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Preliminary report

Tithonia diversifolia saponin–blood lipid interaction and its influence on immune system of normal wistar rats



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ABSTRACT

This study investigated the effect of saponins (20–100 mg/kg) from *Tithonia diversifolia* leaf (STD) on the liver, kidney, heart, lipid profile and hematological parameters of normal rats. The results showed that STD (20–100 mg/kg) though caused a slight increase in the liver, heart and kidney activity of ALT, AST, ALP and GGT ($p < 0.05$), did not result in corresponding increase in the serum level of these enzymes. A significant reduction in the level of triglycerides, LDL and cholesterol, creatinine, urea, LDH, PCV and hemoglobin were observed with a concomitant increase in HDL, white blood cell and lymphocyte. These study demonstrated the role of STD in enhancing immune response and in reducing cholesterol and triglycerides in normal rats at studied dosages.

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1. Introduction

The plant kingdom is an important potential source of effective treatment for various diseases. More than 300,000 species of plants have been reported to display medicinal properties, but only a few have been investigated in any detail for their safety [1]. However, concerns exist about the quality and safety of herbal medicinal products, particularly relating to safety and dosage.

Tithonia diversifolia (Hemsl.) A. Gray popularly known as Mexican sunflower is a perennial shrub of the family Asteraceae [2]. It is native to Central America but very abundant in Nigeria where it is employed for treatment of many ailments because of its diverse pharmacological actions. The decoctions of various parts of this plant have been used in folk medicine for the treatment of malaria, diabetes mellitus, sore throat, liver, menstrual pains and inflammation [3–6]. The ability of the plant to scavenge free radicals and promoting and induction of cellular systems involved in cellular stress defences and adipogenesis in mesenchymal cells has been reported [7]. Most of the pharmacological activities have been attributed to sesquiterpene lactones and some chlorogenic acid derivatives in the leaves of this species [8]. But recently,

saponin and flavonoids content of the leaves have been implicated in their pharmacological potentials [6,9]. Saponins are bio-surfactants composed of a rigid hydrophobic structure of a steroid or triterpenoid type which is linked to one, two or three hydrophilic sugar chains, they are known to possess a wide range of biological activities, such as enhancing cell membrane permeability, regulating nutrient uptake in the intestine, reducing protein digestibility, decreasing serum cholesterol, etc [10,11]. Though repeated dose toxicological study to identify compounds responsible for toxic effects of *T. diversifolia* had been conducted, there is dearth of information on the effect of *Tithonia diversifolia* saponins on biochemical parameters of normal rats. Therefore this research was designed to investigate the *in-vivo* effect of saponin rich fraction of *Tithonia diversifolia* on vital organs, lipid profile and hematological parameters of normal rats.

2. Materials and methods

2.1. Plant materials

The leaves of *T. diversifolia* were collected by the roadside between Ikare and Arigidi Akoko in Ondo State. They were identified, authenticated by Dr. Obembe of the Department of plant science and Biotechnology, Adekunle Ajasin University, Nigeria and kept in the herbarium. The leaves were air dried, pulverized and stored at room temperature in air tight polythene bag prior to use.

