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ACUTE TOXICITY STUDY OF AQUEOUS ALCOHOLIC EXTRACTS OF NYCTANTHES ARBOR TRISTIS, VITEX NEGUNDO, BOSWELLIA SERRATA, CYPERUS SCARIOSUS AND COMMIPHORA MUKUL

MAYA KRISHNA GUPTA
Shri Venkateshwara University
Gajraula, Uttar Pradesh

DR. BHUWANENDRA SINGH
Shri Venkateshwara University
Gajraula, Uttar Pradesh

YASH PRATAP SINGH RANA
SD College of Science Atrauli
Aligarh, Uttar Pradesh

ABSTRACT:

The plants which are used in the acute toxicity study were *Nyctanthes arbor tritis, Vitex negundo, Boswellia serrata, Cyprus scariosus* and *Commiphora mukul* all these plant are known for their Anti-arthiritic activity from various traditional literature further the study of this activity with the synergistic effect will have the higher efficacy than the individual extract so for the safety of all the extract and to study the efficacy the primary aim is to consider the acute toxicity study. Present study was designed for acute toxicity study of different aqueous alcoholic extract of all the five drugs used in the treatment of arthritis which were done *in vivo* where various group of 8 wistar rats. There was no sign of toxicity observed at the dose level of up to 6mg/kg body weight.

1. INTRODUCTION

Rheumatoid Arthritis (RA) is a most frequent inflammation disease which affects huge number of the people world widely. Joint disorders are the main symptom if it is not treated early. It is surely fatal disease increases the risk of life or we can say mortality by two times and average life span is decreased by 5 to 7 years. Rheumatoid Arthritis patients suffer from the other diseases such as the cardiac arrest and the disorder of the renal disturbances. Rheumatoid arthiritis affects the great percentages of disease that is about 1 percent. Main patient for the disease are females as females are more affected by this disease [1]. Various herbal drugs used in the treatment some of them can be used synergistically for the treatment so the aqueous alcoholic extracts of the following drugs were tested for its acute toxicity study:

Commiphora mukul

Guggul (Commiphora mukul), a highly valued botanical medicines has been used for centuaries in ayurveda to treat several ailments. Various extracts of this drug produces guggulipid in that guggulsterone is the active constituent responsible for its therapeutic activity.

Cyperus scariosus

Traditional medicine as defined by WHO refers to the complementary/alternative/non-conventional/indigenous medicine that is developed based on the theories, beliefs and experiences innate to different cultures, whether interpretable or not, used to maintain health, as well as prevent, attenuate or cure physical and mental illnesses [2]. Out of the 7.5 billion world's population, 4.5 billion of them use traditional medicines for primary healthcare. India's population being equivalent to 17.84% of the total world population, around 0.93 billion Indians still use traditional medicines for maintaining primary health [3, 4].

Boswellia serrata

Boswellia serrata (Salai/Salai guggul) (Family: Burseraceae; Genus: *Boswellia*) is a moderate to large sized branching tree that grows in dry mountainous regions of India, Northern Africa and the Middle East [5, 6]. The family of Burseraceae is represented in the plant kingdom with 17 genera and 600 species wide-spread in all tropical regions. There are about 25 known species belonging to Genus *Boswellia*, most of them occur in Arabia, northeastern coast of Africa and India. Since ancient times, three of these species have been considered as 'true Frankincense' producing trees [7, 8].

Vitex negundo

Arthiritis is one of the most common diseases in old geriatric people and occurs in different forms. The most common form is osteoarthiritis which results in trauma and infection in the joints. Arthiritis is a major problem in oldage people and it needs chronic treatment with analgesics. The chronic treatment may cause adverse effects and that may increase further complications. Hence we need some alternative system of natural medicine of to treat arthiritis. *Vitex negundo* Linn. (Verbinaceae) is widely distributed in the region of south Asia and it has anti-inflammatory, antibacterial, antifungal and analgesic properties. This plant is used in the treatment of superficial buises, injuries, sores, and skin infections [9,10].

