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An Evaluation of the Toxic Effects of *Tamarindus indica* Pulp Extract in Albino Rats

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Abstract: The effects of graded doses of *Tamarindus indica* pulp extract on the haematological, toxicological and histopathological indices in rats were evaluated. Hematological parameter was determined using micro-hematocrit method and Neubauer hemocytometer counting chamber. The haematological parameters (PCV, WBC, Lymphocytes and Monocytes) of the treated groups showed no significant difference ($p<0.05$) when compared with the control. Although there was a significant increase ($p<0.05$) ($p<0.01$) in the nuetrophils in group 3 and 4 and decrease ($p<0.05$) ($p<0.01$) in eosinophils at group 4 and 5, respectively. There was no fatality recorded in the acute toxicity tests when the animals received 900-4500 mg kg^{-1} body weight of the extract, however the higher doses of the extract (2700-4500 mg kg^{-1} body weight) exhibited some behavioral changes in the rats such as aggressive scratching of the body and mouth part, anorexia, mild restlessness and sensitive to slight sound. There was no significant difference ($p<0.05$) in the toxicological parameters investigated when compared with the control. The gastro intestinal tract revealed no apparent congestion or hemorrhage while the histopathological examination of liver and kidney showed no visible lesions indicating its non toxic effect to these organs. These results have provided scientific evidence to justify the safety of this plant in tradition medicine.

Key words: *Tamarindus indica*, pulp extract, rats, acute oral toxicity

INTRODUCTION

The orthodox medicine, as currently made available today in Nigeria (as in most African countries), so long as every nook and corner of our rural populations in Africa cannot yet be provided with basic health care needs including full-time resident medical personnel and readily available and affordable drugs, has failed woefully (Elujoba *et al*., 2005). Although wherever, modern health facilities exist, traditional medicine is incomparable. Therefore, the most workable health agenda for Africa is the institutionalization of traditional medicine in parallel (not in complete fusion) with orthodox medicine, within the national health care scheme in order to move the health agenda forward. Effective health agenda for the African continent can never be achieved by orthodox medicine alone unless it is complemented by traditional medicine practice (Elujoba *et al*., 2005).

According to the World Health Organisation (WHO) estimates, about 80% of the world population today relies chiefly on traditional medicines for their primary health care needs and that over 30% of the world plant species have at one time or the other been used for one medicinal purpose or the other for our primary care needs (Akerele *et al*., 1991). Moreover, the increasing use of plant

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extracts in food, cosmetic and pharmaceutical industries demands toxicity risk assessment of various indigenous plant preparations used in the treatment of diseases (Yakubu *et al*., 2005) and suggests that a systemic study of medicinal plants is very important (Nostro *et al*., 2005).

Tamarindus indica pulp is being used among the food ingredients of a popular *Hausa* food in the northwestern part of Nigeria. The plant has also been reported to be among the recipe in hausa folkloric medicine in treatments of cold, fever, stomach disorder, diarrhea and jaundice and as skin cleanser (Doughari, 2006). It is popularly employed on a daily base in the general traditional medicine practice as a drug conveyor, in combination with other herb for treatment of various diseases such as indigestion, constipation. In the native practice, *Tamarindus indica* is applied on inflammations, used as a gargle for sore throat and mixed with salt as a liniment for rheumatism, accelerate expulsion, relieve pains, reduce secondary bacterial infections and promote healing (Fabiyi *et al*., 1993).

The extensive use of this medicinal plant in treatment of various ailments has made it necessary to investigate its toxicity. The present study was therefore undertaken to evaluate, using biochemical and toxicity test, the risk assessments of the aqueous pulp extract of *Tamarindus indica*.

MATERIALS AND METHODS

Collection of Plant Material

Tamarindus indica pulp was obtained around the wild of Sokoto south local government area of Sokoto state, Nigeria. The pulp was identified by the herbarium and a voucher specimen was deposited in the Department of Botany of the Usmanu Danfodiyo University, Sokoto-Nigeria.

Preparation of Extract

The aqueous pulp extract of *Tamarindus indica* was prepared by extraction with hot water in order to simulate the local procedure as described by Akinyole and Olorede (2000). *T. indica* pulp (400 g) was soaked in 2 L of distilled water and boiled for 5 min. This hot decoction was shaken for 10 min and allowed to cool then filtered using Whatman filter paper No. 1. The filtrate was then evaporated under *vacuo* to a brownish gummy residue and stored at 4°C until used. The percentage yield was 25.2% w/w. This portion is referred to as the extract.

