ASSESSMENT OF ACUTE AND SUBACUTE ORAL TOXICITY ELICITED BY ETHANOLIC EXTRACT OF TURNERA APHRODISIACA LEAVES IN ALBINO RATS

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ABSTRACT

Objectives: The medications of plant-based, herb-mineral, and animal sources have been used by the conventional medics to maintain well-being and care for diseases ever since ancient times. The current study aimed to evaluate the acute and subacute toxicities of the ethanolic extract of Turnera aphrodisiaca (TA) leaves in albino rats.

Methods: The acute toxicity studies were carried out where the maximum dosage of 5000 mg/kg body mass was used. The outcome reported for 24 h and singly daily for 2 weeks. The rats were weighed and a range of interpretations, for example, behavior, lesions, mortality, or any indication of sickness, was carried out daily once throughout the study. For the subacute study, four groups of ten animals (female rats) received 10% Tween 20 in distilled H2O (as control), and 250, 500, and 1000 mg/kg of newly developed extracts, correspondingly, every 24th orally for about 4 weeks. At the end of every study, hematological study and biochemical parameters were assessed.

Results: No major variations (p>0.05) were experienced in the comparative organs, body mass, hematological, biochemical parameters, and offensive malfunctions, in comparison to control, with no mortality reported. Hence, the results of the study may direct the outcome that the intermediate-period oral administration of the TA leaves for 4 weeks does not produce toxicity.

Conclusion: Due to these results, we may well wrap up that leaves of TA extract are non-toxic in all doses considered in this study and did not created any obvious signs in the acute and subacute oral toxicity studies.

Keywords: Acute toxicity, Biochemical investigation, Turnera aphrodisiaca, subacute toxicity.

INTRODUCTION

Medicinal plants have been used in all civilizations and cultures; consequently, they have all the time performed an essential purpose globally in health-care systems. In mainly emergent nations, the native procedures of herbal management are components of the traditions and the principal method of remedial treatment. This remediation’s, with a significant scope of efficacy, are collectively established, cheaply feasible and, typically, are the simply accessible foundation [1]. Medicinal plants utilized in customary medications, thus, encompass a significant task in the safeguarding of well-being globally. Due to the undesirable effects, and as well the improvement of confrontation alongside with synthesized medicines, the role of drugs obtained from medicinal plants are fetching admired in emerging nations [2]. Although, the newest studies encompass several remedial plants that had shown undesirable side-effects [3].

Turnera aphrodisiaca (TA) is usually identified as damiana. Leaves of T. aphrodisiaca have been used customarily as a tonic, diuretic, laxative, aphrodisiac, kidney impairment, menstrual, and maternity conditions [4,5]. According to the British herbal pharmacopeia [6] list particular signs for damiana that is associated with impotency due to anxiety neurosis, it includes new signs, for example, atonic constipation, nervous dyspepsia, depression, and coital insufficiency.

Phytochemical information on T. aphrodisiaca specifies that plant comprises cyanoglycoside [6], gonzaltosin I (flavonoid) [7], arbutin [8], damianin [9], and hexacosanol [10]; a volatile oil including α-pinene, β-pinene, p-cymene, and 1,8-cineole [11] and β-sitosterol (phytosterol) [12].

Although, no study has been explained in the earlier studies on the toxic effect of T. aphrodisiaca plants. As a result, in the current study, we designed to examine the toxic effect (both oral acute and sub-acute) of T. aphrodisiaca leaves with a view to boost the self-assurance in safeguarding humans and to treat a range of disorders.

METHODS

Collection and identification of plant material
Leaves of TA were procured from the nearby Suryapet in September 2017. The plant was being recognized and validated by Dr. Ashok Kumar, Taxonomist, Pratishta Institute of Pharmaceutical Sciences, Suryapet, India, and the receipt sample was stored (03/TA/BOT) for further records.

Preparation of sample
The leaves of TA were collected in fresh form and were dried for 3 weeks. It was augmented into a crude powder using mortar and pestle, which was additionally treated with an electric blender to prepare a fine powder.

Sample extraction
Two hundred grams of finely powdered leaves of TA sample were constantly extracted with 0.5 L of 90% C2H5OH [11] for about 48 h using a Soxhlet apparatus by keeping a temperature of 55°C. The extracted sample was then vaporized under decreased pressure by utilizing a rotational evaporator (Modern Scientific Industries) and moreover compacted in an H2O bath at 55°C yield obtained was air-dried.

