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EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF THE SEEDS OF LUFFAACUTANGULA ROXB IN ANIMAL MODEL

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ABSTRACT

Objective: To evaluate the acute anti-inflammatory properties of different doses of ethanolic extracts of leaf of *Luffa acutangula* Roxb on carrageenan induced paw oedema of rats. **Material and methods**: The three doses of *Luffa acutangula* Roxb was extracted by using Ethanol. The anti-inflammatory profile of these extracts was investigated on the basis of paw edema induced by carrageenan. The animals were divided into 5 groups of 6 animals each. Group I served as control. Group II served as standard and Administered Aspirin. Group III and IV and V were treated orally with 50 mg/kg, 100 mg/kg and 200 mg/kg extract body weight respectively. **Results:** All the extracts showed considerable dose dependent activity. However ethanol extract 200mg/kg showed higher activity than other extracts. **Conclusion:** The different doses of leaf extract has anti inflammatory property. Further study needed to put the anti inflammatory Property in a precise way.

KEYWORDS: Inflammation; Carrageenan; Paw edema.

INTRODUCTION

Luffa acutangula (Cucurbitaceae), also known ridge gourd has medicinal values. It is mostly found in India, especially Assam is a site of occurrence of this plant. This plant is cultivated by its seeds in the middle of the every year. Japan, Asia, china, Africa are also the site of its occurrence. It is a medicinal herb which is used to treat asthma, intestinal worms, sinusitis[1], edema, pharyngitis and rhinitis. Leaves part of the plant are usefull in the treatment of amenorrhea, decayed teeth, parasitic, skin diseases, chronic bronchitis[2], inflammation, abscesses, Stems of the plants are useful in the treatment of respiratory difficulties[4] whereas fruit parts are used to relief haemorrhage of bladder, hernia, haemorrhoids, jaundice, menorrhagia, scarlet fever, bronchitis, hacmaturia, leprosy, splenomegaly and syphilis.[1,2] Flowers are very useful part for migraine. From pharmacological view point the plants has anti-tussive, anti-asthmatic, cardiac stimulant, hepatoprotective, hypolipidemic analgesic, anti-inflammatory and anti- emetic activities. From the phytochemical viewpoints leaves comprises flavonoids, saponins and triterpenes where as in fruitsascorbic acid, anthocyanins, flavonoids, triterpenoid, saponins are present. The flowers are rich in flavonoids, the peel contains carotenoids, flavonoids and oleanolic acid(Kao et. al., 2012) whereas polypeptides are reported in Seeds.[3,4,5]

Luffa acutangula Roxb mainly comprises of carbohydrates, carotene, fat, protein, phytin, amino acids, alanine, arginine, cystine, glutamicacid, glycine, hydroxyproline, leucine, serine, tryptophan, pipecolic acid[10], flavonoids and saponins. Luffeine is present in fruits and fruit contain 94.2% water, 1.7% fiber and leaves contain different types of vitamins and minerals. Whereas glycerides of palmitic, stearic and myristic acids are found in seeds, as well as bitter principle Cucurbitacin B, an acid sapogenin, oleanolic acids were also isolated from the seeds of *Luffa acutangula*. The plant also shows presence of oleanane type triterpene saponins-acutoside A, B, C, D, E, F and G.[6,7,8,9]

Inflammation is defined as the local response of living tissue to injury caused by different mediators and is nothing but the immunological reactions to expel out and to minimize the further spread of injurious mediators. Inflammation has different phases: the first phase is caused by an increase of vascular permeability resulting in exudation of fluid from the blood into the interstitial space, the second one by infiltration of leukocytes from the blood into the tissues and the third one by granuloma formation. Accordingly, anti-inflammatory tests have to be divided into those measuring acute inflammation, subacute inflammation and chronic repair processes. In some cases, the screening is directed to test compounds for local application. Predominantly, however, these studies are aimed to find new drugs against inflammations.[10,11,12]

MATERIAL AND METHOD

Collection of Plant Material Plant Material

The Seed of *Luffa acutangula* Roxb were collected from Assam in the region of Nagaon. The plant was identified and authenticated by Dr.Bapan Banik, Asstt. Prof., Deptt. of Herbal Science and Technology, ADP College, Nagaon, Assam. A herbarium specimen **no. ADPHST002/2017-2018** is preserved in the college museum.

