Phytochemical screening and Acute Oral Toxicity Study of *Tamarindus indica* pod extract and its methanol fraction

Nandini S*, Suchetha Kumari N.

* Department of Biochemistry, Shimoga Institute of Medical Sciences, Shimoga-577201, Karnataka, India
* Department of Biochemistry, Nitte University, Mangalore, Karnataka, India

**Article history:**
Received: 17 May, 2014
Accepted: 22 May, 2014
Available online: 28 August, 2014

**Keywords:**
Phytochemical screening, Acute oral toxicity, Hydroalcoholic extract (HAE). Methanol fraction (MF), LD$_{50}$, *Tamarindus indica* L (*T. indica*).

**Corresponding Author:**
Nandini S.*
Email: nandinisham@gmail.com

**Abstract**
*Tamarindus indica* has been used by many ethnic groups as herbal medicine to treat various human ailments. Present study aimed to screen phytochemical constituents of *T. indica* pod and its acute oral toxicity profile. Phytochemical screening of hydroalcoholic (70% alcohol) extract of *T. indica* pod and its different fractions such as hexane, chloroform, ethylacetate and methanol were analysed and are found to contain alkaloids, phenols, flavonoids, phytosterols, tannins, saponins, carbohydrates and diterpenes. Acute toxicity profile of hydroalcoholic extract and its methanol fraction of *T. indica* pod, performed at 2000 mg/kg body weight and 5000 mg/Kg body weight resulted in no mortality or evidence of adverse effects. The oral LD$_{50}$ of HAE and MF of *T. indica* pod was therefore estimated to be higher than 5000 mg/kg BW. During 14 days of observatory period no changes were seen in behavioural pattern, body weights, food and water intake of treated mice compared to the control group. Also there were no any significant elevations observed in the biochemical and haematological parameters in the treated groups when compared to control. Further, histopathological examination revealed normal architecture and no significant adverse effects observed in liver and spleen. Overall findings suggests that HAE and MF of *T. indica* pod were found to be nontoxic and safe upto 5000 mg/Kg body weight.

**Citation:**

All Rights Reserved with Photon.

**Photon Ignitor:** ISJN41728352D707228082014

**1. Introduction**

Plants are one of the most important sources of medicine. Today the large number of drugs in use is derived from plants, like morphine from *Papaver somniferum*, Ashwagandha from *Withania somnifera*, Ephedrine from *Ephedra vulgaris*, Atropine from *Atropa belladonna*, Reserpine from *Rauwolfia serpentina* etc. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability (Sarker et al., 2006; Cragg et al., 2006). Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice. India is a rich treasure of medicinal plants and the indigenous medicinal plants have been used for successful treatment of various diseased conditions like bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhoea, arthritis, emetic syndrome, skin diseases, insect bite etc. and in treatment of ulcers, jaundice, cardiovascular and immunological disorders (Sarker et al., 2006; Janice et al., 2007; Okoli et al., 2009; Conforti et al., 2007).

As per Strobel and Daisy there should be some rationale for plant selection i.e it should have an ethnobotanical history, an unusual longevity and growing in areas of great biodiversity (Subash et al., 2012; Strobel and Daisy, 2003). Plant chosen for my study is *Tamarindus indica* L because of its abundant occurrence and has been found being used in treating several diseases in traditional medicine. *Tamarindus indica* L belongs to the family Fabaceae, a multipurpose tree of which every part finds atleas some use either nutritional
or medicinal (Kumar & Bhattacharya, 2008). It is commonly known as Indian date. The awareness of the role of medicinal plants in health care delivery in developing countries has resulted in researches into traditional medicine, with a view to integrate it with the modern orthodox medicine (Sofowora, 1993). Plants in all facets of life have served available starting material for drug developments (Edeoga et al., 2005). Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites (Prashanth Tiwari et al., 2011) such as tannins, flavonoids, steroids, terpenoids, alkaloids and glycosides. The identification, isolation and concentration of these constituents in plants have shown that each plant activity is due to these active components. These components have both physiological and biochemical effect on the body which often leads to the amelioration of diseases (Kumar et al., 2012).