Experimental animals
Both sexes albino rats (Rattus norvegicus) weighed 140–155 g were utilized for the acute and subacute toxicity studies. Both rats were acquired from animal house, NIN, Hyderabad, India. The rats were adapted to laboratory conditions for 7 days earlier to the experiments. Rats were kept at a room temp, with a 12 h bright/shady cycle and a humidity (55±5) %.
acclimatization, the animals were randomized into a test and control groups were contained independently in sterile polypropylene cages with sterilized paddy husk as a comforter. The rats were fed free entrée to a normal pellet diet and H₂O impromptu. All investigational events were in observance with the Animal Ethical Committee (GPSEIA) and were permitted by the Institutional Ethical Committee.

Acute oral toxicity study

Acute oral toxicity studies were carried out in accordance with guidelines 420 for testing of chemicals of the Organization of Economic Co-operation and Development (OECD) [12]. Both the sexes (earlier fasted for 16 h) rats of 7–8 weeks old were used. Ethanol extract of TA (EETA) was dissolved in Tween 20 (10%) and delivered orally at a sole dose of 5000 mg/kg at a rate of 1.5 mL/kg to sexes of both rats (n=12; 6 males, 6 females), while the control group received only 10% of Tween 20 as a medium. All animals were after that permitted free admission to food and water and were witnessed for 24 h, with exceptional care given to the first 6 h and 1 time each day for 2 weeks for any indications of the acute toxicity. The visible interpretation of lethality, a range of changes in physical manifestation, behavior (salivation and tiredness), and any damage or sickness were conducted 1 time every day for 2 weeks. After 2 weeks, all rats were anesthetized by ip ketamine injection. Using tubes and non-heparinized tubes, blood samples were collected by cardiac pierce into ethylenediaminetetraacetic acid (EDTA) for hematological and biochemical investigation, respectively. Hematological and biochemical analyses were carried out at Sri Surya Diagnostic Centre, Suryapat, Telangana, India. The rats after that were euthanized by ip ketamine injection. The organs, specifically the lung, heart, spleen, liver, and kidney were vigilantly removed and weighed. The comparative organ weight of every rat was estimated as follows:

\[
\text{Comparative organ weight} = \frac{\text{Organ weight (g)}}{\text{Body weight of the animal on sacrifice day (g)}} \times 100
\]

Subacute toxicity studies

Subacute oral toxicity study was performed out in accordance with guideline 407 for testing of chemicals of OECD [12] and WHO guidelines [13]. 10 female rats were used as a test. According to results of acute toxicity studies stated that EETA was non-toxic at a dose intensity of 5000 mg/kg, subacute toxicity studies of EETA at the doses of 250, 500, and 1000 mg/kg body mass were delivered orally to four groups at each 24 h for 4 weeks and controls received the Tween 20 (10%) as a medium with similar volume. A range of toxic indications and examination, for example, body-weight, mortality, and food and H₂O Intake, was supervised.

After 4 weeks, all survived rats were fasted nightly and anesthetized. For determining hematological parameters, the heparinized blood samples were assembled and the serum from non-heparinized blood was collected for identification of blood biochemistry. After the collection of blood and internal organs were detached and weighed, the organs, specifically the lung, heart, spleen, liver, and kidney were vigilantly removed and weighed. The comparative organ weight of every rat was estimated as follows:

\[
\text{Comparative organ weight} = \frac{\text{Organ weight (g)}}{\text{Body weight of the animal on sacrifice day (g)}} \times 100
\]

Weekly body-weight

The body mass of all rats was vigilantly supervised before the beginning of the study, once weekly throughout the study and on day of the sacrifice.

Mortality and toxic indications

The visible interpretation of lethality, diverse changes in physical manifestations, behavior (sleepy, salivation, and tiredness), and damage or sickness were carried 1 time for 4 weeks, particularly the following dosing and up to after 4 h dosing [14].

Comparative organ weight

After 4 weeks, all the rats were anesthetized by an i.p ketamine injection. Using tubes and non-heparinized tubes, blood samples were assembled by cardiac pierce into EDTA for hematological and biochemical investigation, respectively. The rats after that were euthanized and the inner organs (lungs, heart, spleen, liver, and kidney) were detached and weighed to compute the comparative organ weights (by the above formula) and noticed for any sickening injuries.

