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Toxicity study of the aqueous extract of *Tithonia diversifolia* leaves using selected biochemical parameters in rats

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ABSTRACT
*Tithonia diversif*olia has manifold ethnomedicinal uses in traditional settings without much consideration about the possible adverse effects of the consumption of its crude extracts. In this study, effects of repeated oral administration of aqueous extract of *Tithonia diversifolia* leaves (100 and 200 mg/Kg body weight) for seven days on concentrations of serum electrolytes and biomolecules and the activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in serum, heart, liver and kidney of rats were investigated. The extract significantly increased concentrations of serum calcium ion, potasium ion and HDL-cholesterol but reduced serum albumin concentration at both doses administered compared to controls. At 200 mg/Kg body weight, the extract significantly increased alkaline phosphatase activities in the liver and heart. The results of this study suggest that the extract may exert adverse effects on the functions of the liver, heart and kidney.
Keywords: Extract, Heart, Kidney, Liver, *Tithonia diversif*olia, Toxicity

INTRODUCTION
Tithonia diversifolia (Hemsl) A. Gray is an impressive member of the sunflower family, Asteraceae. It is native to Central America and the West Indies, although it has become naturalized around the tropics. It serves various indigenous medicinal uses in many countries. In Nigeria, the decoctions of its various parts are used for the treatment of malaria, diabetes mellitus, sore throat, liver and menstrual pains (1-3). An oral decoction of the leaves and stem is used for the treatment of hepatitis in Taiwan and gastrointestinal disorders in Kenya and Thailand (4). In Costa Rica, the dried leaves are applied externally on wounds (5) while in Cameroon, an infusion of the leaves is used for the treatment of measles (6).

Some of these indigenous medicinal uses have been scientifically authenticated. *T. diversifolia* has been reported to exhibit analgesic and anti-inflammatory properties (2, 7). The antibacterial and antiplasmodial activities of the various parts of the plant have been demonstrated (1, 8-10). The plant has been reported to contain tagitinins A, B, C and F with diversifol, tirotundin, tithonine, and sulphurein (11-13). Tagitinin C, a sesquiterpene lactone, has been reported as the main antiplasmodial constituent of the plant (12).

Due to presumptive treatment of diseases by indigenous people, coupled with the wide use of this plant for the preparation of indigenous medicine, it is necessary to evaluate possible risks that the consumption of crude preparations of various parts of this plant may pose to the health of indigenous people. The present study has been aimed at evaluating the effect of repeated oral administration of the aqueous extract of *Tithonia diversifolia* leaves (the most common indigenous preparation of the plant in Nigeria) on some biochemical parameters in rats which could serve as indices of functions of specific organs.

MATERIALS AND METHODS

Animals and reagents
Fifteen adult male Wistar rats with an average weight of 150 g, obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, were used for this study. They were fed with rat pellet (Bendel Feeds Ltd, Ewu, Nigeria) and given water *ad libitum*. A twelve hour day and

night cycle and temperature of 25 ± 5 °C were maintained in the animal house. The assay kits for total cholesterol and HDL-cholesterol concentrations were obtained from Randox laboratories Ltd. (Co. Antrim, U.K) while all other reagents used for this study were of analytical grade and were prepared in all glass-distilled water.

Plant extract preparation

Tithonia diversifolia leaves were collected in Odo-Okun village, Ilorin, Kwara State, Nigeria. The leaves were air dried at room temperature (25 ± 5 °C) under shade after which they were pulverized into powder. The powder (50 g) was percolated in the 600 ml of distilled water for 48 hr after which it was filtered and evaporated into dryness at 60 °C in a water bath.

Extract administration

The animals were randomly divided into the following groups with 5 rats per group:

Group I: received an appropriate volume of sterile distilled water,

Group II: received the aqueous preparation of the extract (100 mg/kg body weight daily), and

Group III: received the aqueous preparation of the extract (200 mg/kg body weight daily).

The administration of the extract lasted for seven days.

Sample preparation

At the end of the experimental period, venous blood was collected from the experimental animals using the method of Narayanan *et al*. (14). The clotted blood was centrifuged at 3000rpm for 5 minutes (15) and a Pasteur pipette was used to collect the supernatant (i.e. the serum) which was stored frozen until needed for analysis. The heart, liver and kidneys of the animals were quickly isolated, cleaned of blood, weighed, suspended in ice-cold 0.25M sucrose solution (1:5 w/v) and homogenized. The homogenates were kept frozen overnight to ensure maximum release of the enzymes (16).

