

The Toxicological Effects of Aqueous Leaf Extract of *Tithonia diversifolia* Gray in Rats

J.O. Fakunle and M.O. Abatan

Department of Veterinary Physiology and Pharmacology,
Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: The toxicological effects of *Tithonia diversifolia* Gray was evaluated in albino rats using crude aqueous extract administered orally for 14 days. Changes observed in haematological parameters, serum biochemical parameters and histopathological reports were used as indices for toxicosis. The aqueous extract of *T. diversifolia* caused statistically significant ($p < 0.05$) reductions in Packed Cell Volume (PCV), Total Red Blood Cell count (TRBC) and Haemoglobin (Hb) levels at the 200 mg kg⁻¹ b.w. treatment group. It also caused a statistically significant ($p < 0.05$) increase in MCV and MCHC values in both 100 and 200 mg kg⁻¹ b.w. treatment groups. This suggests that this plant may cause macrocytic hyperchromic anaemia in animals consuming them. The serum biochemistry revealed that the aqueous crude extract of *T. diversifolia* at the dosage of 100 and 200 mg kg⁻¹ b.w. caused a statistically significant ($p < 0.05$), increase in Alkaline Phosphatase (ALP), Alanine amino Transferase (ALT) and Aspartate amino Transferase (AST) levels. This may be indicative of hepatocellular damage which is further confirmed by a statistically significant ($p < 0.05$) increase in the Total Bilirubin (TB), reflected in the histopathologic lesions of multifocal vacuolar degeneration, necrosis and thinning of the hepatic cord in the centrilobular region. The marked congestion of the renal capillaries with diffuse degeneration and necrosis of the tubular epithelium, also with focal area of perivascular lymphocytic infiltration observed in the histopathology of the kidney can be explained with the statistically significant ($p < 0.05$) increase observed in the values of Total Protein (TP) and Albumin (ALB).

Key words: *Tithonia diversifolia*, toxicity, extract, rats

INTRODUCTION

Tithonia diversifolia is a plant that has been commonly used for diverse medicinal purposes in most parts of the world. The antiplasmodial activity of *T. diversifolia* was reported by (Goffin *et al.*, 2002) who isolated lactone targitin C showing activity against plasmodium organism and that it is also cytotoxic (Madureira *et al.*, 2002) reported the activity of *T. diversifolia* against chloroquine resistant *Plasmodium falciparum* and also reported the hepatic schizontocidal activity (Manobjyoti *et al.*, 1996) reported the artemisinin structure of the mature stem of *T. diversifolia*.

Bork *et al.* (1996) observed that *T. diversifolia* has an anti-inflammatory and antibacterial activity and this was further confirmed by the work of (Rungeler *et al.*, 1998). Tona *et al.* (1998, 1999) observed the antibacterial and antiamebic properties of the aqueous extract of *T. diversifolia*.

Hamowia and Saffaf (1994), corroborated by the work of (Jayawardena *et al.*, 2000), reported that the development and hatchability of gastrointestinal

nematode eggs in goat faeces were reduced with increasing concentrations of *T. diversifolia* leaf extract.

Hamowia and Saffaf (1994) further reported on the antitumor activity of *T. diversifolia*. Gu *et al.* (2002) reported that sesquiterpenoids from *T. diversifolia* have potential cancer chemopreventive activity (Miura *et al.*, 2002) also reported that it exhibits antidiabetic effects as its aqueous extract improves the blood glucose level of KK-Ay mice by reducing insulin resistance.

Considering the numerous medicinal uses of this plant, this study is aimed at determining if there is any toxic potential in the plant *T. diversifolia*.

MATERIALS AND METHODS

The animals used in this study were twenty-four healthy rats of the wistar albino strain, ages between 12-16 weeks and weighing between 150-250 g and are of both sexes. They were maintained at the Experimental Animal House of The Faculty of Veterinary Medicine, University of Ibadan. They were kept in metal rat cages

and fed rat cubes (Guinea Feeds Nigeria Limited) and allowed free access to clean fresh water *ad libitum*.

