

Full Length Research Paper

Biochemical and toxicological studies of aqueous extract of *Tithonia diversifolia* (Hemsl.) leaves in wister albino rats.

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The effects of aqueous extracts of *Tithonia diversifolia* leaves on some biochemical indices such as body weight, blood parameters and liver functions were investigated in albino male wister rats. The safe dosage of the aqueous extract of *T. diversifolia* leaves were daily injected intraperitoneally ($P \leq 0.05$) to a group of Wister strain rats over a period of 7 and 14 days respectively. During these periods the daily body weights of the control and treated rats showed significant difference ($P \leq 0.05$). Also, the differences in the WBC and PCV values between the two groups were statistically significant ($P \leq 0.05$). The serum level of glutamate pyruvate transaminase (GPT) increased significantly ($P \leq 0.05$) in the experimental group while that of glutamate oxaloacetate transaminase (GOT) did not show any significant change ($P \leq 0.05$) compared to the control animals. The study showed that the aqueous extract of *T. diversifolia* even at a safe dose affects the liver functions index, and some haematological parameters.

Key words: *T. diversifolia*, Maximum Tolerated Dosage (MTD), Lethal Dosage (LD₅₀).

INTRODUCTION

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being, and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). Furthermore an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998).

Tithonia diversifolia (Hemsl.) A. Gray asteraceae also known as Mexican Sunflower, tree marigold, shrub sunflower or Japanese sunflower (English), "sepeleba" (Yo-

ruba), pua renga (Cook Island), Kavakava (Cook Island Ma'uke) and matala (Niue) belongs to the family of Asteraceae (Compositae). It was introduced into West Africa as an ornamental plant but it has become a weed of field crop, waste areas and roadsides (Akobundu and Agyakwa, 1998). Its synonyms are *Tithonia rotundifolia*, *Scenecio cineraria* and *Tagetes erecta* (Rai and Acharya 1999)

Although the plant is yet to be comprehensively studied it is known to be used in folk medicine to treat various illnesses including malaria, diarrhea, inflammation, haematomas, as well as bacterial and parasitic infections (Wanjau et al., 1997; Tona et al., 1998; Rungeler, et al., 1998; Kuo and Chen, 1998; Goffin et al., 2002; Gu et al., 2002) In addition infusion from its leaves has been used for subduing swelling, dissolving lumps and treating enteritis and gastritis in local folk medicine (Tona et al., 1998). However Rai and Acharya (1999) showed that *T. diversifolia* have antimycotic activity against *Fusarium Oxysporum* and *Erichophyton in vitro*. Ether extract from aerial parts of the plant has been shown to have good antiplas-

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modial activity against three strains of *Plasmodium falciparum* *in vitro* and against *Plasmodium berghei* *in vivo* (Bidla et al., 2004; Elufioye and Agbedahunsi, 2004). The plant has also proven to be effective in treating cramps and gastrointestinal disorder. *In vitro* studies revealed that *T. diversifolia* contains some constituents or compounds capable of acting as an anti-tumor agent (Gu et al., 2002). A reliable reversed phase high performance liquid chromatographic method revealed the presence of Tagitinin C and A, an antiplasmodial sesquiterpenes lactones (Goffin et al., 2002). Also, an antiproliferation bioassay performed with human colon cancer cells showed the presence of three new sesquiterpenoids; 2-alpha-hydroxy-tirofundin, tithofolinolide and 3-alpha-acetoxydiversifolol, along with eight known sesquiterpene lactones some of which are, 3 beta-acetoxy-8 beta-Isobutyroxyreynosin, tagitinin A and tirofundin in an ethyl acetate extract of the aerial parts of *T. diversifolia* (Gu et al., 2002)

In Nigeria, the crude extract of *T. diversifolia* has been extensively used locally as antimalaria without possible recourse to its possible deleterious effect, although few reports on its pharmacological values and toxicological effects have been documented. (Elufioye and Agbedahunsi, 2004))

Against this backdrop our intentions in this work was to quantified some biochemical measured effects- body weights, liver function and hematological parameters in order to examine the response of animals during administration of safe dosage of the plant's extracts.

MATERIALS AND METHODS

Host animals: Fifty male Wister strain rats weighing between 30.6 - 40.3 g were procured from a random bred colony in the animal house of National Veterinary Research Institute, Vom, Nigeria. The rats were caged singly in the metabolic laboratory at $29 \pm 2^\circ\text{C}$. They were allowed to acclimatize for 8 weeks during which the weight gained was between 96.5 - 114.5 g. During this period the rats were fed on rats-chow diets and water *ad-libitum*.

Plant collection and authentication: The plant was authenticated and voucher specimen was deposited at the botany unit of the Babcock University. Fresh leaves of the plant were collected from vegetation within Babcock University campus.

Preparation of extract: Tri- replicate of the plant (50 g) previously dried in an oven and milled into powder using electric blender/mill grater (model MS-223, Taiwan) was soaked in 300 ml-distilled water for 24 h. The resultant mixture was filtered with cheesecloth and the filtrate concentrated under reduced pressure at 40°C for 20 min using a rotary evaporator (Gallenkamp UK). The resulting residue (5.13 g) called the aqueous extracts were stored at 4°C . The residue was re-constituted in distilled water to obtain the various concentrations used for the toxicological and biochemical tests.