According to Ayurveda, *T. indica* Linn. is used in the treatment of asthma, inflammations, biliousness, dysentery, vaginal and uterine complaints, burning sensation and other conditions (Kirtikar and Basu, 1933; Prashanth et al., 2010). *T. indica*, is widely used in traditional medicine in Africa for the treatment of gastrointestinal disorders, gonococci, fever, jaundice and dysentery (Samina et al., 2008; Kheraro and Adam, 1974; Kobayashi et al., 1996; Ferrara, 2005). Several reports indicate that extracts of Tamarind exhibited antimicrobial (Doughari, 2006), anti-inflammatory and analgesic (Anuama et al., 2012), anti-diabetic (Maiti et al., 2004), antibacterial (Abukaker et al., 2008), antifungal (Pouset et al., 1989), hypcholesterolemic (Maruf et al., 2006), antiulcer (Pankaj et al., 2011), anti-oxidant (Narendra et al., 2009), anthelmintic (Mallikarjuna et al., 2011) and other pharmacological activities. Present study includes the qualitative phytochemical analysis of the bioactive constituents of the HAE and its different fractions of *T. indica* pod and also its acute toxicity profile.

### 2. Objective of Research

Main aim of our research is to provide the evidence that the phytoconstituents present in *T. indica* pod is a potential source of natural antioxidants, and this justifies its use in traditional medicine and also to prove it as toxicologically safe for *in vivo* use.

### 3. Materials and Methods

#### 3.1. Collection of Plant Material

*Tamarindus indica* fruits were collected in January 2012 from Horticulture Dept garden, Shimoga. The plant material is identified as *T. indica* Linn by Dr. K.G. Bhat, Dept of Botany, Poorna Prajna College, Udupi, Karnataka after examination of the specimen.

#### 3.2. Preparation & extraction of plant material

Tamarind pods were thoroughly washed under running tap water and air dried. The fruit pulp was separated, chopped and dried in hot air oven at 40°C for 2 days. The shells, fibres and seeds were separated, dried as above and powdered. Soft part of the pod (Pulp) was macerated with hydroalcohol and extracted using shaker incubator for 48 hours. Hard part (Shell, fibres and Seeds) were powdered and extracted using soxhlet apparatus (hot percolation) with hydroalcohol. Later the macerate was filtered through muslin cloth. Both filtrate and extract obtained by maceration and soxhlet extraction respectively were pooled and evaporated using Rotary flash evaporator. Colour, consistency and percentage yield of HAE is reported in table1.

#### 3.3. Fractionation

Fractionation was carried out by suspending HAE and the consecutive fractions in water separately and partitioning with different organic solvents (n-hexane, chloroform, ethyl acetate and methanol) in the order of increasing polarity by using separating funnel according to standard procedures (Yamin et al., 2011). All extracts were stored in an airtight container at 4°C till further use. Colour, consistency and percentage yield of different fractions of HAE are reported in table1.

#### 3.4 Qualitative phytochemical analysis:

Phytochemical tests were carried out for all, the extract and its different fractions of *T.indica* pod were carried out as per the standard methods (Prashant Tiwari et al., 2011; Khandelwal et al., 1995; Subash et al., 2012).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract</th>
<th>Colour &amp; Consistency</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydro-alcoholic extract (HAE)</td>
<td>Dark brown and non-sticky</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>n-hexane fraction</td>
<td>Dark green and oily</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform fraction</td>
<td>Greenish black and sticky</td>
<td>0.14</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate fraction</td>
<td>Blackish brown and non-sticky</td>
<td>0.8</td>
</tr>
</tbody>
</table>
3.4.1 Test for alkaloids
Extract and its different fractions were dissolved individually in dilute hydrochloric acid and filtered. Each of the 1ml filtrate was measured into four test tubes and was treated with 2 drops of the following reagents.

* Mayer’s test (Potassium Mercuric Iodide): Formation of yellow coloured precipitate indicates the presence of alkaloids.

* Wagner’s test (Iodine in Potassium Iodide): A reddish-brown precipitate indicates the presence of alkaloids.

* Dragendorff’s test (Solution of Potassium Bismuth Iodide): A red precipitate indicates the presence of alkaloids.

* Hager’s test (Saturated picric acid solution): Formation of yellow coloured precipitate indicates the presence of alkaloids.

3.4.2 Test for Glycosides
The extract and their fractions were hydrolyzed with dilute Hydrochloric acid and the hydrolysate was subjected to glycoside tests.