Hematological parameters

Following the collection of blood from cardiac pierce into comprising EDTA tubes, different parameters were assessed at Sri Surya Diagnostic Centre, Suryapat, Telangana, India. The hematological parameters such as hemoglobin, red blood cells (RBCs), white blood cells (WBCs), differential WBCs, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, red blood cell distribution width (RDW), platelet distribution width (PDW), platelet large cell ratio, mean platelet volume, and procalcitonin were assessed [15].

| Parameter | Unit | Control | EETA (5000 mg/kg) |
|-----------|------|---------|------------------|
| Hb        | g/L  | 14.07±0.05 | 14.47±0.12        |
| RBCs      | 10⁹/L| 6.52±0.52 | 6.52±0.52         |
| PCV       | L/L  | 0.45±0.05 | 0.45±0.05         |
| MCV       | fl   | 55.22±0.76 | 55.22±0.76        |
| MCH       | Pg   | 18.02±0.41 | 18.02±0.41        |
| MCHC      | g/L  | 327.02±4.12 | 327.02±4.12      |
| WBCs      | 10⁹/L| 9.11±0.9  | 9.11±0.9          |
| Neutrophils | %  | 12.11±1.2 | 12.11±1.2        |
| Lymphocytes | % | 82.22±1.7 | 82.22±1.7        |
| Monocytes | %  | 3.21±0.71 | 3.21±0.71         |
| Eosinophils | % | 1.29±0.12 | 1.29±0.12        |
| Platelet count | 10¹²/L | 856.00±16.31 | 856.00±16.31 |

Values are expressed as mean±SD (n=6). p>0.05., EETA: Ethanolic extract of Turnera aphrodisiaca

Hematological parameters

Table 1: The comparative organ mass of rats treated with a sole dose of EETA for 2 weeks

Table 2: The outcome of hematological parameters in the acute oral toxicity studies

Table 3: The outcome of EETA on biochemical parameters in acute oral-toxicity studies
The treated animals were evaluated based on the intake of water and meal for the whole study with no unusual outcome as compared to control.

Comparative organ weight

Comparative organ weights of 4-week treated animals are depicted in Table 5. The comparative organ mass of every organ reported at the examination in the processed groups did not demonstrate major alterations (p>0.05) as compared to control.

Hematological parameters

The outcomes of subacute management of EETA on hematological parameters are depicted in Table 6. Mainly hematological parameters, for example, hemoglobin, total RBCs, WBCs, RDW, lymphocytes, monocytes, neutrophils, and platelet count, in treated animals were not considerably unlike as of the control.

Biochemical analysis

The outcome of subacute management of EETA on biochemical parameters is depicted in Table 7. The EETA had no consequence on the serum electrolytes (e.g., sodium, chloride, etc.). Parameters, such as urea, uric acid, and creatinine, did not disclose at all major alterations. No statistically noteworthy variations in the parameters of liver function, for example, alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase were reported. Furthermore, no pertinent modifications were established in globulin, albumin, and total protein.

DISCUSSION

For hundreds of years, plant-based medicaments and their preparations have been believed designated as harmless and useful owing to their slight adverse implications. This statement might have prejudiced the haphazard exploit of these preparations to a huge scope among the rural population. The treatments are typically operated over an extended period devoid of appropriate dosage surveillance by the professionals and need of responsiveness of the toxic effects that may produce from such formulations [16-18]. Consequently, systematic awareness against oral-toxicity is greatly desirable, which will not just assist spot the doses that might be used consequently, however, as well to divulge the potential clinical alterations induced by agents in the study. Despite the pharmacological advantages of the TA, comprehensive information concerning subacute toxicities of this remedial plant is deficient. Therefore, the current study was performed to estimate and emphasize the acute and subacute toxicity of TA leaves in albino rats. Furthermore, the dosage of 5000 mg/kg of EETA leaves had no side effect on the treated animal’s up to 2 weeks of surveillance. There were no major alterations in the mass and organs of the rats.

Subacute toxicity assessment

Body weight weekly

A week’s time, body mass was established on the early day of each week of four groups. With control, 1st one was EETA of 250 mg/kg, IIst EETA of 500 mg/kg, and final as EETA of 1000 mg/kg. No major alterations in the body mass were noticed (Table 4).

Clinical observation and lethality

Everyday oral management of EETA for 4 weeks did not create any sign of toxicity in animals, together with the maximum dose experienced at 1000 mg/kg body mass. No mortality or apparent clinical indications were identified in any group during the study. Not at all, the animals displayed indications of toxicities in their salvation, fur, eyes, and skin.