One kg of the air-dried seeds were blended to a fine powder and dried. The dried powder of the seed were defatted with petroleum ether and then extracted with 70% ethanol using soxhlet apparatus. The extracts was concentrated under reduced pressure using Rotaflash evaporator and stored in airtight container in refrigerator below 10ºC. 70% ethanolic extract which was used for pharmacological investigations, *in-vitro* and *in-vivo* anti-inflammatory studies, after subjecting it to preliminary qualitative phytochemical studies and quantification of total phenol, flavonoid and tannin the percentage yield of corresponding extracts were calculated.[13]

Phytochemical screening

The primary screening was done on petroleum ether, 70% ethanolic and aqueous extract of *Luffa acutangula* Roxb seed for qualitative identification of phytoconstituents contained. From the above screening was done to determine the presence of sterols, phonolic compounds, flavonoids and saponins respectively.[14]

Chemicals

i. Aspirin fine powder (HGI)
ii. Vehicles: Carboxymethyl cellulose (1%).
iii. carrageenan (sigma)
iv. histamine (fluka)

Animals

Wister albino rats (weighing 150-200g) of either sex were used in this study. They were procured from local market of Guwahati, Assam, India. The animals for the experiment were kept for one week under laboratory conditions in polypropylene cages at a temp of 27°C ± 2°C for 12 hrs. dark/ light cycle and maintain the food heigenity. Food and water were supplied regularly except during the experimental period. After the experiments, the used animals were kept separately from others in the animal house to enable observation for development of any complication and to prevent them from being employed for the study on any subsequent day. The Institutional Animal Ethics Committee of GIPS, Guwahati, approved the pharmacological and acute toxicity protocol. Ethical clearance for handling the animals was obtained from the Institutional animal ethical committee prior to the beginning of the project work, the registration.

1. Carrageenan induced rat paw edema

In the following studies, albino rats were divided into 7 groups of 6 animals each; i.e. One group is used as control, one as standard (Aspirin100mg/kg), one 50mg/kg Aspirin, three groups of *Luffa acutangula rox b*ethanolic extracts of doses 100mg/kg, 150mg/kg, 200mg/kg and a combination dose of Aspirin 50mg/kg and ethanolic extract of *Luffa acutangula rox b* (100mg/kg). The method devised by **Winter Risley and Nuss (1962)** was adopted. Carrageenan (1%) in 0.9% Sodium chloride solution was taken and a volume of such solution was injected into the sub-plantar tissues of the hind paw of rat without any anaesthesia. The rats were divided into 7 groups. Among the 7 groups the control group received 1% Carboxy methylcellulose, the standard group received Aspirin 100mg/kg, p.o., the other group of Aspirin received a sub-therapeutic dose of 50mg/kg, p.o, and the test groups received 100mg/kg150mg/kg, 200mg/kg *Luffa acutangula rox b*ethanolic extract respectively. The last test group was given a combination dose of 50 mg/kg Aspirin +100mg/kg EELAS.[15]

The standard drug and the test drugs were administered 1hr before the injection of Carrageenan. As the EELAS was water soluble, it was suspended in 1% Carboxy methylcellulose. These drugs were administered orally with the help of a rat feeding canula fitted to a 1ml tuberculin syringe, one hour prior to Carrageenan injection, keeping the volume of medicament upto 1ml. The paw volume was measured in unanaesthesised rats by water displacement method using Ugo Basile Plethysmometer (7140), once just before administration of the injection and subsequently each hour after injection till 4 hours. The volume of paw edema was recorded as the difference between the paw volume measured 4 hours after carrageenan injection and the initial paw volume taken just before Carrageenan injection.[16]

RESULTS

Successive soxhlet extraction of seed of *Luffa acutangula rox b* yield 4.3% for petroleum ether extract, 31% for 70% ethanolic extract and 16.55% for aqueous extract.

