Toxicity Analysis of Tapinantis Globiferus in Swiss Albino Mice

Nigerian Institute for Trypanosomiasis Research (NITR) No.1 Surame Road, Kaduna state, Nigeria. 
Nigerian Institute for Trypanosomiasis Research (NITR) No.1 Surame Road, Kaduna state, Nigeria.
Corresponding author: J. A. Abedo,

Abstract: Medicinal plants with their derivatives play vital roles in the health of humans as well as animals and have long been known to possess biological activity. The plant species Tapinantis globiferus, commonly known as mistletoe, is widely used in traditional medicine for the treatments of several diseases such as asthma, cancer, chronic fever, tinnitus, inflammations, dyspepsia, epilepsy, cough etc. The present studies aimed to evaluate acute toxicity of T. globiferus by using lorke’s method as well as to determine the after effects on biochemical parameters and histopathological alterations. The study was conducted on albino rats in two phases. In the first phase, animals were divided into 4 groups of 3 animals each. The first three groups (ABC and D) were treated intraperitoneally with 10, 100 and 1000 mg/kg body weight with methanolic leaf extract of T. globiferus respectively. While the fourth group which served as control was administered normal saline. All the results were observed for adverse clinical signs and mortality in 24, 72 hrs and up to 2 weeks. The second phase involves another three groups of mice 3 per group were administered increased dosage of the extract; 1600, 2900 and 5000 mg/kg body weight respectively. Toxicity test showed there were no major clinical signs and mortality observed in most of the experimental groups. However mild behavioural and clinical changes seen in phase 2 treated animals were hyperactivity and diarrhoea. Also, there were no significant changes in biochemical parameters and histopathological alterations. This study shows that although T. globiferus a leaf extract is safe acutely, its long-term use needs to be ascertain.

Keywords: Tapinantis globiferus, toxicity, phytochemical, biochemical, histopathological.

I. Introduction

The world has shown considerable attention on medicinal plants as alternative for orthodox medicines in recent years. The review on some medicinal plants clearly validates the effectiveness and reliability of ethnomedical knowledge and traditional uses of these plants species in managing diseases[1,2]. Present estimates indicate that about eighty percent of the world’s population rely either directly or indirectly on traditional medicine for health care delivery[3].

The interest directed to the discovery of new biologically-active molecules by the pharmaceutical industries into adaption of herbal extracts of plants for use in several ailments and afflictions[4,5]. Herbal plants produce and contain a variety of chemical substances with varied physiological effects. They are huge reservoir of various chemical substances with potential therapeutic properties [6, 7]. Herbal medicine is also the oldest form of health care known to mankind.

The main reason for the increasingly-popular use of plant derived medicine is that they are readily available and relatively safer than common syntheticalternatives, offering profound therapeutic benefits and alongside affordable treatment. However, not all medicinal plants are safe for consumption in their crude form, therefore the level of toxicity needs to be ascertained hence toxicity studies.

Tapinantis globiferus (Family- Loranthaceae) Commonly known as mistletoe in English, afomoonisanain Yoruba and kauchi in Hausa is a parasitic plant which grows on various species of Trees such as Kola, Citrus, Pakaibiglobosa, Combretum, Acacia, Aloe and Terminalia as host plants [8]. It is widely spread but commonly found in North Central Namibia and rainforestsof Nigeria. The leaves of Tapinantis globiferus are generally ovate, rounded at the apex and is about 7 cm long and 3 cm broad with irregular pinnately arranged lateral extract of the leaves was evaluated in mice to assess its safety or otherwise, since the findings are important considering the usage of the plants by human beings[9].
II. Materials and Methods

2.1 Plant Materials Collection:
The plant, *Terminalia globiferus* was found growing on *Punika biglobosa* Trees located at Unguwan Rimi area of Kaduna state. Mallam Musa identified the collected plant at the Herbarium unit of Department of Botany Faculty of life science, Ahmadu Bello University Zaria, Nigeria with a voucher no. 900107.