Table 4: The outcome of EETA leaves on body mass of rats (g) at several days

| Group     | Doses               | Weight 1st week | Weight 2nd week | Weight 3rd week | Weight 4th week |
|-----------|---------------------|----------------|----------------|----------------|----------------|
| Control   | -                   | 133.1±3.01     | 137.1±6.14     | 143.4±6.18     | 146.2±6.87     |
| I         | 250 mg/kg           | 136.3±4.11     | 143.1±6.48     | 147.3±6.23     | 152.5±6.72     |
| II        | 500 mg/kg           | 141.2±5.22     | 142.1±5.31     | 151.1±5.91     | 158.8±5.82     |
| III       | 1000 mg/kg          | 142.2±4.21     | 142.1±4.72     | 151.1±4.68     | 152.2±3.02     |

Table 5: The comparative organ mass of rats treated with different doses of EETA for 4 weeks

| Organ   | Control (250 mg/kg) | EETA (500 mg/kg) | EETA (1000 mg/kg) |
|---------|---------------------|------------------|-------------------|
| Heart   | 0.44±0.11           | 0.42±0.03        | 0.41±0.03         |
| Liver   | 3.04±0.12           | 3.25±0.16        | 3.15±0.03         |
| Spleen  | 0.31±0.03           | 0.32±0.02        | 0.28±0.04         |
| Kidney  | 0.75±0.03           | 0.76±0.02        | 0.72±0.01         |
| Lungs   | 0.86±0.05           | 0.94±0.08        | 0.85±0.03         |

Values are expressed as mean±SD (n=10). p>0.05. EETA: Ethanolic extract of Turnera aphrodisiaca

The outcome of subacute management of EETA leaves on body mass of rats (g) at several days

The outcomes of subacute management of EETA leaves on body mass were noticed (Table 1). In the current study, it was observed that no major alteration in the hematological and biochemical requirements demonstrated no major alteration in the physiological conditions. The alterations emerged merely in two treated animals. The rest of the animals (8 rats) revealed no modifications in their histopathology. The LD₅₀ of TA was, consequently, anticipated to be in excess of 5000 mg/kg.

Biochemical estimations

Blood samples were centrifuged at 4000 rcf for 5 min. The serum obtained was analyzed at Sri Surya Diagnostic Centre, Suryapet, Telangana, India, for a range of parameters such as Na⁺, K⁺, Cl⁻, urea, uric acid, creatinine, albumin, globulin, albumin-globulin ratio, total protein, and total bilirubin [15].

Statistical analysis

All estimations were reported as the mean±SD and the outcome was analyzed by one-way ANOVA ensued by Tukey’s multiple comparison tests using GraphPad Prism (GraphPad, CA, USA).

RESULTS

Acute-oral toxicity study

EETA at a dose of 5000 mg/kg created no toxic outcome on the behavioral rebulbs of the treated animals (solo dose) and witnessed for 2 weeks. There were no indications of modifications in the behavior forms, skin, eyes, salivation, and diarrhea of the animals. Nor lethality neither major mass loss was experienced. There were usually no noteworthy variations pragmatic in the comparative organ mass (Table 1). In the current study, it was observed that no major alteration in the hematological and biochemical requirements in the EETA treated group compared to the control group (Tables 2 and 3). Even though several variations have been observed; however, the hematological and biochemical requirements demonstrated no major alteration in the physiological conditions. The alterations emerged merely in two treated animals. The rest of the animals (8 rats) revealed no modifications in their histopathology. The LD₅₀ of TA was, consequently, anticipated to be in excess of 5000 mg/kg.

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addition in the meal and H2O ingestion is measured as being liable for expansion and increase in body mass.

The outcome of the present study demonstrated no major modifications in the comparative organ-weight of control and test groups, which exhibited such that not any of the organs were harmfully impacted, neither shown any indications of toxicity during the investigation.

The hematological variables can be utilized to establish the blood involving the tasks of plant extract. The extort reported an insignificant distinction on the RBC index that proposed that TA does not influence the morphology, erythropoiesis, or osmotic tenderness of RBCs [21]. The hematological variables can be utilized to establish the blood involving the tasks of plant extract. The extort reported an insignificant distinction on the RBC index that proposed that TA does not influence the morphology, erythropoiesis, or osmotic tenderness of RBCs [21].

CONCLUSION

In view of these results, we may well wrap up that leaves of TA extract are non-toxic in all doses considered in this study and did not created any obvious signs in the acute and subacute oral toxicity studies. Moreover, the information of acute and subacute studies of toxicity on leaves of TA was acquired so that to augment the assurance in its well-being to humans for the employment in the preparation of medications. Further studies as well require additional experimental endeavors, for example, subchronic toxicity studies, the outcome of the extract on the fetuses, and their breeding competence to inclusive protection outline of TA.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Nil.

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