Biochemical assays

Concentrations of serum sodium and potassium ions were determined by flame photometry using the Jenway Clinical PFP7 flame photometer (17). Serum urea concentration was estimated by the diacetylmonoxime assay (18). Serum creatinine concentration was determined using Jaffe's reaction (19). Serum albumin concentration was estimated using the albumin-bromocresol green reaction method (20). Serum phosphate ion and calcium ion concentrations were determined by the methods of Goldenberg *et al*. (21) and Sarkar and Chauhan (22) respectively. Alkaline phosphatase (ALP) activity was determined by the method of Wright et al (23) while the activities of aspartate and alanine aminotransferases (AST and ALT respectively) were assayed as per Reitman and Frankel (24). Serum total cholesterol and HDL-cholesterol concentrations were determined by the methods of Frederickson *et al* (25) and Albers *et al* (26) respectively.

Statistical analysis

Data were analyzed using Duncan multiple range test following one-way analysis of variance (ANOVA) using SPSS 10.0 computer software package (SPSS Inc., Chicago, U.S.A). Differences at $P < 0.05$ were considered significant.

RESULTS

Serum electrolytes and biomolecules

The extract did not significantly alter ($P > 0.05$) serum concentrations of sodium ion, phosphate ion, urea, creatinine, and total cholesterol at both doses administered compared to controls (Tables 1 and 2). However, the extract significantly increased ($P < 0.05$) serum concentrations of calcium ion, potassium ion and HDL-cholesterol but significantly reduced ($P < 0.05$) serum albumin concentration at both administered doses compared to controls (Tables 1 and 2).

Enzyme activities

The two doses of extract administered in this study had no significant effect ($P > 0.05$) on Kidney ALP and serum AST and ALT activities while the dose of 200 mg/Kg body weight significantly increased ($P<0.05$) heart and liver ALP activities compared to controls (Table 3).

DISCUSSION

Increased serum HDL-cholesterol concentration without alteration in the total cholesterol concentration at both doses of *T. diversifolia* leaf extract suggest its likelyhood of protecting against diseases such as atherosclerosis. Since the plant is used to treat diabetes mellitus in folkmedicine, this may be one of the possible mechanisms by which it alleviates complications of diabetes e.g cardiovascular disease (27). However, the increased serum potassium ion concentration may be a threat to normal heart function. Increased serum potassium ion level may be attributed to kidney dysfunction possibly by a defective mechanism of tubular potassium excretion (28). This may lead to abnormal heart rhythms and impairment in the functions of smooth and skeletal muscles in various organs (28).

Increased serum calcium ion concentration in the

Table 1: *Effects of aqueous extract of T. diversifolia leaves on serum electrolytes*

Groups	Concentration (mmol/L)			
	Na^+	K^+	Ca^{2+}	PO_4^{3-}
Control	142.80 ± 1.24[a]	4.10 ± 0.11[a]	1.87 ± 0.06[a]	1.10 ± 0.13[a]
100 mg/Kg	141.80 ± 2.27[a]	4.72 ± 0.21[b]	2.13 ± 0.07[b]	0.82 ± 0.07[a]
200 mg/Kg	145.00 ± 1.52[a]	4.88 ± 0.06[b]	2.13 ± 0.07[b]	1.04 ± 0.08[a]

Values are mean ± SEM. Values in the same column with different alphabet superscripts are significantly different at P < 0.05.

Table 2: *Effects of aqueous extract of T. diversifolia leaves on serum biomolecules*

Groups	Urea Conc. (mmol/L)	Creatinine Conc. (µmol/L)	Albumin Conc. (g/L)	HDL-Chl Conc. (mmol/L)	Total Chl Conc. (mmol/L)
Control	8.44 ± 0.44[a]	69.80 ± 1.50[a]	31.60 ± 0.75[a]	0.800 ± 0.07[a]	2.38 ± 0.29[a]
100 mg/Kg	7.92 ± 0.50[a]	68.60 ± 3.22[a]	26.00 ± 1.52[b]	1.020 ± 0.07[b]	2.40 ± 0.21[a]
200 mg/Kg	8.80 ± 0.47[a]	76.80 ± 5.32[a]	26.00 ± 1.48[b]	1.080 ± 0.09[b]	2.32 ± 0.32[a]

Values are mean ± SEM. Values in the same column with different alphabet superscripts are significantly different at P < 0.05.
HDL-Chl = HDL- cholesterol ; Total Chl = Total cholesterol.