Experimental Animal Model

Adult albino rats weighing 201.6±65 g body weight (b.w) were obtained from the Animal Farmhouse of the Zoology Department of Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were allowed acclimatize for seven days having access to water and food *ad libitum*. They were randomized to one of the following experimental groups, with five animals per group:

Group 1: Normal rats chow and distilled water only (control group).
Group 2: Normal rats chow and 900 mg kg^{-1} body weight of the extract.
Group 3: Normal rats chow and 1800 mg kg^{-1} body weight of the extract.
Group 4: Normal rats chow and 2700 mg kg^{-1} body weight of the extract.
Group 5: Normal rats chow and 3600 mg kg^{-1} body weight of the extract.
Group 6: Normal rats chow and 4500 mg kg^{-1} body weight of the extract.

This research was carried out in the Small Animal Laboratory of Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria in June/July 2006.

Sub-Acute Toxicity Studies

The rats were treated orally with pulp extract with the aid of a blunt end cannula daily for 28 days. The control group was given distilled water of corresponding volume with the largest volume

of the extract administered. They were allowed free access to feed and water. They were observed for acute toxicity signs like behavioral changes and death over 24 h period.

Blood Analysis

Blood samples were collected via cardiac punctured and transferred into two sample bottles one with anticoagulant for haematological analysis and the other in plain labeled dry sample bottles for biochemical analysis.

Markers/Indices of Toxicity

Aspartate Amino Transferase (AST)

AST was measured according to the method of Reitman and Frankel (1957) as described by Sini *et al*. (2006) by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine. The maximum absorbance at 546 nm is proportional to the concentration of AST in the sample.

Alanine Amino Transferase (ALT)

ALT was measured according to the method of Reitman and Frankel (1957) as described by Sini *et al*. (2006) by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl hydrazine which absorbs maximally at 546 nm with the absorbance proportional to the concentration of ALT in the sample.

Alkaline Phosphatase Determination

Alkaline phosphatase was determined according to the method of King and Armstrong (1980). Alkaline phosphatase is measured by monitoring the concentration of phosphate formed with p-nitro phenyl phosphate that absorbs maximally at 405 nm.

Bilirubin Determination

The colorimetric method of Sini *et al*. (2006) was adopted. Direct (conjugated) Bilirubin reacts with diazotised sulphanic acid in alkaline medium to form a blue color complex with maximum absorbtion at 546 nm. The intensity of the color is proportional to the conjugated Bilirubin in the sample. The total Bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotised sulphanic acid that absorbs maximally at 578 nm. The absorbance is proportional to the concentration of total bilirubin in the sample.

Albumin Determination

This was determined according to the method of Grant (1987) based on the quantitative binding to the indicator bromocresol (BCG). The albumin-BCG-complex absorbs maximally at 578 nm the absorbance is directly proportional to the concentration of albumin in the sample.

Total Protein Determination

This was determined using the method of Kumar *et al*. (2004). Cupric ion reacts with proteins in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein in the sample.

Haematological Parameters Determination

The determination of Pack Cell Volume (PCV) was by the micro-hematocrit method, Whole Blood Count (WBC), lymphocytes, nuetrophils, monocytes, eosinophils and basophils were determined using improved Neubauer hemocytometer counting chamber as described by Dacie and Lewis (1991).

Histopathological Studies

Histopathology examinations were carried out on the liver (site for biotransformation) and kidney (site for excretion) from the rats. They were fixed in 10% formalin solution. Thin cryostat sections were stained and examined under light microscope at high (x 400 objective) power magnification.

Statistics

Data collected in the study were expressed as means±SEM and subjected to analysis of variance (ANOVA) for accessing statistical significance.

RESULTS

Effects of Aqueous Pulp Extract of *T. indica* on Hematological Indices

Table1 shows the effects of the pulp extract haematological indices. There was no significant difference in the haematological parameters of the treated such as PCV, WBC, Lymphocytes and Monocytes of all the different concentrations of the extract administered as compared with the control. Although there was a significant increase in nuetrophils in group 3 ($p<0.05$) and group 4 ($p<0.01$) while decrease in eosinophils at group 4 ($p<0.05$) and group 5 ($p<0.01$).

Effects of Aqueous Pulp Extract of *T. indica* on Toxicity Markers

The results of the sub-acute toxicity study of *Tamarindus indica* pulp extract shown in Table 2 indicates no fatality in the acute toxicity tests in animals given 900-4500 mg kg^{-1} body weight of the extract, although the rats given higher doses (2700-4500 mg kg^{-1} body weight) exhibited some behavioral changes such as scratching of the body, mouth and head parts, anorexia, sensitive to slight sound and restlessness about 10-15 min after oral administration of the extract. The total protein, Albumin, Serum bilirubin, AST and ALT were not significantly different from that of the control. Serum alkaline phosphate increased in treated groups though not significantly compared to the control.