2.2 Preparation of methanolic Plant Extract of *T. globiferus*
The fresh leaves of the collected plant materials were air dried until crispy. The dried plant was grounded into a powder form using a laboratory blender. Five hundred (500g) of the dried sample was macerated in 500ml of methanol in a conical flask for 72hrs. The menstruum was filtered using no. 1 Whatman’s filter paper. The collected filtrate was concentrated using a rotary evaporator. It was exposed to dryness and stored in a refrigerator at 4°C until required (10).

Chemicals: The chemicals used in the course of this research were of certified analytic grade.

2.3 Experimental Animals
Albino rats weighing about 180-220 g, aged 8-12 weeks, of both sexes were obtained from the animal house of Nigerian Institute for Trypanosomiases Research Kaduna. The animals were kept in clean polypropylene cages in well-ventilated house condition (Temperature: 28-31°C; humidity: 50-55%) a 12h natural light cycle with litter changed every week fed growers mesh and water ad libitum. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1998).

2.4 Phytochemical Test

2.4.1 Test for Alkaloids
One gram of the sample was boiled with water, followed by addition of 10 ml hydrochloric acid, added to the sample while boiling on a water bath and was filtered after boiling. The pH of the filtrate was adjusted with ammonia to about 6-7; 3 drops of Picric acid, Mayer’s reagent and Wagner’s reagent were added separately to about 0.5 ml of the filtrate in three different test tubes. Formation of turbidity, creamy white and reddish brown precipitate observed, indicates the presence of alkaloids (11).

2.4.2 Test for Anthraquinones
5 ml Chloroform was added to 0.5 g of the extract. The resulting mixture was shaken for 5 mins and filtered; equal volume of 10% ammonia (NH4OH) solution was added to the filtrate. The presence of bright pink, red or violet colouration in the ammoniacal layer indicated the presence of combined anthraquinones (11).

2.4.3 Test for Flavonoids
5 ml dilute ammonia (NH4OH) solution was added to a portion of the methanolic filtrate of the plant extract followed by addition of 0.5 ml concentrated H2SO4. A yellow coloration was observed in each extract indicating the presence of flavonoids (12).

2.4.4 Test for Saponins
One gram of the extract was boiled at 50°C with 10 ml of distilled water in a water bath for 10 mins. The mixture was filtered, allowed to cool and shaken vigorously for 2 mins, frothing indicates the presence of saponin in the filtrate (13).

2.4.5 Test for Cardiac Glycosides (Keller-Killian’s)
5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution, 1ml of concentrated sulphuric acid(H2SO4) was also added in drops. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed indicating the presence of cardiac glycosides (12).

2.4.6 Test for Tannins
Test with Iron salts: One gram of the extract was separately boiled with 20 ml of distilled water for five minutes at 50°C in a water bath and filtered, while hot. 1ml of the cool filtrate was distilled to 5 ml with distilled water and 3 drops of 10% ferric chloride were added. Bluish-black or brownish-green precipitate indicated the presence of tannins. (14).
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2.7 Test for Phlobatannins
Two grams of the extract was boiled with 5ml of distilled water and filtered. 5cm³ of 1% aqueous hydrochloric acid (HCl) was added to the filtrate. The observance of a reddish precipitate indicates the presence of phlobatannins (14).

2.7 Test for Terpenes
5ml of the extract was diluted with 5ml distilled water unto which 1cm³ of acetic anhydride was added, and finally 1cm³ of concentrated H₂SO₄ was mixed into the test tube to form a layer underneath. A reddish-violet colour indicates the presence of terpenes (11).

2.5 Determination of acute toxicity (LD₅₀)
The acute toxicity studies of methanolic leave extract of Tapinantus globiferus using Lorke’s method (15) with slight modification. The experiment was carried in albino rats in two phases as follows: the first phase comprises three groups (A B C) of four mice each were administered 100, 500 and 1000 mg/kg intraperitoneally with methanolic extract (of) Tapinantus globiferus intraperitoneally. While the fourth group in both phases (D) served as control and were only administered phosphate buffer saline. The animals were observed for signs of toxicity and mortality for 12, 24, 48, 72 h and for 2 weeks. In the second phase, the three groups of 3 mice (DEF) were each administered doses of 1500, 2900 and 5000mg / kg body weight of the extract respectively. They were also monitored as described in phase one after administration of different doses of the extract. The lethal dose (LD₃₀) was determined.