Preparation of leaf extracts and administration to rats:

The leaves of the *T. diversifolia* Gray was collected from the Faculty of Agriculture area, at the University of Ibadan in September 2003 and identification made with the Department of Botany of University of Ibadan, Ibadan, Nigeria.

The leaves were harvested freshly for the preparation of the extract daily. One hundred gram of the plant leaves were weighed and macerated using an electric blender (Philip). One thousand milliliters of clean water was added to ensure proper maceration. Thereafter, the solution was filtered using filter paper to produce aqueous crude extract of *T. diversifolia*.

The animals were divided into four groups A, B, C and D, each consisting of 6 animals per group which were administered with doses of 100, 200 and 400 mg kg⁻¹, respectively with the last group D on control given ordinary water. The fresh sample of the aqueous extract of *T. diversifolia* Gray were administered to the animals orally using stomach canula on a daily basis for 14 days.

Sample collection and processing: Blood was collected by cardiac puncture from diethylether anaesthetized rats, into heparinized and non-heparinized (for serum collection) bottles for haematological and serum biochemical studies respectively. For the haematological studies the PCV was done according to the method described by Schalm *et al.* (1975), Rbc by the haematocytometry method as described by Jain (1986) and the erythrocyte indices were obtained by calculation.

Total Protein (TP) was analysed by the Biuret reaction (Gornall *et al.*, 1949), the bilirubin by diazo reaction (Jendrassik and Goff, 1938; Nosslin, 1960; Michealson, 1961); Alkaline Phosphatase (ALP), Aspartate amino Transferase (AST) and alanine amino transferase were determined according to the improved methods by Sigma (1985).

Histopathological technique: Samples from the Liver, spleen, kidney and lung were isolated and fixed in 10% buffered formalin and then dehydrated in ethanol (70-100%), cleared in xylene and embedded in paraffin.

Tissue sections were examined under a light microscope after staining with Haematoxylin and Eosin (H&E) dye (Culling, 1963; Lillie, 1965).

Statistical analysis: Results are expressed as the mean±standard error of the mean. Significant differences between means were determined by the student' t-test (Bradford, 1991; Bailey, 1992).

RESULTS

Effects of crude extract of *T. diversifolia* gray on haematological parameters of rats: The aqueous extract of *T. diversifolia* at the dosage of 200 mg kg⁻¹ b.w. caused significant (p<0.05) reduction in the Packed Cell Volume (PCV) and Haemoglobin (Hb) concentration (Plate 1).

It also caused a statistically significant (p<0.05) reduction in RBC count at 200 mg kg⁻¹ b.w. dosage (Table 1).

The result of the erythrocyte indices showed that *T. diversifolia* extract at 100 and 400 mg kg⁻¹ doses caused a statistically significant (p<0.05) increase in the values of MCV (Table 1).

Effects of crude extract of *T. diversifolia* on serum biochemistry of rats: The aqueous crude extract of *T. diversifolia* caused a statistically significant (p<0.05) increase in the levels of serum Total Proteins (TP) and Albumin (ALB) at the dosage of 400 mg kg⁻¹ b.w.



Plate 1: *Tithonia diversifolia* plant

Table 1: Effects of *T. diversifolia* on haematological parameters of rats (Mean±standard error of the mean)

Dosages	PCV	HB	RBC	MCV	MCH	MCHC	WBC	LYMP	NEUT
100	32.5±0.4	10.6±0.2	4.30±0.9	81.0±22*	26.5±7.5	32.5±0.5*	10.80±0.1	85.0±4.1	15.0±7.1
200	26.5±12.7*	8.4±4.4*	3.70±1.2*	66.0±15	20.5±6.5	32.5±0.5*	8.40±0.4	66.0±3.7	34.5±3.7
400	34.0±4.9	10.7±1.3	4.53±0.8	78.7±20*	25.0±4.5	31.7±0.8	11.80±1.4	75.5±9.6	24.0±9.3
Control	37.3±1.5	12.0±0.4	5.70±0.4	66.3±4.7	21.3±1.6	32.3±0.4	9.35±1.4	74.3±3.8	25.7±3.9