Nyctanthes arbor tristis

Nyctanthes arbor tristis Linn. (Harsingar) is widely used as a decoction in the Ayurvedic system of medicine for treatment of sciatica and arthritis, but it has not yet been screened scientifically. In the present study, the water soluble portion of the alcoholic extract of the leaves of *Nyctanthes arbor tristis* (NAT) was screened for the presence of anti-inflammatory activity.[11]

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh samples of plants were collected from in the month September, 2018 and were authenticated through Sri Sai Ayurvedic Medical College and Hospital Aligarh having the specimen no. RKT14360 and the herbarium were preserved. Plants and its parts were washed thoroughly to remove impurities and few samples were dried in hot air oven at 55˚C. Then grounded to yield fine powder which was further subjected for the extraction processes.

2.2 Preparation of plant extract

The extraction process was carried out by Hot Continuous Extraction (Soxhlet) where the solvent aqueous alcoholic is used for all the drugs which was in the ratio water:alcohol (1:1)[12].

The yield of aqueous alcoholic extract after the extraction was found to be **2.5%**.

2.3 Experimental animals

For acute toxicity study

30 male Swiss albino mice of body weight from 25-30 g were procured from Mangalaytan University Aligarh. The animals were housed in polypropylene cages in air conditioned room with controlled temperature and alternating 12 hour periods of light and dark were maintained. The animals were acclimatized to standard laboratory conditions prior to experimentation. The guidelines issued by Institutional Animal Ethics Committee of Mangalaytan University Aligarh.

2.4 Acute Toxicity study

Acute Toxicity study of all five plant extracts was carried out by using mice as the experimental model. The study was carried out to assess the acute toxicity of the plant extract made by the extraction of plant material with continuous hot extraction (Soxhletion). The study was carried out as per the details laid down in OECD guidelines 420 viz, fixed dose procedure (Evident toxicity). More clinical observation such as condition of fur, damage area of skin, subcutaneous swelling or lumps, abdominal detention, eye dullness, eye opacity, pupil diameter, ptosis (drooping of upper eyelid), colour and condition of faeces, wetness or soiling of perineum, condition of teeth and breathing abnormalities should be recorded as indication of toxicity [13].

2.5 Composition of diet

The animals were fed on the standard pellet diet, and water was given *ad libitum*. The standard pellet diet comprised 20% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates).

3. RESULT AND DISCUSSION

3.1 For Acute Toxicity Study

Protocol:-

1. Animal species/ strain	Albino Swiss mice.
2. Sex	Male.
3. Body weight	24–30.
4. Animal procured from	Mangalaytan University Aligarh
5. No. of doses groups	12
6. Animals per group	5
7. Route of administration	Oral via gauge.
8. Vehicle of administration	Distilled water
9. Volume of administration	Not more than 1ml as combined volume of plant sample and vehicle.
10. Dosing details	Refer to dosing table (Table 1).
11. Observation period	14 days post dose and 7 days prior to dosing.

1. Clinical Observation

Assessment of the behavior of animals was carried out by general observations of each animal on a daily basis from the stage of dosing to the end of the study as compared to control. Any changes or abnormalities recorded could be an indication of toxicity. The test animals at all dose levels showed no significant changes in behavior before and after the administration of an oral dose of whole plant powder as slurry. The clinical observation detailed below is in general for the plant material under investigation[14,15].

1. Condition of fur	Normal

2. Damage area of skin	Normal
3. Subcutaneous swelling or lumps	Normal
4. Abdominal detention	Normal
5. Eye dullness	Normal
6. Eye opacity	Normal
7. Pupil diameter	Normal
8. Ptosis (drooping of upper eyelid).	Normal
9. Colour and condition of faeces	Normal
10. Wetness or soiling of perineum	Nil
11. Condition of teeth	Normal
12. Breathing abnormalities	Normal

2. Body weight changes

Body weight is an important factor to monitor the health of the animal. The loss of body is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10% or more reduction in body weight, is considered to be a toxic dose. It is considered to be the dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes. All the animals from treated groups did not show any significant decrease in body weight for all the 14 days as compared with the 0 day it thus indicating no signs of toxicity (Table 2).

3. Food and water consumption

There was no significant change in food and water consumption (Table 3 and 4).

4. Mortality

Mortality is the main criterion in assessing the acute toxicity (LD50) of a drug. There was no mortality recorded even at the highest dose level i.e.6g/kg body weight of all the groups.

From the results of this study it is observed that there is no significant change in body weight, food and water consumption by the male Wistar rats from all the dose groups. There was no mortality recorded even at the highest dose level i.e.6g/kg body weight, which proves that the aqueous alcoholic extracts of all drugs have no toxic effect in male Wistar rats. The results have indicated that this plant is safe.