2.6 Blood Biochemical Determinations
The level of biochemical analysis such as aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine were performed with the blood collected from cardiac puncture of rats using sterile 5ml needle and syringe the sample was allowed to clot and was centrifuged at 5000RMP for 10 min to recover the serum, this was used to evaluate biochemical parameters (16). The procedure was performed in order to determine the function of the livers and kidneys of the treated and control groups.

2.7 Animal dissection, organs weight measurement and tissue sampling
At the end of the experiment, animals of each group were sacrificed after been anaesthetia with chloroform, they were carefully laid on a clean sheet place on the board and pinned on four extremities to the corkboard and was dissected with a surgical scissors from the neck to pubis to open the peritoneum. Then, 3–4 mm wide strips of tissue samples liver, kidney, spleen and lungs were carefully detached from the tissue of the opened peritoneum using a forceps. Each of these organs where cleaned in normal saline, weighed separately with a precision automatic internal calibration CX series balance and transferred to a specimen bottle containing 10% formalin (16).

2-8 Histopathological Examinations
Histological examination on tissue of organs (liver and kidney) fixed in 10% formaldehdy, described above were washed for several hours under tap water and dehydrated with different grades of alcohol at various time interval and finally in absolute alcohol. The tissues were cleared in xylene and embedded in Paraffin wax. Tissue blocks were sectioned (at) 5 μm thick and (thick, stained with) stained with Haematoxylin and Eosin (H & E) and examined under (a) light microscope (10 and X40) in order to verify alterations in the tissues samples such as (such as; extent of deformation,) extent of deformation, infiltration of cell, protein formation, tubules necrosis.

III. Results
3.1 Phytochemical test
The results of the phytochemical screening (Table 1) revealed the methanolic extract of leaves of T. globiferus indicated the presence of medicinally constituents such as; alkaloids, Terpenoids, Tannins, saponins, cardiac glycosides, flavonoids, anthraquinones and phlobatannins.

Toxicity test
There were no signs of acute toxicity observed with any of the animals treated in the first phase. However the main signs of activity observed in the second phase was hyperactivity with the group administered with 1500 mg/kg/wt. while mild diarrhoea was observed with the group given 5000 mg/kg/wt. No mortality was recorded after observation for 4 hrs, then 12, 24, 36, 48 hrs up to a period two (2) weeks of experimental duration (Table 2).
Serum biochemical test

The evaluation of serum biochemical parameters have significant importance to toxicological changes produced by toxicants. This is because of the body’s response to clinical signs and symptoms. To assess the possible toxic effects of drugs, evaluation of hepatic and renal function is primarily preferred as these organs are functionally predisposed. In the present liver and kidney function tests, there were no significant changes in the serum level of ALT, AST, ALP and creatinine and plasma urea (BUN) in animal groups treated with various doses of methanolic leaf extracts of *T. globiferus* (Table 3) in comparison to the controls. Also there was no statistically significant differences (p>0.05) noted in organ weights between the *T. globiferus* extract treated and control groups, except for the liver and kidney of animals treated 1500, 2900 and 5000 mg (Table 4). This could also be connected to histopathological findings observed (Plate 1-10).

Statistical Analysis

Data from organ weight, serum biochemical level, subjected to the Analysis of Variance (ANOVA). The levels of significance were considered at P<0.05 and data was expressed as Mean ± SEM.