Effects of Aqueous Pulp Extract of *T. indica* on Histopathology

Histopathological examinations of liver and kidney in Fig. 1-4 revealed no visible changes on the liver and kidney of the rats administered between 900-4500 mg kg^{-1} body weight orally of aqueous

Table 1: Effects of aqueous pulp extract of *T. indica* extract on hematological indices

	Group					
	1	2	3	4	5	6
Parameters	Control	900 (mg kg^{-1})	1800 (mg kg^{-1})	2700 (mg kg^{-1})	3600 (mg kg^{-1})	4500 (mg kg^{-1})
PCV (%)	35.00±0.1	33.1±0.1	34.5±1.3	33.0±1.0	34.0±0.3	33.5±0.5
WBC (10^3)	3.25±0.1	3.0±1.2	2.7±0.2	3.4±1.4	3.0±0.2	25.0±0.4
Lymphocytes (%)	33.00±2.9	30.0±0.3	31.2±2.7	26.0±4.3	45.0±11.4	51.0±1.2
Nuetrophiles (%)	55.10±2.7	62.0±0.1	63.2±2.3*	66.8±2.3**	57.4±2.1	51.0±1.1
Monocytes (%)	9.00±0.6	5.0±0.5	6.0±0.3	6.0±2.3	6.5±0.1	6.7±0.8
Eosinophils (%)	5.00±0.3	3.0±0.3	4.5±0.1	2.5±0.9*	2.0±0.4**	3.3±0.4
Basophils (%)	NP	NP	NP	NP	NP	NP

*: $p<0.05$ when compared with control, **: $p<0.01$ when compared with the control, NP: Not Present

Table 2: Effect of *T. indica* pulp extract on the blood chemistry

Parameter	Total		Total	Conjugated			
Dose	protein	Albumin	bilirubin	bilirubin	AST	ALT	ALP
(mg kg^{-1})	mg dL^{-1}				U L^{-1}		
Control	9.4±0.24	4.5±0.29	0.38±0.08	0.14±0.14	33.8±6.11	17.3±0.32	40.5±0.55
900	8.0±0.17	3.7±0.40	0.32±0.01	0.14±0.30	30.3±4.30	14.8±1.41	64.5±14.51
1800	8.0±0.33	3.6±0.29	0.43±0.16	0.22±1.12	32.8±6.52	15.3±1.47	68.5±22.50
2700	10.8±0.77	4.6±0.37	0.32±0.02	0.14±0.10	28.3±6.44	14.3±1.50	51.5±6.56
3600	9.7±0.34	5.5±0.44	0.43±0.16	0.14±4.11	21.0±2.31	14.3±3.10	61.0±9.08
4500	10.4±0.27	5.4±0.55	0.43±0.16	0.22±1.16	31.3±3.70	14.8±1.53	63.0±5.57

Fig. 1: Histopathological slides 1 (liver of control) (x 400)

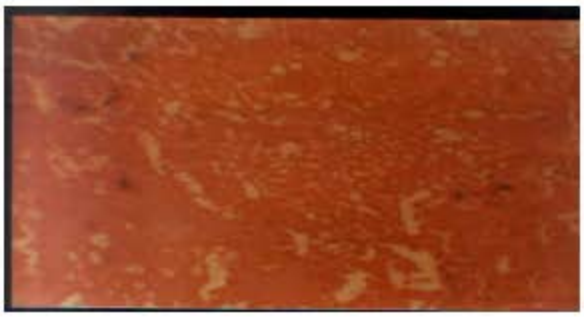

Fig. 2: Histopathological slides 2 (liver of group 6 given 4500 mg kg^{-1} of extract orally) (x 400)

Fig. 3: Histopathological slides 3 (kidney of control) (x 400)

Fig. 4: Histopathological slides 4 (kidney of group 6 given 4500 mg kg^{-1} of extract orally) (x 400)

pulp extract of *T. indica*. Physical examination of the intestines shows no apparent congestion or hemorrhage in the gastro intestinal tract.

DISCUSSION

The PCV is used as an index of anemia and measures the rates at which red cells are added to and withdrawn from circulation by synthesis and breakdown respectively. Simultaneous measurement of the total and differential leukocyte counts, are of value in confirming or eliminating a tentative diagnosis and aid in making a more accurate prognosis (Coles, 1986). In the present study, there was no significant difference in the haematological parameters of the treated groups such as PCV, WBC, Lymphocytes and Monocytes as compared with the control, however there were significant differences in nuetrophils and eosinophils of some groups as compared to control.