Table 3: *Effects of aqueous extract of T. diversifolia leaves on enzyme activities*

Groups	Enyme activities (IU/L)				
	Serum ALT	Serum AST	Heart ALP	Liver ALP	Kidney ALP
Control	36.20 ± 9.37[a]	109.80 ± 19.94[a]	57.75 ± 9.98[a]	53.75 ± 2.56[a]	1800.00 ± 197.87[a]
100 mg/Kg	29.80 ± 3.65[a]	81.40 ± 20.59[a]	73.00 ± 9.06[a]	53.00 ± 4.81[a]	1707.00 ± 316.48[a]
200 mg/Kg	40.20 ± 2.35[a]	93.00 ± 13.36[a]	86.75 ± 13.62[b]	103.50±17.00[b]	1781.50 ± 136.23[a]

Values are mean ± SEM. Values in the same column with different alphabet superscripts are significantly different at P < 0.05.

extract treated mice suggests increased intestinal absorption of calcium (29). This may result from increased conversion of vitamin D to the active form, 1,25-dihydroxyvitamin D3, which has been reported to be the primary hormone that mediates calcium absorption in the intestine (30). Vitamin D is first hydroxylated in the liver to 25- hydroxyvitamin D3 which is further hydroxylated in the kidney to 1, 25-hydroxyvitamin D3 by hydroxylases (31). 1, 25-dihydroxy vitamin D3 has been reported to stimulate the production of the transport protein necessary for calcium transport across the epithelium of the small intestine (31). Thus, the extract may possess the potential of causing an imbalance in homeostatic regulation of calcium ion in subjects. However, hypocalcemia is a common feature of malaria (32, 33). Ability of the extract to increase serum calcium level suggests one of the possible mechanisms by which it alleviates complications of malaria, being a disease for which it is also used in folkmedicine.

Reduction in serum albumin concentration by the extract suggests impairment in the role of the liver to synthsize the protein (20). Decrease in serum albumin level has adverse consequences. Albumin in conjunction with other plasma proteins (being large colloidal molecules) cannot diffuse through the thin capillary wall membranes as most other plasma solutes. Thus they are entrapped in the vascular system and exert a colloidal osmotic pressure, which serves to maintain a normal blood volume and normal water content in the interstitial fluid and the tissues (20). Albumin fraction is the most important in maintaining this normal colloidal osmotic or oncotic pressure in blood. Thus decrease in serum albumin concentration, if not checked, implies that water will diffuse from the blood vessels and enter interstitial fluid and the tissues, leading to accumulation of water in such tissues (20).

Alkaline phosphatase, alanine and aspartate aminotransferases in tissues and blood are important marker enzymes which are used to assess the integrity of the cell membrane, cytosolic activity and cell death (34, 35). Increased ALP activities in the heart and liver suggests the possibility of the extract causing membrane damage in these organs at higher concentrations or longer period of exposure of the animals to the extract than those used in this study, though there was no alteration in serum AST and ALT activities at the concentrations used in this study. This observation is reflective of the response of the cellular systems to offset the stress imposed on the enzyme by exposure to the extract which may result from the inhibition of the enzyme activity *in situ* (36). Increased ALP activity is needed during stress to produce adequate amount of phosphate groups for oxidative phosphorylation to generate ATP which, in turn, is required for the phosphorylation of some biomolecules like ethanolamine and choline to form phosphatidyl ethanolamine and phosphatidyl choline, which are vital phospholipid components of the plasma membrane (37), thereby trying to stabilize the integrity of the membrane.

Presumptive treatment is common in traditional medicine practice. Indiscriminate consumption of the crude extracts of *T. diversifolia* leaves may cause grave consequences. The results of the present study suggest that the administration of aqueous extract of *T. diversifolia* leaves may adversely affect liver, heart and kidney functions. Thus indiscriminate use of the plant should be discouraged.