### Table 1: phytochemical screening of *Tapinantus globiferus*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloids</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Cardiac glycosides</th>
<th>Terpenoids</th>
<th>Anthraquinones</th>
<th>Tannins</th>
<th>Phlobatanins</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T.globiferus</em></td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) = present, (-) = absent

### Table 2: Effect of acute toxicity test of *T. globiferus* administered to experimental rats

<table>
<thead>
<tr>
<th>Doses(mg/kg/wt)</th>
<th>ALT(U/I)</th>
<th>AST(U/I)</th>
<th>ALP(U/I)</th>
<th>CRETININE</th>
<th>UREA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>27.01 ± 2.33(^a)</td>
<td>126.66±34.41(^a)</td>
<td>96.23±17.28(^a)</td>
<td>0.65 ± 0.21(^d)</td>
<td>21.13 ± 2.44(^a)</td>
</tr>
<tr>
<td>100</td>
<td>26.25 ± 2.43(^a)</td>
<td>127.25 ± 18.12(^a)</td>
<td>96.60 ± 16.55(^a)</td>
<td>0.64 ± 0.04(^a)</td>
<td>19.46 ± 3.80(^a)</td>
</tr>
<tr>
<td>1000</td>
<td>23.20±2.33(^a)</td>
<td>130.42±13.22(^a)</td>
<td>94.57±61.62(^a)</td>
<td>0.660±0.100(^d)</td>
<td>20.63±7.2(^a)</td>
</tr>
<tr>
<td>1500</td>
<td>26.10 ± 2.21(^a)</td>
<td>115.50±16.70(^a)</td>
<td>92.25 ± 16.77(^a)</td>
<td>0.69 ± 0.00(^a)</td>
<td>19.88 ± 2.68(^a)</td>
</tr>
<tr>
<td>2900</td>
<td>24.31±2.10(^a)</td>
<td>121.23±26.30(^a)</td>
<td>94.22±10.21(^a)</td>
<td>0.62±0.04(^a)</td>
<td>20.70±1.00(^a)</td>
</tr>
<tr>
<td>5000</td>
<td>28.43±2.00(^a)</td>
<td>113.23±24.13(^a)</td>
<td>92.21±21.52(^a)</td>
<td>0.61±0.80(^a)</td>
<td>20.87±21.20(^a)</td>
</tr>
<tr>
<td>Control</td>
<td>27.63±2.43(^a)</td>
<td>136.67±24.49(^a)</td>
<td>96.83±17.68(^a)</td>
<td>0.70±0.03(^d)</td>
<td>20.08±2.38(^a)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard Deviation, different superscripts in the same column indicates values differ significantly (p < 0.05).
## Table 4: Weight of some visceral organs of experimental rat after treatment with *T. globiferus*

<table>
<thead>
<tr>
<th>Dose (mg/kg b.wt)</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.2130±0.2000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2557±0.3733&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3920±0.0163&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2434±0.1207&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>0.3870±0.0141&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3270±0.5910&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3997±0.0202&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3302±0.3275&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>1000</td>
<td>0.2890±0.1160&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0701±0.3434&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3900±0.1120&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3651±0.0000&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>1500</td>
<td>0.4730±0.2641&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8700±0.5910&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4922±0.0000&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5400±0.1300&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2900</td>
<td>0.3200±0.0846&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7900±0.2000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5020±0.8080&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2504±0.5162&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5000</td>
<td>0.3999±0.2116&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1000±0.2232&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5221±0.2312&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5882±0.1410&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.2460±0.0291&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2040±0.2353&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4420±0.0556&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2920±0.1631&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard Deviation, different superscripts in the same column indicates values differ significantly (p < 0.05).

**Plate A.** Kidney section of group administered with 100mg showing normal tubular and glomerulus

**Plate B.** Kidney section of group given 1000mg/kg/wt. showing normal tubular and glomerulus
Plate C. Kidney section of group administered 1500 mg/kg showing slight tubular necrosis with lymphocyte hyperplasia

Plate D. Kidney section of group administered with 2900mg/kg/wt. showing moderate tubular and glomerular necrosis

Plate E. Kidney section of group administered with 5000mg/kg showing slight lymphocyte hypeplasia with sinusoids congestion
Plate F. Kidney section of group given normal saline showing Normal Kidney (control)

