Y. Xie and X.S. Li *et al*., 2017. Antihyperglycemic, antihyperlipidemic and antioxidant effects of standard ethanol extract of *Bombax ceiba* leaves in high-fat-diet-and streptozotocin-induced type 2 diabetic rats. Chin. J. Nat. Med., 15: 168-177.
29. Rahmouni, F., L. Hamdaoui, R. Badraoui and T. Rebai, 2017. Protective effects of *Teucrium polium* aqueous extract and ascorbic acid on hematological and some biochemical parameters against carbon tetrachloride (CCl_4) induced toxicity in rats. Biomed. Pharmacother., 91: 43-48.
30. Amado, J.R.R., A.L. Prada, J.C.E. Arranz, R.P. Roses and H.M. Quevedo *et al*., 2016. Antioxidant and hepatoprotective activity of a new tablets formulation from *Tamarindus indica* L. Evidence-Based Complement. Altern. Med. 10.1155/2016/3918219.

31. Noonan, S.C. and G.P. Savage, 1999. Oxalate content of foods and its effect on humans. Asian Pac. J. Clin. Nutr., 8: 64-74.
32. Asadollahi, A., H. Sarir, A. Omidi and M.M. Torbati, 2014. Hepatoprotective potential of prosopis farcta beans extracts against acetaminophen-induced hepatotoxicity in wister rats. Int. J. Prev. Med., 5: 1281-1285.
33. Kamisan, F.H., F. Yahya, N.A. Ismail, S.S. Din and S.S. Mamat *et al*., 2013. Hepatoprotective activity of methanol extract of *Melastoma malabathricum* leaf in rats. J. Acupuncture Meridian Stud., 6: 52-55.
34. Durairaj, V., G. Shakya and R. Rajagopalan, 2014. Antihyperlipidemic effect of wheatgrass on alcohol and ΔPUFA induced liver toxicity in male albino Wistar rats. Int. J. Pharm. Pharm. Sci., 6: 547-551.
35. Oboh, G., A.J. Akinyemi, I.S. Oyeleye and K. Williamsnelson, 2016. Protective effect of phenolic extracts from two species of miracle berry leaves (*Thaumatococcus daniellii* and *Megaphrynium macrostachyum*) on some pro-oxidant induced oxidative stress in rat pancreas *in vitro*. J. Applied Pharm. Sci., 6: 118-124.