In tissues, the principle function of nuetrophilic granulocytes is phagicytosis of small particle. These functions are primarily that of matured segmented nuetrophils (although metamyelocyte and band nuetrophils have some phagocytic ability) and are associated with inflammatory conditions found in large numbers in tissue infected with pyogenic microorganisms such as staphylococci, streptococci and corynebacteria. In addition to their phagocytic capabilities, nuetrophils elaborate powerful proteolytic enzymes that react within the cell to destroy phagocytosed particles or may be liberated and function outside the cell body (Coles, 1986). Significant increase in nuetrophils in group 3 ($p<0.05$) and group 4 ($p<0.01$) may be attributed to the changes in the number of circulating nuetrophils as a result of systemic infection, localized infection or non infectious disease usually those that stimulate a stress reaction (included in this category are metabolic disturbance, drugs and toxic chemicals). Also tissue destruction, irrespective of its cause, will produce an increased in number of circulating nuetrophils (Coles, 1986).

Eosinophils plays a role in fighting viral infections (which is evident from the abundance of RNAses they contain within their granules), helminth (worm) colonization, allergic response, fibrin removal in inflammation, disease severity and are considered the main effector cells in asthma pathogenesis. Eosinophils may be slightly elevated in the presence of certain parasites (Lee and Lee, 2005). An increase in eosinophils (eosinophilia) is typical with parasitic infestation of the intestines, a collagen vascular disease (such as rheumatoid arthritis), malignant diseases such as Hodgkin's Disease, extensive skin diseases (such as exfoliative dermatitis), Addison's Disease and with the use of certain drugs such as penicillin (Wills and Karp, 2004). Eosinophilia in the control group could be a reflection of hypersensitivity in parasitic conditions or allergic reactions (such as asthma, urticaria, dermatitis and food allergies etc.) or in the recovery stage of some acute infection. Since there was no apparent disorder such as dermatitis or asthma, this increase in eosinophils can be attributed to parasitic infection especially as there was no initial antihelmentic medication prior to the start of the experiment. Decrease in eosinophils of the pulp extract treated groups especially at group 4 ($p<0.05$) and group 5 ($p<0.01$) could be the result of the anti allergic properties of the plant extract as compared to that of the control. Another possible cause of eosinopenia in these treated groups could be stress (Coles, 1986; Lee and Lee, 2005).

Plasma proteins are the most readily obtainable protein available in the animal and also occupy a central and dominant position in the metabolism of protein because of their intimate relation to metabolism in the liver and their interactions with other tissues in the body (Coles, 1986). This close relationship of plasma proteins can be used to examine specific biochemical functions and the general status of the body's protein metabolism. The result of this study indicated that there was no significant ($p<0.05$) alteration in both serum total protein and albumin of the pulp extract treated group as compared to the control respectively.

Serum ALP and Bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Coles, 1986; Murugesh *et al*., 2005). Effective control of alkaline phosphatase (ALP) and bilirubin levels points towards improved secretory mechanism of the hepatic cell. The result of the effect of this extract on serum ALP and Bilirubin (Conjugated and Total) revealed no significant difference ($p<0.05$) between the treated and control group. This can also be supported by the report that *T. indica* is used in the treatment of bile disorders (Doughari, 2006). Slight elevated of serum ALP as shown in the treated groups could be as a result of cholestasis, regardless of the cause (Coles, 1986).

The determination of enzyme levels such as AST and ALT is largely used in the assessment of liver damage. Necrosis or membrane damage releases these enzymes into circulation and therefore can be measured in serum. High levels of AST indicate liver damage, such as that due to viral infection as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in the liver (Murugesh *et al*., 2005). In the present study, there was no significant difference ($p<0.05$) in the serum AST and ALT of the pulp extract treated groups as compared to that of the control though slight increase in the enzymes were seen in the control. Histopathological studies (Figure 1) showed no signs of visible lesion and physical examination of the intestines revealed there was no apparent congestion or any sign of hemorrhage. Conclusively, this might imply that the pulp extract of *T. indica* does not have effect on hepatic secretions, biochemical functions and enzyme activity respectively. This can also be supported by the report that *T. indica* is used in the treatment of bile disorders (Morton, 1987).

This is to our knowledge the first report on the effects of *Tamarindus indica* aqueous pulp extract on some hematological and toxicological indices. The absence of death at the highest dose of 4500 mg kg^{-1} could be an indication of some margin of safety.

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