Plate G. Liver section of group given 100 mg/kg showing normal hepatocytes

Plate H. Liver of group administered with 1000 mg/kg wt. showing normal hepatocytes
Plate I. Liver section of group given 1500mg/kg showing slight lymphocyte hyperplasia

Plate J. Section of liver given 2900 mg/kg showing vascular congestion and slight necrosis

Plate K. Liver section of groups administered with 5000mg/kg showing moderate vacuolation with necrosis and vascular congestion
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IV. Discussion

Various medicinal plants and botanical drugs have been widely adapted worldwide as precursors for synthesis of important drugs for treating various human afflictions. However only a few of these herbal medicines have been studied intensely (17). (Therefore better understanding on the acute and chronic side effect of these medicinal plants will contribute towards better and vital information in regards to human health care) Thus, the acute and chronic side effects of these medicinal plants contribution to vital information to human health care (18).

In traditional medicine, Tapinanthus globiferus is used for the treatment of various diseases such as stomach ache, hypertension, chronic fever, tinnitus, asthma, inflammations, dyspepsia, relief pain, epilepsy, cough, and cancer (9,19). Phytochemicals (Research shows that phytochemical can have either positive or negative effects on an animal. The presence of active components such as alkaloids, saponins, flavonoids, cardiac glycoside in different amounts in the plant may have been responsible for the plants high activity for treatment of various ailments mentioned earlier (20,21). Alkaloids are used as medications, recreational drugs or in entheogenic rituals e.g. the local anaesthetic and stimulants such as cocaine, caffeine, analgesic morphine and the anti-malarial drug quinine (22). Also Flavonoids are hydroxylated phenolic substances, responsible for their therapeutic potency against wide array of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with the bacterial cell wall. Phytochemicals are thought to have both prooxidant and antioxidant effects on the body (23). While the antioxidant protects the tissues and organs, the prooxidant damages the tissue.

Alkaloids can block ion channels or interfere with neurotransmission producing hallucination, convulsion vomiting and even death (24). Diterpene gossypol blocks phosphorylation and is very toxic, spinasterol from spinach interferes with animal hormone reaction (25). Plant containing cytogenic glycosides can liberate cyanide which blocks cytochrome C-oxidase thus, becoming potentially poisonous (26).

In the present study, the methanolic leaf extracts of T. globiferus did not induce lethality in mice within 24hrs and no mortality was recorded during the course of the experiment. The LD₅₀ of the extract to be greater than 5000 mg/kg is thought to be safe as suggested by Lorke (1983) Hence, the plant extract can be assumed to be safe. However, mild signs of toxicity observed with the group treated with higher doses (Phase-2 groups) were hyperactivity and mild diarrhoea. Elevated serum levels of enzymes produced by the liver or kidney might be indications to their spillage into the blood stream as a result of necrosis of the tissues. In the present test there was no significant difference in serum biochemical tests in groups of animals administered extract compared with the control group. This indicates that the methanolic extract does not possess significant toxic effect.

There was no significant change in the actual organ weights of liver and kidney seen in all animals treated with methanol leaf extract of T. globiferus. The photomicrograph of Liver and Kidney sections treated with methanol leaf extract at (at higher doses) higher doses revealed some mild histopathological changes which were not considered treatment based. This changes could also be due to the presence of bioactive compounds

Plate L. Liver section of group give normal saline showing normal liver.
such as alkaloids, tannins, saponins, flavonoids, oxalate, and glycosides (27,28) which were not completely dissolve in the solvent of extraction.

V. Conclusion

The results of the acute toxicity studies showed that a dose of up to 5000 mg/kg body weight of methanolic leaf extract of Tapinanthus globiferus administered intraperitoneally appeared to be non-toxic.

The Swiss albino rats treated with 100,500 and 1000 mg/kg did not present any significant changes to weights of organs weight, biochemical and gross structure of kidney and liver compared to the control (non-treated). However, further research is necessary in order to conclude if the slight alteration observed in histological observations made on kidney and liver of Phase-2 groups treated with higher doses are reversible. Although, this change appears to be non-significant with regard to the liver and kidney function biochemical parameters.

References