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REFERENCES

1. T. O. Elufioye and J. M. Agbedahunsi. Antimalarial activities of Tithonia diversifolia (Asteraceae) and Crossopteryx febrifuga (Rubiaceae) on mice in vivo. *J Ethnopharmacol*. **93 (2-3):** 167-171 (2004).
2. V. B. Owoyele, C. O. Wuraola, A. O. Soladoye and S. B. Olaleye. Studies on the anti-inflammatory and analgesic properties of Tithonia diversifolia leaf extract. *J Ethnopharmacol*. **90(2-3):** 317-321 (2004).
3. D.O. Moronkola, I. A. Ogunwande, T. M. Walker, W. N. Setzer and I. O. Oyewole. Identification of the main volatile compounds in the leaf and flower of *Tithonia diversifolia* (Hemsl) Gray. J Nat Med. **61:** 63-66 (2007).
4. T. Johns, G. M. Faubert, J.O. Kokwaro, R.L.A. Mahunnah and E.K. Kimanani. Anti-giardial activity of gastrointestinal remedies of the LUO of East Africa. *J Ethnopharmacol*. **46:** 17 – 23 (1995).
5. Kuo YH, Chen CH (1997). Diversifolol, a novel rearranged Eudesmane sesquiterpenes from the leaves of *Tithonia diversifolia*. *Chem Pharm Bull.* **45:** 1223 – 1224.
6. L. Kamdem, H.M. Messi, N.A. Ndongo, C. Mbi, A.P. Njikam, and S. Elobo. Ethnobotanical investigations carried out in Mouloundon (Eastern province) and Zoetele (Southern province). *Rev Sci Technol.* (Health SCI SER), **3/4:** 59 – 68 (1986).
7. P. Rungeler, G. Lyss, V. Castro, G. Mora, H.L. Pahl and I. Merfort. Study of three sesquiterpene lactones from *Tithonia diversifolia* on their anti-inflammatory activity using the transcription factor NF-kappa B and enzymes of the arachidonic acid pathway as targets. *Planta Med.* **64:** 588 – 593 (1998).
8. .M. Bork, M.C. Schmitz, C. Weimann, M. Kist, M. Heinrich. Nahua Indian medicinal plants (Mexico): Inhibitory activity on NF-kb as an anti-inflammatory model and antibacterial effects. *Phytomed.* **3:** 263 – 269 (1996).
9. M.C. Madureira, A.P. Martins, M. Gomes, J. Paiva, A.P. Cunha and V. Rosario. Antimalarial activity of medicinal plants used in traditional medicine in S. Tome and Principe islands. *J Ethnopharmacol.* **81:** 23 – 29 (2002).
10. C. A. Obafemi, T. O. Sulaimon, D. A. Akinpelu and T. A. Olugbade. Antimicrobial activity of extracts and a germacranolide-type sesquiterpene lactone from *Tithonia diversifolia* leaf extract. *Afr J Biotechnol*. **5 (12):** 1254-1258 (2006).
11. N.C.Baruah, R.P. Sharman, K.P. Madhusudanan and G. Thyagarajan. Sesquiterpene lactones of *Tithonia diversifolia* stereochemistry of the tagitinins and related compounds. *J Org Chem.* **44:** 1831–1835 (1979).
12. E. Goffin, E. Ziemons, P. De Mol, M. de Céu De Madureira, A.P. Martins, A.P. Da Cunha, G. Philippe, M. Tits, L. Angenot and M. Fredrich. *In vitro* antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: Tagitinin C. Planta Medica 68, 543–545 (2002).
13. J.Q. Gu, J.J. Gills, E.J. Park, E. Mata-Greenwood, M.E. Hawthorne, F. Axelrod, P.I. Charez, H.H. Fong, R.G. Methta, J.M. Pezzuto, J. Kinghor. Sesquiterpenes from *Tithonia diversifolia* with potential cancer chemopreventive activity. *J Nat Prod.* 65: 532 – 536 (2002).
14. C.R. Narayanan, D.D. Joshi and A.M. Mujumdar. Hypoglycemic action of *Bougainvillea spectabilis* leaves*. Curr Sci.* **53:** 579-581 (1984).
15. S.I. Ogbu, and E.I. Okechukwu. The effect of storage temperature prior to separation on plasma and serum potassium. *J. Medical Lab. Sci.***10:** 1-4, (2001).
16. E.O. Ngaha, M.A. Akanji, and M.A. Madusolomo. Studies on correlation between chloroquine –induced tissue damage and serum changes in rats. *Experimentia*. **45:** 143 (1989).
17. N.W. Tietz, E.L. Pruden and O. Siggaard–Anderson. In: G. A. Burtis and E. R. Ashwood eds. Tietz textbook of Clinical Chemistry. W.B. Saunders Company, London; 1354-1374 (1994).
18. M.P. Veniamin and C. Vakirtzi–Lemonias. Chemical basis of the carbamidodiacetyl micro-method for estimation of urea, citrulline and carbamyl derivatives. *Clin Chem.* **16:** 3-6 (1970).
19. G.H. Cook. Factors influencing the assay of creatinine. *Ann Clin Bioch.* **12:** 219-232 (1975).
20. G.H. Grant and J.F. Kachmar. In: N.W. Tietz ed. Fundamental of clinical Chemistry", 3rd Edition. W.B. Saunders Company, Philadelphia; 298- 320 (1987).
21. H. Goldenberg and A. Fernandez. Simplified method for the estimation of inorganic phosphorus in body fluids. ***Clin Chem.* 12(12):** 871-882 (1966).

