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Phytochemical analysis and acute toxicity studies of *Artemisia annua* in Swiss albino mice

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Abstract

Artemisia annua also known as sweet Annie or Wormwood belonging to the family Asteraceae and the genus *Artemisia*, widely distributed in Asia, Europe and North America. The main chemical constituent obtained from *Artemisia annua* is a hydrophobic sesquiterpene lactone called Artemisinin which is responsible for the anti malarial action. The per cent extractability of the extract of *Artemisia annua* was 4.89 per cent. The extract of whole plant of *Artemisia annua* was greenish brown in colour, solid and sticky in consistency, bitter in taste and pleasant in odour. Phytochemical analysis (qualitative) revealed presence of alkaloids, flavonoids, saponin, tannins, steroids, glycosides and phenols in the twelve different solvent extracts of *Artemisia annua*. The hydro-ethanolic plant extract of *Artemisia annua* was administered orally upto 5000 mg/kg body weight in swiss mice was shown no lethality or toxic reactions at any of the doses of *Artemisia annua* extract. The absence of toxicity symptoms suggest that *Artemisia annua* was non toxic and was well tolerated at the doses employed in this study.

Keywords: *Artemisia annua*, extractability, phytochemical analysis, acute toxicity studies, mice

Introduction

Artemisia annua has been used in traditional chinese medicine for treatment of malaria and fever because it contains artemisinin, a sesquiterpen found and in enough amounts to be used as a therapeutic agent. *Artemisia annua* also known as sweet annie or wormwood belonging to the family asteraceae of the genus *artemisia*, widely distributed in asia, europe and north america. Artemisinin is most important class of antimalarials because it is potent rapid acting blood schizonticides effective against multi drug resistant parasites and hence can be used to treat severe malaria (potawale *et al.* 2008) [10].

In 1971, extraction of aerial parts of *artemisia annua* with low-boiling solvents like diethylether, produced a compound mixture that had antimalarial properties on infected mice and monkeys. Artemisinin is now available commercially in china and vietnam as an antimalarial drug efficacious against drug resistant strains of *plasmodium malariae*, the malaria parasite. A semi synthetic drug based onartemisinin (artemether) has been recently registered in africa as paluther. The artemisinin also has toxic effect, even on *artemisia annua*, and is a candidate for natural herbicide (duke *et al.* 1987 and chen *et al.* 1991) [5, 4].

Artemisia annua, an annual herb has fern-like leaves, bright yellow flowers and a camphor-like scent. Its height averages about 2 m tall. The plant has a single stem, alternating branches with alternating leaves which range 2.5-5cm in length. It is cross-pollinated by wind or insects. It is a diploid plant with chromosome number, 2n=36. Artemisinin may be extracted using a low boiling point solvent such as diethyl ether and is found in the glandular trichomes of the leaves, stems and infloresces. It is concentrated in the upper portions of plant within new growth (potawale *et al.* 2008) [10].

The present study was undertaken to evaluate the hydroethanolic extract of *artemisia annua*, phytochemical screening and acute toxicity studies on swiss albino mice.

Materials and Methods

The present study was carried out in the Department of Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola (Maharashtra State).

1. Preparation of extract and determination of per cent extractability

Artemisia annua plant was purchased from local market of Akola District of Maharashtra. All the procured plant materials were identified and authenticated from expert botanist, Department of Botany, Shri Shivaji Science College, Akola (M.S).

The freshly prepared dried powder of whole plant (25 g) of *Artemisia annua* was immersed in hydro-ethanolic solution (40 % distilled water + 60 % ethanol) in a flask stoppered tightly with cotton plug and was kept at room temperature for 48 hours at 150 rpm in an orbital shaker. The contents of the flask were filtered through muslin cloth. The residue left in the flask was rinsed with little quantity of hydro-alcoholic solvent and filtered through the muslin cloth. The filtrate thus obtained was filtered through Whatman filter paper No. 1. Final filtrate, so obtained was transferred to previously weighed large petri dish and was kept for evaporation of solvent at room temperature. After complete evaporation, the petri dish was once again weighed to know the amount of extract for determination of per cent extractability. The extract was stored in airtight screw cap vials and kept in the desiccators until further use during the study.

2. Phytochemical analysis

The freshly prepared plant extract of *Artemisia annua* was subjected for phytochemical analysis (qualitative) for identification of different phytoconstituents in twelve different solvents viz. acetic acid, acetone, benzene, chloroform, distilled water, ethyl acetate, ethanol, hexane, hydro-ethanol, methanol, petroleum ether and xylene for the presence of the active phytochemical constituents as per various test such as test for alkaloids (Dragendroff's reagent, Mayer's reagent (Potassium mercuric iodide reagent and Wagner's reagent), test for reducing sugars (Fehling's solution test and Benedict's reagent), test for glycosides (Benedict's reagent and Fehling's reagent), test for tannins (Ferric chloride test and Lead acetate test), test for sterol (Salkowski's reaction), test for anthraquinones (Borntrager's test), test for flavonoids, test for proteins (Biuret test and Xanthoprotein test), test for amino acids (Ninhydrine test), test for saponins (Foam test) and test of phenolics (Roberts *et al.*, 1981) [11].