PCV = Packed Cell Volume (%), Hb = Haemoglobin concentration (g dL⁻¹), RBC = Red Blood Cell (×10⁶ mm⁻³), WBC = White Blood Cell (×10³ mm⁻³), LYMP = Lymphocyte, NEUT = Neutrophil, MCV = Mean Corpuscular Volume (fl), MCH = Mean Corpuscular Haemoglobin (pg), MCHC = Mean Corpuscular Haemoglobin Concentration (%), * = Superscripted figures are statistically significant at p<0.05

Table 2: Effects of *T. diversifolia* on serum biochemistry of rats (mean±standard error of mean)

Dosages	TB	ALP	AST	ALT	TP	ALB	TRIG	UREA	GLUC
100	0.56±0.1	675.0±58	301.0±31*	116.0±4.8	6.86±0.4	2.83±0.2	129.00±20	52.00±13	79±12
400	0.60±0.1*	981.0±2.8*	268.0±7.4	125.0±3.7*	7.95±0.3*	2.85±0.2*	99.50±14	72.00±3.3	86±22.5
Control	0.47±0.03	490.0±113	220.3±14	110.7±7.2	6.53±0.4	2.53±0.1	97.67±7.8	52.67±7.8	78±19.0

TB = Total bilirubin ($\mu\text{m L}^{-1}$), ALP = Alkaline Phosphatase (U L^{-1}), AST = Aspartate aminotransferase (U L^{-1}), ALB = Albumin (g dL^{-1}), TP = Total Protein (g dL^{-1}), GLUC = Glucose (mg dL^{-1}), TRIG = Triglycende (mg dL^{-1}), UREA = Urea (millimole L^{-1}), * = Superscripted figures are significant

It also caused significant ($p<0.05$) increase in values of serum enzymes ALP at the dosage of $400 \text{ mg kg}^{-1} \text{ b.w.}$, while the $100 \text{ mg kg}^{-1} \text{ b.w.}$ dose level produce a statistically significant ($p<0.05$) increase in the value of serum enzyme AST. The $400 \text{ mg kg}^{-1} \text{ b.w.}$ dose level produce a statistically significant ($p<0.05$) increase in the serum total bilirubin level (Table 2).

DISCUSSION

In this study, the aqueous crude extract of *T. diversifolia* caused a statistically significant ($p<0.05$) reductions in the Packed Cell Volume (PCV), Haemoglobin concentration (Hb) and Red Blood Cell count (RBC). This implies that the aqueous crude extract of *T. diversifolia* has adverse effects on the erythron of rats and may cause anaemia which is macrocytic hyperchromic. This form of anaemia is normally regenerative (Guyton and Hall, 1994).

The aqueous crude extract of *T. diversifolia* caused an elevation in the levels of the serum biochemical parameters ALP, AST, ALT and bilirubin. The elevated serum bilirubin level is an indication of hepatocellular damage.

Obstruction to flow of bile as a result of the occlusion of the sinusoids due to swelling of the hepatocytes are reported to cause a rise in the level of ALP (Kaneko, 1980; Duncan *et al.*, 1994). Coppock *et al.* (1989) reported that ALP level increases in toxic liver failure.

The increase observed in the serum ALT level may indicate hepatic necrosis (Cornelius, 1989).

The hepatotoxic nature of *T. diversifolia* is further confirmed by the generalized hepatocellular necrosis observed in the histopathological lesions (Fig. 1).

The aqueous crude extract of *T. diversifolia* also caused a statistically significant ($p<0.05$) increase in the level of serum Total Protein (TP) and Albumin (ALB) (Table 2).

This may indicate an incompetent ability of the kidney and this is further confirmed by the histopathology of the kidney that reveals the diffuse degeneration and necrosis of the tubular epithelia cells of the kidney (Fig. 2).