* Modified Borntrager’s Test: Hydrolysates were treated with ferric chloride solution and heated in boiling water bath for about 5 mins separately. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. Rose pink or cherry red colour in ammonia layer indicates the presence of anthraquinone glycoside.

* Legal’s Test: Hydrolysates were treated separately with sodium nitroprusside in pyridine and Sodium hydroxide. Formation of pink to red colour indicates the presence of cardiac glycosides.

3.4.3 Test for Phenols
* Ferric chloride Test: Extract and their fractions were treated with few drops of neutral ferric chloride solution (5%). The formation of bluish black colour indicates the presence of phenols.

3.4.4 Detection of Flavonoids
* Alkaline reagent Test: The extract and their fractions were treated with few drops of sodium hydroxide solution separately. Formation of intense yellow colour, which turned colourless on addition of few drops of dilute acid, indicates the presence of flavonoids.

* Lead acetate Test: The extract and their fractions were treated separately with few drops of 10% lead acetate solution. The formation of yellow precipitate confirms the presence of flavonoids.

* Shinoda Test: The extract and their fractions were treated separately with few fragments of magnesium metal separately, followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta colour indicates the presence of flavonoid.

3.4.5 Test for Phytosterols
* Liberman buchard’s Test: The extract and their fractions were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The formation of brown coloured ring at the junction confirms the presence of steroids.

* Salkowski’s Test: The extract and their fractions were treated with chloroform and filtered. The filtrates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand.
Appearance of red or violet colour at the interface indicates the presence of triterpenes.

3.4.6 Test for Tannins
* Gelatin Test: To the test material, 1% gelatin in sodium chloride solution was added. Formation of white precipitate indicates the presence of tannins.

3.4.7 Test for Saponins
* Froth Test: The extract and their fractions were diluted with 20 ml of distilled water separately and further shaken for 15 mins in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicates the presence of saponin.

3.4.8 Test for Carbohydrates
Total alcoholic extracts and their fractions were dissolved individually in 5ml of distilled water, shaken vigorously and filtered. The filtrates were used to test the presence of carbohydrates.

* Molisch Test: Filtrates were treated with 1-2 drops of alcoholic α-naphthol solution in a test tube and 2 ml concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.
* Benedict’s test: Filtrates were treated with Benedict’s reagent and heated in water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.
* Fehling’s Test: Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling’s A and Fehling’s B solutions. Red precipitate formed indicates the presence of carbohydrates.
* Barfoed’s Test: Filtrates were treated with Barfoed’s reagent and heated in water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.

3.4.9 Test for Diterpenes
Copper acetate test: The extract and their fractions were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Tests</th>
<th>HAE &amp; its fractions of T. indica pod</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>HAE</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Diterpenes</td>
<td>-</td>
</tr>
</tbody>
</table>

HAE-hydroalcoholic extract, HF- hexane fraction, CF- Chloroform fraction, EAF-Ethylacetate fraction, MF- methanol fraction .+: indicates the presence of constituent by the given assay, - : represents the constituents not detected by the specified assay method.

3.5 Experimental animals
Healthy adult female Swiss albino mice (8 weeks) used for the acute oral toxicity study were bred and reared at the Institutional Animal House. The animals were housed in polypropylene cages with stainless steel grill tops and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions 5 days prior to treatment. The temperature in the animal room was maintained between 25 ± 3 °C with a relative humidity of 30-70% and illumination cycle set to 12 h light and 12 h dark. The animals were fed with commercially available standard pellet chow and unlimited supply of drinking water.

3.6 Acute Oral Toxicity
Thirty animals were randomly allocated into five groups of five animals each (n=5). Animals were fasted for 3-4 hours before treatment. Following the period of fasting, the fasted body wt of each animal was determined and the dose was calculated according to their body weight. Group I served as control which were administered with distilled water orally in a single dose using gavage. Group II and III were administered with HAE and MF of T. indica pod dissolved in water at a dose of 2000 mg/kg body weight respectively whereas Group IV and V were administered with the same test substances but at 5000 mg/kg body weight respectively. After the administration of test substance, food was withheld further for an hour. The volume given was
not more than 1ml/100g body weight. Limit test was performed as per OECD guidelines 425 (OECD Test Guideline 425).

Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 2 hours), and daily thereafter, for a total of 14 days. Animals were weighed weekly thereafter and observed for the next 14 days for any delayed toxic effects. The visual observations included changes in the skin and fur, eyes and mucous membranes and behavioural pattern. Attention was given to parameters like grooming, hyperactivity, convulsions, tremors, salivation, lethargy, diarrhoea, loss of righting reflex, sleep and coma (Bandawane et al., 2010; Ecobichon DJ., 1997).

3.7 Haematological Parameters: At the end of experimental period, the animals were euthanized, blood samples collected through cardiac puncture into EDTA tube and counts of WBC, RBC and levels of Haemoglobin were recorded using Hematology Analyser (ERMA PCE 210 VET).

3.8 Biochemical parameters
Blood collected in gel tube is centrifuged at 3000 rpm for 10 minutes. Serum separated is used for biochemical estimation viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum bilirubin contents by using commercially available reagent kits (Span Diagnostics, Surat, India).

3.9 Statistical analysis
5 rodents are used as per the guidelines provided by OECD 425 and the results were expressed as mean ± standard error of mean. P>0.05; Non significant.

4. Results

Phytochemical screening
HAE of T. indica pod gave an yield of 370g per kg powdered material, which adds up to 37% w/w. HAE on fractionation gives 0.9%HF, 0.14% CF, 0.8% EF, 82.32%MF and 2%MIF. Phytochemical screening of HAE showed the presence of flavonoids, phytosterols, carbohydrates, and diterpenes. HF-phytosterols and carbohydrates, CF-phenols, flavonoids, carbohydrates and diterpenes, EF- alkaloids, phenols, flavonoids, carbohydrates and diterpenes whereas MF revealed the presence of phenols, flavonoids, tannins, saponins, carbohydrates and diterpenes (Table 2).

Acute oral toxicity studies of both HAE and MF of T.indica pod at 2000 mg/kg body weight and 5000 mg/kg body weight exhibited no toxic effects or mortality in mice.

Food and water intake of treated group was found comparable to the control group without showing significant alteration (p>0.05) in body weight (Table 3) after 14 days.

Table 3: Effect of HAE and MF of T. indica pod on body weight in mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial weight (gms)</th>
<th>Final weight (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (dist.water)</td>
<td>26.4 ± 1.21</td>
<td>27.4 ± 0.91</td>
</tr>
<tr>
<td>HAE (2000 mg/kg)</td>
<td>27.3±0.95</td>
<td>28.1±1.3</td>
</tr>
<tr>
<td>MF (2000 mg/kg)</td>
<td>25.9±1.02</td>
<td>26.4±1.8</td>
</tr>
<tr>
<td>HAE (5000 mg/kg)</td>
<td>28.±2.1</td>
<td>28.7±2.01</td>
</tr>
<tr>
<td>MF (5000 mg/kg)</td>
<td>26.8±1.5</td>
<td>27.1±0.8</td>
</tr>
</tbody>
</table>

Serum biomarkers of liver damage such as AST, ALT, ALP and Bilirubin recorded non significant (p>0.05) alterations in all the treated groups when compared to control (Table 4).

Table 4: Effect of HAE and MF of T. indica pod on biochemical parameters in mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (dist.water)</td>
<td>48.4 ± 2.98</td>
<td>15.6± 0.64</td>
<td>68.5±1.3</td>
<td>0.84 ±0.2</td>
</tr>
<tr>
<td>HAE (2000 mg/kg)</td>
<td>49.28±4.2</td>
<td>16.2±2.3</td>
<td>69.85±2.5</td>
<td>0.87±0.23</td>
</tr>
<tr>
<td>MF (2000 mg/kg)</td>
<td>52.45±2.8</td>
<td>17.4±2.8</td>
<td>71.5±1.8</td>
<td>0.95 ±0.18</td>
</tr>
<tr>
<td>HAE (5000 mg/kg)</td>
<td>51.36±3.5</td>
<td>16.8±3.2</td>
<td>70.3±4.6</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>MF (5000 mg/kg)</td>
<td>54.8±1.9</td>
<td>18.5±1.6</td>
<td>74.38±3.4</td>
<td>1.0 ±0.25</td>
</tr>
</tbody>
</table>

The hematological parameters (RBC, WBC, Hb) did not record any significant alterations (p>0.05) in any of the treated groups when compared to control (Table 5).