22. B.C. Sarkar and U.P. Chauhan. A new method for determining micro quantities of calcium in biological materials. *Anal Biochem.* **20(1):** 155-166 (1967).
23. P.J. Wright, P.D. Leathwood and D.T. Plummer. Enzymes in rat urine. Alkaline phosphatase. *Enzymologia*. **42:** 317-327 (1972).
24. S. Reitman and S. Frankel. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Pathol.* **28:** 56-63 (1957).
25. D.S. Fredrick, R. I. Levy and R. S. Lees. Fat transport in lipoproteins – An integrated approach to mechanisms and disorders. *N. Engl J Med.* **276:**148 – 156 (1967).
26. J.J. Albers, G.R. Warmick and M.C. Cheung. Quantitation of high density lipoproteins. *Lipids.***13:** 926-932 (1978).
27. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA*. **241(19):** 2035-2038 (1979).
28 P. Kes . Hyperkalemia: a potentially lethal clinical condition. *Acta clin Croat.* **40:** 215-225 (2001).
29. B.G. Katzung, *Basic and clinical pharmacology,* (McGraw-Hill Medical Publishing Division, New York, 2006); 706.
30. J.S. Garrow, W.P.T. James and A. Ralph. *Human nutrition and dietetics,* (Churchill Livingstone, Edinburgh, 2002).
31. H.M. Baker. *Nutrition and dietetics for health care*, (Churchill Livingstone, Edinburgh, 2002) 28.
32. M.R. Prabha , P. Pereira, N. Chowta and B.M. Hegde. Clinical implications of hypocalcemia in malaria. *Indian J Med Res.* **108:** 62-65 (1998).
33. A. St**.** John, T.M. Davis, T.Q. Binh, L.T. Thu, J.R. Dyer and T.K. Anh. Mineral homoeostasis in acute renal failure complicating severe falciparum malaria. *J Clin Endocrinol Metab*. **80:** 2761-2767 (1995).
34. M.A. Akanji, O.A. Olagoke, and O.B Oloyede. Effect of chronic consumption of metabisulphite on the integrity of rat kidney cellular system. *Toxicol.* **81:** 173-179 (1993).
35. P.J. Wright, and D.T. Plummer. The use of urinary enzyme measurement to detect renal damage caused by nephrotoxic compounds. *Biochem. Pharmacol.* **23:** 65-73 (1974).
36. S. O. Malomo, A.S. Daramola, and E.A. Balogun. Some serum and tissue enzyme changes in mice infected with *Plasmodium yoelii nigeriensis* before and after administration of halofantrine hydrochloride. *Nig J Biochem Mol Biol.* **10:** 71-77 (1995).
37. A.L. Lehninger. *Principles of biochemistry,* (Worth Publishers Inc., USA,1982) 318.