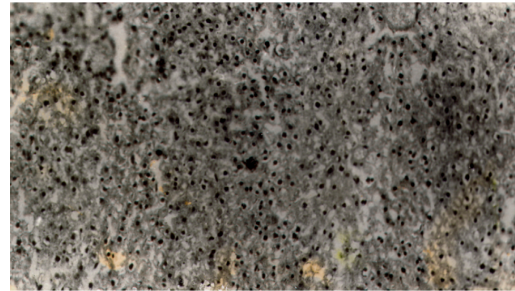


Fig. 1: Section of the liver showing massive generalized coagulative necrosis (H&E stain. Mag. X 16). Effect of crude extract of *T. diversifolia* at $400 \text{ mg kg}^{-1} \text{ b.w}$

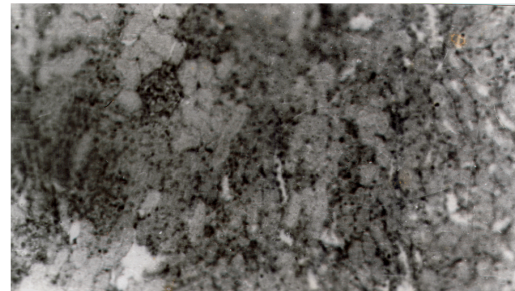


Fig. 2: Section of the kidney showing diffuse degeneration and necrosis of the tubular epithelial cells. There is also pale cytoplasm and karyolytic nuclei (H&E stain. Mag X16). Effect of crude extract of *T. diversifolia* at $400 \text{ mg kg}^{-1} \text{ b.w}$

CONCLUSION

It is thereby deduced from the above that despite numerous therapeutic claims of various medicinal usages of the plant, *T. diversifolia* has very significant toxic potential that should not be ignored.

REFERENCES

- Bailey, N.T., 1992. Statistical Methods in Biology. 2nd Edn. Cambridge University Press, Cambridge.