Table 5: Effect of HAE and MF of T. indica pod on hematological parameters in mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>WBC (10^3 cells/µl)</th>
<th>RBC (10^5 cells/µl)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (Dist Water)</td>
<td>5.65 ±0.56</td>
<td>11.36 ±0.93</td>
<td>14.12 ±0.85</td>
</tr>
<tr>
<td>HAE (2000mg/kg)</td>
<td>5.26 ± 0.74</td>
<td>11.14 ± 0.54</td>
<td>13.98 ± 0.36</td>
</tr>
<tr>
<td>MF (2000mg/kg)</td>
<td>5.94 ± 0.43</td>
<td>10.8 ± 0.46</td>
<td>13.34 ± 0.64</td>
</tr>
<tr>
<td>HAE (5000mg/kg)</td>
<td>5.11 ± 0.81</td>
<td>11.00 ± 0.74</td>
<td>13.76 ± 0.32</td>
</tr>
<tr>
<td>MF (5000mg/kg)</td>
<td>6.01 ± 0.32</td>
<td>10.44 ± 0.91</td>
<td>13.04 ± 0.28</td>
</tr>
</tbody>
</table>
Organ weights of liver, kidney, spleen did not record any significant alterations (P>0.05) in the treated groups when compared to control (Table 6). A detailed examination of histology of liver and spleen were performed. It did not show observable changes in cellular morphology of the organs of treated groups when compared to the control group.

Table 6: Effect of HAE and MF on organ weight in mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Liver (gms)</th>
<th>Spleen (gms)</th>
<th>Kidney (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (Dist Water)</td>
<td>1.34±0.21</td>
<td>0.10±0.02</td>
<td>0.36±0.08</td>
</tr>
<tr>
<td>HAE (2000mg/kg)</td>
<td>1.36±0.12</td>
<td>0.11±0.02</td>
<td>0.41±0.09</td>
</tr>
<tr>
<td>MF (2000mg/kg)</td>
<td>1.29±0.01</td>
<td>0.12±0.022</td>
<td>0.36±0.07</td>
</tr>
<tr>
<td>HAE (5000mg/kg)</td>
<td>1.33±0.05</td>
<td>0.13±0.02</td>
<td>0.38±0.06</td>
</tr>
<tr>
<td>MF (5000mg/kg)</td>
<td>1.26±0.07</td>
<td>0.14±0.01</td>
<td>0.44±0.04</td>
</tr>
</tbody>
</table>