Acute toxicity test: The acute toxicity of the extract was tested on 30 male Wister Albino rats divided into 5 groups of 6 rats each with each group receiving different dose of 50, 80, 100, 120 and 140 mg/kg body weight as described by Miller and Tainter (1944). The number of deaths in each group was recorded within 24 h. The lethal dosage was estimated from the graph of percentage mortality against log-dose of the extract.

Administration of extract: Sets of rats ($n = 5$ each) were divided into experimental and control groups for two separate treatments. Rats in group A were administered with daily oral dose of 10 ml of distilled water for 7 days to serve as control for 7 days administration. Those in group B were similarly treated for 14 days to serve as control for 14 days administration. While groups C and D were injected daily with 100 mg/kg of aqueous extract intraperitoneally for 7 and 14 days respectively. The animals were fed normally while a visible change in their skin, weights and locomotory activities was recorded daily after each treatment for the period of the experiment.

Clinical examinations: The animals in groups A, B, C and D were anaesthetized in mild chloroform while venous blood was collected with a 2 ml syringe from each (treated and control) into EDTA bottles and centrifuged at 6000 rpm to collect the serum, and stored frozen at -4°C for further analysis.

Part of the blood was put into heparinized capillary tubes to determine the packed cell volumes (PCV) using haematocrit centrifuge and reader. White blood cell (WBC) counts were determined by mixing 0.1 ml blood with 3.8 ml Thurk's fluid solution and smeared on a microscope slide. The number of WBC was expressed as a percentage of the total red blood cells. In the serum collected both GOT and GPT were measured by assiduously following the method of Reitman and Frankel (1957) as described in the Randox (2006) diagnostic Kit used

Statistical analysis: The results are expressed as mean values \pm S.E.M of five replicates. Data were subjected to Chi square and Student's t-test where necessary for statistical analysis. Results were considered significant with $P \leq 0.05$.

RESULTS AND DISCUSSION

The LD_{50} was found to be 120 mg/Kg body weight while the Maximum Tolerated Dosage (MTD) regarded as a maximum safe dosage for administration was found to be 100 mg/kg body weight from the Acute Toxicity text. The body weight in the experimental animals was reduced to 96 ± 17.22 indicating a mean decrease of 6 g (5.9%) within one week and 92 ± 15.34 , that is, a mean decrease of 10 g (9.8%) on day 14 of treatment (Tables 1). This reduction in body weight was significantly different when compared with the control ($P \leq 0.05$). The decrease in the body weight could be due either to the effect of the extract on the internal organs or to the general discomfort which led to a low feeding rate in the treated animals (Brodie et al., 1970). Although slight lesions noticed on the skin of the experimental animals during administration of the crude extract might have affected their consumption rates due to the general discomfort suffered by the animals which was evident in the reduction of their locomotion and general activities. The PCV values and WBC counts also dropped significantly in the experimental animals (Table 2), although, this was more pronounced in the 14-day administration. The significant decrease in PCV value ($\chi^2 = 18.3$, $df = 1$, $P \leq 0.05$) may indicate destructive effect of the extract on RBC. The WBC also dropped significantly ($\chi^2 = 4782.01$, $df = 1$, $P \leq 0.05$) in the treated animals and this may also indicate the mobilization of WBC to the surroundings of injuries

Table 1. Comparison of body weight between control and experimental animals.

Control animals	Weight at time of administration (g)	weight on day 7 (g)	Weight on day 14 (g)	Weight loss on day 7 (g)	Weight loss on day 14 (g)	% weight loss on day 7	% weight loss on day 14
Mean ± SEM	101 ± 19.65	99 ± 17.34	93 ± 15.44	2	8	1.98 ± 0.07	7.9 ± 1.05
Treated animals							
Mean ± SEM (P≤0.05)	102 ± 25.62	96 ± 17.22	92 ± 15.34	6	10	5.89 ± 0.19	9.8 ± 1.24

Table 2. Comparison of PCV Values and WBC Control to Experimental Animals (n=5).

PCV (%)		WBC / cu.mm	
Mean ± SEM	37.92 ± 2.1	Control animals	7550 ± 393.5
		Treated animals	2300 ± 242.4
Mean ± SEM	32.5 ± 1.92		
❖ P≤0.05			

Table 3. Comparison of GOT and GPT values of Control to Experimental Animals.

*Enzyme activity (IU/L)				
Days	Glutamate pyruvate transaminase		Glutamate oxaloacetate transaminase	
	Control (Distilled water)	Treated	Control (Distilled water)	Treated
* Mean ± SEM				
7	19.84 ± 1.43	23.66 ± 1.65	17.49 ± 1.28	17.98 ± 1.32
14	22.74 ± 1.05	24.41 ± 1.23	18.05 ± 1.19	17.99 ± 1.06

*Mean ± SEM = values for 5 rats.

sustained. This shows that prolonged administration of the extract may have toxic effect on the number of formed elements of blood given an indication of progressive hemolysis or hemorrhages. Our findings further showed that the administration of the extracts for 7 and 14 days produced significant increase in the serum level of glutamate pyruvate transaminase (GPT) as compared to the control ($P \leq 0.05$). In contrast, no significant change was observed in the level of glutamate oxaloacetate transaminase (GOT) ($P \geq 0.05$) (Table 3). Previous findings have reported that GOT is a more reliable marker of liver integrity than GPT (Tietz, 1987). Hence the observed significant increase in the activity of GPT alone may be extrahepatic origin perhaps as a result of leak-ages from damaged or necrotic tissues. (Demetriou et al., 1974; Wells et al., 1986; Bennet and Plum, 1996).

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