- Bork, P.M., M.L. Schmitz, Weimann, M. Kist and M. Heinrich, 1996. Ethnobotanical Study of Anti-Inflammatory and Antibacterial Activity in Some Selected Plants. *Phytomedicine*, 3: 263-269.
- Bradford, H.A. and I.D. Hill, 1991. Principles of Medical Statistics. 12th Edn. Edward Arnold, London.
- Coppock, R., J. Reynolds and W.B. Buck, 1989. Acute Aflatoxicosis in Feeder pigs resulting from Improper Storage of Corn. *J. Am. Vet. Med. Assoc.*, 195: 1380-1381.
- Cornelius, C.E., 1989. Serum Enzyme Activities and Other Markers for Detecting Hepatic Necrosis, Cholestasis or Carcinoma. In: *Clinical Bioch. Dom. Anim.* 4th Edn. Academy Press, pp: 381-386.
- Culling, C.F.A., 1963. Handbook of Histopathological Techniques (Including Museum Technique). 2nd Edn. Butterworth and Co., London.
- Duncan, J.R., K.W. Prasse and E.A. Mahaffey, 1994. *Vet. Lab. Med. (Clin. Path)* 3rd Edn. Iowa State Univ. Press.
- Goffin, E., E. Ziemons, De Mol, P. de Madureira, C. Mdo, A.P. Martins, A.P. da Cunha, G. Philippe, M. Tits, L. Angenot and M. Frederich, 2002. *In vitro* antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent; targitinin C. *Planta Med.*, 68: 543-545.
- Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of Serum protein by means of biuret reagent. *J. Biol. Chem.*, 177: 751-755.
- Gu, J.Q., J.J. Gills, E.J. Park, E.J. Mata-Greenwood, E. Greenwood, M.E. Hawthorne, F. Axelrod, P.I. Chavez, H.H.S. Fong, R.G. Mehta, J.M. Pezzuto and A.D. Kinghorn, 2002. Sesquiterpenoids from *Tithonia divers.* To lia with Potential cancer Chemo preventive activity. *J. Nat. Product.*, 65: 532-536.
- Guyton, A.C. and J.E. Hall, 1994. *Textbook of Medical Physiology*. 9th Edn. W.B. Saunders Co., Philadelphia.
- Hamowia, A.M. and A.M. Saffaf, 1994. Pharmacological studies of *vernonia amygdalina* (Del) and *Tithonia diversitolia* (gray). *Vet. Med. J. Giza*, 42: 91-97.
- Jain, N.C., 1986. *Schalm's Veterinary Haematology*. 4th Edn. Lea and Fabiger, Philadelphia, pp: 564-575.
- Jayawardena, V.P., A.S.B. Rajaguru and H.P.M. Gunasena, 2000. Nutritive and Medicinal Values of *Erythrina* and *Tithonia* sp. Proceedings of the Eleventh National Workshop on Multipurpose Trees, Kandy, Sri-Lanka, pp: 72-77.
- Jendrassik, L. and P. Goff, 1938. Vere infacilte photometrische Methoden zur bestimmung des blut bilirubins. *Biochemistry*, 297: 81-84.
- Kaneko, J.J., 1980. Serum Proteins and the Dysproteinemias. In: Kaneko, J.J. (Ed.). *Clin. Bioch. of Dom. Animals*. 3rd Edn. Orland, Fla. American Press.
- Lillie, R.D., 1965. *Histopathologic Technic and Practical Histochemistry*. 3rd Edn. Blakiston Division, McGraw-Hill Book Co., New York.
- Madureira Mdo, C., A.P. Martins, M. Gomes, J. Paiva, A.P. Cunha and V. Rosario, 2002. Antimalaria Activity of Medicinal plants used in traditional Medicine in S. Tono and Principe Islands. *J. Ethnopharm.*, 81: 23-29.
- Manobjyoti, B., N.C. Barua, A.C. Ghosh and M. Bordoloi, 1996. An artemisinic acid analogue from *Tithonia diversifolia*. *Phytochemistry*, 41: 557-559.
- Michealson, M., 1961. Bilirubin determination in serum and Urine Scand. *J. Clin. Lab. Invest. (Suppl. 56)*, 13: 1.
- Miura, T., K. Furuta, A. Yasuda, N. Iwamoto, M. Kato, E. Ishihara, T. Ishida and K. Tanigawa, 2002. Antidiabetic Effects of Nitobeniku in KK-Ay Diabetic mice. *Am. J. Chiese Med.*, 30: 81-86.
- Nosslin, B., 1960. The direct diazo reaction of Bile pigments in serum scand. *J. Clin. Lab. Invest. (Suppl. 49)*: 12-14.
- Rungeler, P., G. Lyee, V. Castro, G. Mora, H.L. Pahl and I. Merfort, 1988. study of three sesquiterpene Lactones from *Tithonia diversitolia* on their antinflammatoty activity. *Plant Med.*, 64: 588-593.
- Schalm, O.W., N.C. Jain and E.J. Carroll, 1975. *Veterinary Haematology*. 3rd Edn. Lea and Febiger, Philadelphia, pp: 221-227.
- Sigma, D., 1985. *Quantitative Colorimetric Determination in Serum, Plasma or Cerebrospinal Fluid (Procedure No. 505)*.
- Tona, I., K. Kambu, N. Ngimbi, K. Cimanga and A.J. Vlietinck, 1998. Antiamoebic and Phytochemical, Screening of some Congolese Medicinal Plants. *J. Ethnopharmacol.*, 61: 57-65.
- Tona, I., K. Kambu, K. Mesia, K. Cimanga, S. Apers, T. De Bruyne, L. Pieters, J. Tottle and A.J. Vlietinck, 1999. Biological Screening of traditional Preparations from Some Medicinal Plants used as Antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*, 6: 59-66.
- Smith, B.P., 1996. *Large Animal Internal Medicine*. 2nd Edn. Baltimore, Mosby Yagi, A. and H. Haraguchi, 1997. Protective effects of terpenoids against oxidative stresses. *Recent Research Development in Phytochemistry*, Gakuen-cho, Fukuyama, 1: 11-24.