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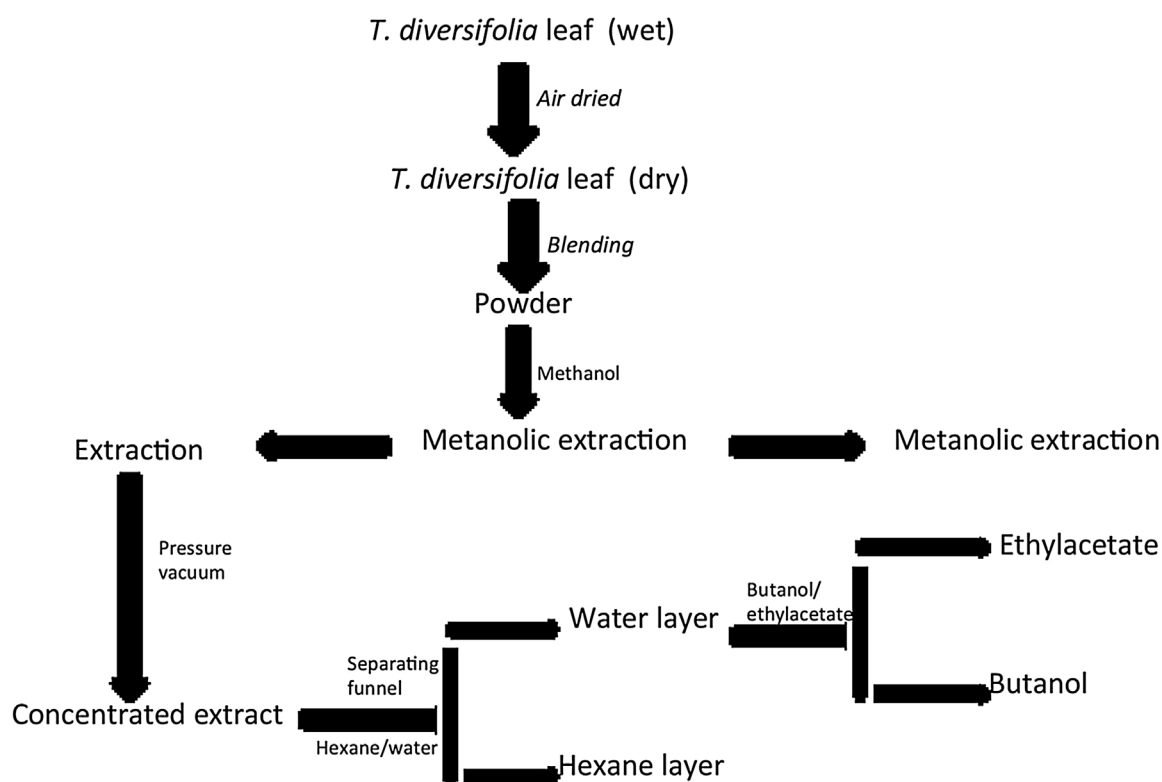


Fig. 1. Flowchart for saponin extraction.

2.2. Extraction and isolation of saponins from *T. diversifolia* leaf

Saponins were extracted as described by [12]. Hundred grams (100 g) ground sample was extracted with 2000 ml of methanol for 48 h. The methanolic extract was concentrated using a rotary evaporator and partitioned with hexane and water (1:2, v/v). After a thorough shaking, the mixture was allowed to stand overnight and the water layer was concentrated and partitioned between ethyl acetate and *n*-butanol (1:3, v/v). The ethylacetate fraction was concentrated to obtain saponin-rich fraction used for this experiment (Fig. 1).

2.3. Animals

Thirty male wistar rats of average weight 145 ± 14 g were obtained from our own breeding colony managed by Dr. Adetokunbo of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko. They were allowed to acclimatize to experimental condition for two weeks. They were housed in clean cages and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$ with dark/light cycle 12/12 h). They were fed *ad libitum* on rat pellets (Top Feeds, Nigeria) and water. The principles of Laboratory Animal care (NIH Publication 85–93, revised 1985) were followed throughout the duration of the experiment.

2.4. Experimental procedure

Male wistar rats ($n=30$) were divided equally into 6 groups. Group I (control), group II–VI (test group) were administered with saponin from *T. diversifolia* at doses of 20, 40, 60, 80, 100 mg/kg body weight respectively for 21 days. After which the rats were sacrificed by cervical dislocation and the blood collected into serum bottles and prepared as described by [13]. The tissues (liver, heart, kidney) were removed and prepared as previously described

[12]. The activity of ALT, AST, ALP and GGT were assayed by Randox commercial kit.

2.5. Statistical analysis

The data are expressed as mean $\pm$ SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) using SPSS version 17. Differences were considered to be statistically significant when $p < 0.05$. Graphpad prism was used to plot the graph.

3. Results

3.1. Effect of STD on kidney of normal rats

The effect of STD on kidney function of normal rat was investigated and is shown in Tables 1 and 2 and Fig. 3. Serum creatinine and urea together with the activity of ALT, AST and ALP were used to assess the effect of STD on kidney function. STD at 20 and 40 mg/kg did not alter the serum creatinine level but decreased (20–100 mg/kg) the serum urea level when compared

Table 1
Effect of saponins from *T. diversifolia* leaf on serum creatinine level of normal rats.

Group	Creatinine (mg/dl)
Control	200.97 ± 32.34^a
Saponin (20 mg/kg)	200.22 ± 18.52^a
Saponin (40 mg/kg)	188.16 ± 17.17^a
Saponin (60 mg/kg)	165.91 ± 25.77^b
Saponin (80 mg/kg)	177.41 ± 25.31^b
Saponin (100 mg/kg)	178.54 ± 29.91^b

Results are presented as means $\pm$ SEM of five (5) determinations. Values carrying different superscript are significantly different ($P < 0.05$). Values with the same superscript do not differ significantly from each other.

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