4. CONCLUSION

From the results of this study it is observed that there is no significant change in body weight, food and water consumption by the Wistar rats from all the dose groups. There was no mortality recorded even at the highest dose level i.e.5g/kg body weight, which proves that aqueous alcoholic extracts of *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* have no toxic effect in Wistar rats. The results have indicated that the aueous alcoholic extract of *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* plant is safe and can be used for efficacy studies for different activities at the effective dose of 600 mg/kg.

Table 1: Dose regimen of acute toxicity study of aqueous alcoholic extracts of all the five drugs:

Group	Sex	Extract	Dose. g/kg. body weight	No. of animal used	Total volume administered
I	Male	NYCTANTHES ARBOR TRISTIS	0.5	5	0.5
II	Male	NYCTANTHES ARBOR TRISTIS	2	5	0.5
III	Male	NYCTANTHES ARBOR TRISTIS	6	5	0.5
IV	Male	VITEX NEGUNDO	0.5	5	0.5
V	Male	VITEX NEGUNDO	2	5	0.5
VI	Male	VITEX NEGUNDO	6	5	0.5
VII	Male	BOSWELLIA SERRATA	0.5	5	0.5

VIII	Male	BOSWELLIA SERRATA	2	5	0.5
IX	Male	BOSWELLIA SERRATA	6	5	0.5
X	Male	CYPERUS SCARIOSUS	0.5	5	0.5
XI	Male	CYPERUS SCARIOSUS	2	5	0.5
XII	Male	CYPERUS SCARIOSUS	6	5	0.5
XIII	Male	COMMIPHORA MUKUL	0.5	5	0.5
XIV	Male	COMMIPHORA MUKUL	2	5	0.5
XV	Male	COMMIPHORA MUKUL	6	5	0.5

Note: Aqueous-alcoholic extract dissolved in distilled water prior to the administration orally.

Table 2: Acute toxicity study of aqueous alcoholic extracts of *Nyctanthes arbor tritis, Vitex negundo, Boswellia serrata, Cyprus scariosus* and *Commiphora mukul* Body weight (gm).

Days	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
0	29.8	24	30	30	30	30	24	30	24.2	30	30	30	24.8	29.9	26
1	29.4	24.2	29.9	29.9	29.9	30	24.2	29.9	24.8	29.5	29.9	30	24.2	29.9	25.8
2	29.8	24.8	29.9	30	29.9	29.9	24.8	29.9	24.2	29.8	29.9	29.9	25	29.8	25.9
3	29.8	24.2	29.9	30	30	29.9	24.2	29.9	25	29.4	30	29.9	24.6	30	25.4
4	29.8	25	29.8	29.8	29.8	29.8	25	29.8	24.6	29.8	29.8	29.8	24.7	28.9	25.7
5	30	24.6	29.6	29.8	30	30	24.6	29.6	24.7	29.8	30	30	25	30	25
6	30	24.7	29.5	30	28.9	28.9	24.7	29.5	30	29.8	28.9	28.9	25.4	29.6	25
7	30	25	30	30	30	30	25	30	30	30	30	30	25.7	29.5	25.4
8	29.9	25.4	30	30	29.9	29.9	25.4	30	30	30	29.9	29.9	25	30	25.7
9	30	25.7	30	29.2	30	30	25.7	30	29.2	30	30	30	25.4	30	25
10	29.9	25	29.9	29.5	29.9	29.9	25	29.9	29.5	29.5	29.9	29.9	25.7	29.9	25.4
11	30	25.9	30	29.7	30	30	25.9	29.8	29.7	30	30	30	25	29.8	25.6
12	28.9	26	28.9	29.8	29.8	28.9	26	29.6	29.8	30	29.8	28.9	25.9	29.6	25.7
13	30	25.8	30	29.9	29.9	30	25.8	29.5	29.9	29	29.9	30	26	29.5	26.2
14	30	25.9	30	30	30	30	25.9	29.9	30	30	30	30	26	30	26.3

Note: Group(I, II, III)- *Nyctanthes arbor tritis* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(IV, V, VI)- *Vitex negundo* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(VII, VIII, IX)- *Boswellia serrata* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(X, XI, XII)- *Cyprus scariosus* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(XIII, XIV, XV)- *Commiphora mukul* aqueous alcoholic extract (0.5, 2, 6 mg/kg).