5. Discussion

Extraction, fractionation and acute toxicity studies have been performed on different parts of the plant such as leaf, seed, pulp, bark, etc. but not on the whole fruit (pod) as such. Our method of extraction has given the highest yield (37%) when compared to the previous work. Abukaker et al has reported aqueous extract of T. indica pulp has given a yield of 25.2%. As per Narendra Vyas et al ethanolic extract of T. indica seed’s yield was 9.3%. According to Anupama et al methanolic extract of seed has given 8% yield. Deepti et al has reported the methanolic extract of T. indica seeds yield was 17.9 %. Phytotherapeutic products from medicinal plants have become universally popular in primary healthcare, particularly in developing countries, and some have been mistakenly regarded as safe just because they are a natural source (Gesler WM., 1992; Vaghasiya et al., 2011; Subramanion et al., 2011). Emphasis on toxicology has shifted from studying the mere toxicity of chemicals to the evaluation of its safety and natural products are no exception to it. All medicines or pharmaceuticals irrespective of their source must be evaluated for its efficacy and safety before its intended use in humans or animals (Arunkumar HS., 2005). Hence acute oral toxicity of HAE & MF of T. indica pod is performed in mice as it determines the safety of the drug. Based on historical research, the oral route of administration is the most convenient and commonly used one, when studying acute toxicity. The absorption might be slow, but this method costs less and is painless to the animals. Since the crude extract is administered orally, the animals should be fasted before taking the dose because food and other chemicals in the digestive tract may affect the reaction of the compound. Although there is a problem regarding extrapolating animal’s data to humans’, a study has shown that mice give better prediction for human acute lethal dose compared to rats (Walum et al., 1995). All the procedures were performed based on the appropriate OECD guidelines (OECD-420, 2001). In this study, the mice in the control and treated groups were administrated with vehicle, HAE and MF, respectively. The mice were monitored daily until day fourteen for any toxic signs and mortality. During the 14 days period of acute toxicity evaluation, mice which are orally administrated with HAE and MF at single dose of 2000 mg/Kg and 5000 mg/kg respectively showed no overt signs of distress, and there were no observable symptoms of either toxicity or death. All of the mice gained weight and displayed no significant changes in behaviour. Apart from that, the physical appearance such as skin, fur and eyes were found to be normal whereas body weight of all mice were increased (Table 3). Increase in body weight of test animals were not statistically significant when compared to control which indicates that the administration of HAE and MF has negligible level of toxicity on growth of the animals. Furthermore, determination of food intake and water consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drugs tested (Steven et al., 1994; Iversen et al., 2003). In this study, the food intake and water consumption also was not affected by the administration of HAE and MF of T. indica and it did not induce appetite suppression and had no deleterious effects and hence this complies that there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen CD., 2001). Generally, the alterations of body weight gain of mice would reflect the toxicity after exposure to the toxic substances (Carol et al., 1995). The body weight changes are indicators of adverse effects of drugs and chemicals and it will be significant if the body weight loss occurred is more than 10% from the initial weight (Raza et al., 2002; Teo et al., 2002). This study reckoned that HAE and MF of T. indica pod extracts do not cause acute toxicity effects and an LD₅₀ value greater than 5000 mg/kg. In principle, the limit test method is not intended for determining a precise LD₅₀ value, but it serves as a suggestion for classifying the crude extract based on the expectation at which dose level the animals are expected to survive (Roopashree et al., 2009). According to the chemical labelling and classification of acute systemic toxicity recommended by OECD, HAE and MF of T. indica pod was assigned class 5 status (LD₅₀ > 5000 mg/kg BW) which was the lowest toxicity class.
Research Highlights

Extraction of the fruit is done employing two methods so as to elute the maximum constituents. Crude obtained is subjected to fractionation using different organic solvents in the order of increasing polarity. Phytochemical screening of HAE and its different fractions has been done. Acute toxicity of HAE and its high yielding fraction, MF has been conducted at different doses viz. 2000 mg/Kg and 5000 mg/Kg BW during the research.

Recommendations

There is a need to perform the extraction of shell, seed and pulp separately and to screen them qualitatively as well as quantitatively for their phytoconstituents, also to perform acute toxicity profile of the individual extract separately, as it is said that mild and moderate behavioural changes were observed such as fatigue, malaise, confusion and anorexia when rats were administered with 3000 mg/Kg and 5000mg/Kg BW of ethanolic pulp extract of T. indica which may be attributed to secondary metabolite content of the extract such as saponin according to Abubakar et al (2010).

A detailed experimental analysis of its sub-acute and chronic toxicity studies is essential for further support of this drug in different species of rodents.

Funding and Policy aspects

Present work is self sponsored and not supported by any funding agency/ source.

Conclusion

Present study provides valuable data on the phytoconstituents of the whole fruit, which justifies its use in curing various diseases since ancient times. It also demonstrated HAE and the high yielding MF of T. indica pod is toxicologically safe upto 5000 mg/Kg BW for in vivo use and its LD_{50} is found to be more than 5000mg/Kg BW. According to the chemical labelling and classification of acute systemic toxicity recommended by OECD, HAE and MF of T. indica pod was assigned class 5 status (LD_{50} > 5000 mg/kg BW) which was the lowest toxicity class.

Acknowledgement

I Thank Dr. Kuppast, The Principal; Dr Shrirdhar Shetty, HOD, Dept of Pharmaceutical Chemistry, and Dr. G.N. Murthy, Professor, Dept of Pharmacognosy, National College of Pharmacy, Shimoga, Karnataka, India for providing the necessary support and guidance.

References


Phytochemical screening and acute toxicity study of ethanolic extract of *Alpinia galanga* in rodents. International Journal of Medical Research & Health Sciences. 2(1), 93-100.


Vaghasiya Y.K., Shukla V.J., Chanda S.V., 2011. Acute oral toxicity study of Pluchea arguta boiss extract in mice. Journal of Pharmacology and Toxicology. 6, 113-123.