Preliminary phytochemical screening of Seed

From preliminary phytochemical screening of the seed of *Luffa acutangula* Roxb it is seen quantitatively that 70% ethanolic extract contains greater concentration of polyphenolic components like phenols, flavonoids and tannins. Hence it is was selected for further study.

In the present study the effects of various doses of ethanolic extract of Luffa acutangula rox b seedhave been observed against a model of acute inflammation (carrageenan induced) and compared with that of Aspirin as a standard anti-inflammatory drug. The increase in paw volume was calculated by subtracting the initial paw volume from final paw volume and the mean with

standard deviation was calculated. The percentage of decrease in paw volume was calculated by the formulae given by **Diniz et al(1978).**

Table 1: Mean % Inhibition Of Carrageenan Induced Rat Paw Edema.

Group→ Time ↓	Control	standard	50mg extract	100mg extract	200mg extract	50mgaspirin +50mg extract
0hr	0.71±0.03	0.69±0.07	0.98±0.06	0.99±0.07	0.95±0.06	0.74±0.08
1hr	1.18±0.06	0.85±0.06	1.45±0.05	1.32±0.06	1.2±0.08	1.17±0.09
2hr	1.30±0.03	0.83±0.06	1.44±0.06	1.3±0.06	1.18±0.08	1.15±0.09
3hr	1.35±0.02	0.83±0.06	1.42±0.05	1.25±0.08	1.17±0.07	1.13±0.08
4hr	1.37±0.06	0.82±0.06	1.4±0.05	1.19±0.14	1.13±0.08	1.01±0.08
%inhibition of paw edema	-	80.30	36.36	69.69	72.72	59.09
P value	>0.05*	<0.05***	<0.05**	<0.05**	<0.05***	<0.05**

DISCUSSION

There is a no. of phytoconstituents present in plant foods having beneficial activity except its food value though India is paying less attention in this regards. The currents study is taken to evaluate the anti-inflammatory activity of various doses of luffa acutangula rox b seed extract, where asprin 100mg/kg was used as standard drug for the study of anti-inflammatory activity. Various doses of EELAS were used for the study of anti-inflammatory activity, e.g. 50mg/kg, 100mg/kg, 200mg/kg EELAS and a combination dose of EELAS 50 mg/kg + 50mg/kg Aspirin p.o. The 50 mg/kg, 100 mg/kg, 200mg/kg EELAS showed 36.36%, 69.69%, 72.72% inhibition of paw edema respectively. But the combination dose of 50mg/kg Aspirin and 50mg/kg EELAS produced 59.09% inhibition of paw edema which is quite high compared to the single dose of 50mg/kg. Thus when 50mg/kg aspirin combines with the minimum dose of test drug, the % of inhibition of paw edema is good and the effect is satisfactory. The % of inhibition of highest dose of test drug (200mg/kg) is nearly equal to standard drug aspirin (100mg/kg).

CONCLUSION

From the evaluated result it can be concluded that *Luffa acutangula* Roxb seed has significant anti-inflammatory activity and is very good antioxidants. From these results and discussion we have concluded that higher dose 200mg/kg EELAS showed 72.72% inhibition of paw oedema. But the combination dose of 50mg/kg Aspirin and 50mg/kg EELAS produced 59.09% inhibition of paw edema which is quite high compared to the Single dose of 50mg/kg. Thus when 50mg/kg aspirin combines with the minimum does of test drug, the % of inhibition of paw edema is good and the effect is satisfactory. The % of inhibition of highest dose of test drug (200mg/kg) is nearly equal to standard drug aspirin (100mg/kg).

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