3. Acute toxicity study

Acute toxicity study was performed according to the OECD-423 guidelines. Swiss albino mice (20 - 25 g) from either sex were used. The mice were procured from the recognized CPCSEA authorized laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS Akola. The experimental protocol was approved from IAEC of

PGIVAS, Akola. The animals were administered with 50, 300, 2000 and 5000 mg/kg BW of extract of *Artemisia annua* orally (p.o). The animals were observed for 24 hours, then for further 14 days for deaths and manifestation of toxic effects. Like agility, muscular tremors and convulsions were observed and recorded.

Results and Discussions

Ethanol (60 %) is one of most suitable solvent for extraction of crude plant material therefore, in present study it was used for extracting whole plant of *Artemisia annua* which gave the average yield of 4.89 g extract obtained from 100 g whole plant of *Artemisia annua* powder. The per cent extractability of the extract of *Artemisia annua* was 4.89 per cent. The extract of whole plant of *Artemisia annua* was greenish brown in colour, solid and sticky in consistency, bitter in taste and pleasant in odour. Per cent extractability and some of physical properties of extracts are shown in Table 1.

Table 1: Per cent extractability and physical properties of *Artemisia annua* plant extract

| Solvent used | Per cent Extractability | Colour | Consistency | Odour |
|--------------|-------------------------|----------------|----------------|----------|
| Ethanol 60% | 4.89 % | Greenish brown | Solid & sticky | Pleasant |

Phytochemical (Qualitative) analysis of plant extract of *Artemisia annua* was carried out to know the active constituents present in the above mentioned plant. Phytochemical analysis of *Artemisia annua* was shown in Table 2. In phytochemical analysis (qualitative), *Artemisia annua* was extracted in twelve different solvent. The results of the phytochemical analysis are depicted in Table 2.

Alkaloids, flavonoids, saponin, tannins, steroids, glycosides and phenols were found in the twelve different solvent extracts of *Artemisia annua*. In related studies Ajah and Eteng (2010) [2], Kumar and Upadhyaya (2013) [8], Owuna *et al.* (2013) [9] and Enas *et al.* (2015) [6] reviewed qualitative estimations of *Artemisia annua* with phytochemical studies and reported several compounds of confirmed biological activity such as tannins, alkaloids, flavonoids, saponin, steroids, glycosides and phenols from *Artemisia annua*, which are in agreement with the present study.

Table 2: Phytochemical analysis (qualitative) of extract of *Artemisia annua* plant.

| Solvent used | Active principle | | | | | | | | | | |
|-----------------|------------------|--------------|----------------|------------|---------|----------|--------|--------|----------------|-------------|-----------|
| | Alkaloides | Flavon oides | Anthra quinine | Amino acid | Protein | Sap onin | Tanins | Sterol | Reducing sugar | Glyco sides | Phenolics |
| Acetic acid | - | - | - | - | - | + | - | - | - | - | + |
| Acetone | - | + | - | - | - | - | - | - | - | - | + |
| Benzene | - | - | - | - | - | + | - | ++ | - | ++ | + |
| Chloroform | + | - | - | - | - | + | + | ++ | - | + | +++ |
| Ethyl acetate | - | - | - | - | - | - | - | + | - | - | - |
| Ethanol | +++ | - | - | - | - | - | + | + | - | - | + |
| Hexane | - | - | - | - | - | + | - | +++ | - | + | - |
| Hydroethanol | ++ | ++ | - | - | - | ++ | + | +++ | - | ++ | ++ |
| Methanol | + | + | - | - | - | - | - | ++ | - | - | + |
| Petroleum ether | - | + | - | - | - | ++ | - | +++ | - | + | - |
| Xylene | - | +++ | - | - | - | + | +++ | - | - | - | +++ |
| Water | - | - | - | - | - | - | - | - | - | - | - |

- Nil
+ Mild
++ Moderate
+++ Abundance

In acute toxicity study, the hydro-ethanolic plant extract of *Artemisia annua* was administered orally at 50, 300, 2000 and 5000 mg/kg body weight to set of six mice per group. There was no lethality or toxic reaction found at any of the doses of *Artemisia annua* extract. The absence of toxicity symptoms suggest that *Artemisia annua* was non toxic and was well tolerated at the doses employed in this study. In similar studies Ahmed (2008) ^[1] reported that oral administration of the *Artemisia annua* extracts in doses from 1000 to 5000 mg/kg did not produce significant acute toxic effects on the experimental mice.

The phenolics contains of plant is indicates powerful antioxidants property. The presence of saponins protects plant from microbial pathogens. The presence of flavonoids in the plant shows anti-inflammatory effect in the same way as the non-steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins (Berknow, 1992) ^[3]. These bioactive agents (flavonoids and saponins) have the ability to inhibit pain perception and can also serve as anti inflammatory agents (Facino *et al.* 1994) ^[7]. Further studies may reveal the mechanisms of action responsible for the analgesic, anti inflammatory and hepatoprotective activity of *Artemisia annua*.

The results of the present study revealed that the extract has large class of phytoconstituents, which may be responsible for many pharmacological activities. However, further work is required to investigate all extracts of plant parts of *Artemisia annua* for various pharmacological activities.

Conflict of Interest: All authors declare no conflict of interest.

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