Table 3. Acute toxicity study of aqueous alcoholic extracts of *Nyctanthes arbor tritis, Vitex negundo, Boswellia serrata, Cyprus scariosus* and *Commiphora mukul* –Food intake (gm).

Days	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
0	20.3	20	16	20	20	15	22	17	20	19	17	17	19.3	22	17
1	17	15	17	20	21	15	18	16.5	18	15	20	16.2	20	18	19
2	19	17	17	19	22	17	20.5	17	15	19	17	17	19.2	22	17.2
3	15.5	20.5	16	20	18	19	23	16.5	19	15	20.1	16.2	20.1	18	19
4	18	15	16.3	18	20	15.3	20.5	16.3	20	18	15.5	16	18.3	20.2	15.5
5	22	15	18	15.5	23	16	21	18	20	22	15.3	18.5	15	23.5	16
6	15.5	18	19	19.5	20	19.5	19	19	18.5	15.5	18	19.5	19.5	20	19.5
7	22	20	19	20.2	21	20.2	21.2	19.1	20.1	22	20	19	20	21	20
8	20.3	20.5	18.5	20	19	20	19	18	20	20	20	18	20	19.5	20.3
9	21.2	18.2	16	18	21	17	19	16	18	21	18	16.5	18	21.5	17
10	17	16	20.3	20.2	19	19	22.2	20	19.2	17	16	20.5	20	19.5	19.5
11	21	20	20	20	22.3	20	19.5	20	20	17	16.3	20	20.2	19	19
12	18	20	18	18.5	19	20.5	21	20	18	21	20	20	20.1	22	20.5
13	20	19.3	19	19.3	21.2	19	20.3	18.5	16.1	18.1	20.2	18	18	19.2	20
14	21.5	17	17.3	20	19	18	20.3	19.5	20.5	20.3	19.2	19.1	19.2	21.5	19.2

Note: Group(I, II, III)- *Nyctanthes arbor tritis* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(IV, V, VI)- *Vitex negundo* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(VII, VIII, IX)- *Boswellia serrata* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(X, XI, XII)- *Cyprus scariosus* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(XIII, XIV, XV)- *Commiphora mukul* aqueous alcoholic extract (0.5, 2, 6 mg/kg).

Table 4: Acute toxicity study of aqueous alcoholic extracts of *Nyctanthes arbor tritis, Vitex negundo, Boswellia serrata, Cyprus scariosus* and *Commiphora mukul* – Water intake (ml).

Days	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
0	190	200	190	220	190	180	220	170	190	215	190	185	190	200	200
1	185	110	180	200	200	190	200	175	200	210	200	190	200	190	220
2	200	190	180	190	200	160	190	195	200	190	200	170	200	210	210
3	195	200	150	210	220	200	210	205	220	210	215	195	220	190	190
4	185	200	190	190	210	220	190	190	210	185	195	220	210	170	210
5	215	150	180	170	190	180	170	195	190	170	190	180	190	190	210
6	145	170	200	190	210	190	190	200	210	195	205	190	210	200	205
7	210	130	190	200	210	200	200	195	210	205	210	200	210	200	210
8	205	205	170	185	205	195	200	195	200	200	205	190	205	190	170
9	185	190	150	190	210	190	190	190	210	180	210	190	210	200	190
10	180	160	200	200	180	190	200	200	180	200	180	190	180	220	200
11	200	200	190	220	200	210	220	200	200	210	200	210	200	180	185
12	190	180	170	180	180	180	180	185	180	180	180	180	180	190	160
13	180	190	190	190	180	190	190	185	180	190	180	190	180	210	200
14	205	170	190	210	170	190	210	205	170	205	170	190	170	185	180

Note: Group(I, II, III)- *Nyctanthes arbor tritis* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(IV, V, VI)- *Vitex negundo* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(VII, VIII, IX)- *Boswellia serrata* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(X, XI, XII)- *Cyprus scariosus* aqueous alcoholic extract (0.5, 2, 6 mg/kg), Group(XIII, XIV, XV)- *Commiphora mukul* aqueous alcoholic extract (0.5, 2, 6 mg/kg).

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