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Study of skeletal muscle relaxant activity of ethanolic extract of *Oryza sativa* var. Joha rice and *Citrus macroptera* var. Annamensis; two indigenous medicinal plants of Assam

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Abstract

Assam is the major state in North-East India and one of the richest biodiversity zones of the world having thousands of natural herbs and medicinal plants but a few are explored yet. The present study is aimed to explore two indigenous medicinal plants of Assam *Oryza sativa* var. Joha Rice and *Citrus macroptera* var. Annamensis for its skeletal muscle relaxant activity. The skeletal muscle relaxant activity was studied after pretreatment of ethanolic extract of *Citrus macroptera* var. Annamensis (EECM) and ethanolic extract of *Oryza sativa* var. Joha Rice (EEJR) with (250 mg/kg, p.o and 500 mg/kg, p.o) for 7 days in mice. The Rota-Rod Test and Grip Strength Test was used to study the muscle relaxant activity. Ethanolic extract of *Citrus macroptera* (EECM) fruit peels showed significant activity in Rota rod as well as Grip strength test but of ethanolic extract of *Oryza sativa* var. Joha rice (EEJR) showed non-significant activity in Rota rod as well as Grip strength test instead it increases performance in both dose level.

Keywords: Assam, indigenous medicinal plants, *Oryza sativa* var. Joha rice, *Citrus macroptera* var

Introduction

Assam is the major state of India in North-East region, one of the richest biodiversity zones of the world consisting tropical rainforest, deciduous forests, riverine grasslands and numerous wetlands [1]. Assam is a land of thousands of natural herbs and medicinal plants due to its unique geographical location, abundant of fertile soil, friendly climate and high rain fall. Assam shares biggest contribution to world as Assam Tea (*Camellia assamica*). Moreover, it is found 300 medicinal plants have been identified but only about 5-10% of the plants and herbs are currently utilized [2] and the rest hold a vast potential and rest have vast potential thousands are till un-explored due to of knowledge, space and adequate facility.

So, by giving emphasis on research on herbal resources as part of organic medicines and food have immense potential to get marketed at the present scenario and can make Assam one of the excellent place to Collect Herbal Plants in India for making Ayurvedic, Herbal and Neutral medicines for benefit of human civilization.

There are some important literatures available for study of herbal resources of Assam. Bhattacharya PC *et al.* [3] mentioned some important rare about 30 medicinal plants of Assam, due to lack of knowledge, the general people are not aware of their uses and potentialities. Most of them are growing and dying unused, some are being destroyed willfully by the people for the purpose of animal feeding, fuel etc. and some are being burnt and cut down in Jungal. In another literature by Das NJ *et al.* [4] mentioned 31 ethno medicinal herbs of Kamrup district of Assam. Taid TC *et al.* [5] surveyed 21 medicinal plants in Dhemaji District of Assam. Sarma SK *et al.* [6] surveyed Medicinal plants used by Bodo tribe of Nalbari district in Assam. Barukial J *et al.* [7] surveyed ethnomedicinal plants used by the people of Golaghat district, Assam. Mondal P *et al.* [8] surveyed Herbal medicines useful for the treatment of diabetes in North-East India. Nath M *et al.* [9] surveyed Medicinal plants used in major diseases by Dimasa tribe of Barak Valley. Pandey A *et al.* [10] surveyed Medicinal Plants used by of peoples in Jorhat districts of Assam. Swargiary A *et al.* [11] surveyed Anti-diabetic Medicinal plants used by Local people of Kokrajhar District of Bodoland Territorial Council of Assam. Buragohain J *et al.* [12] surveyed ethnomedicinal plants used in skin diseases by some Indo-Mongoloid communities of Assam.

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Most of the literature involves in surveying the medicinal plants used by local people for medicinal purposes. It is necessary to screen them scientifically. Phytochemical and pharmacological evaluation of these will produce scientific evidence for uses of various diseases and will bring awareness among scientific society as well as local communities.

The present study is aimed to explore two indigenous medicinal plants of Assam *Oryza sativa* var. Joha Rice and *Citrus macroptera* var. Annamensis for its skeletal muscle relaxant activity.

Citrus macroptera var. Annamensis belongs to the family of Rutaceae is indigenous to north eastern Indian States like Assam, Meghalaya, Manipur and Sylhet division of Bangladesh and mainly commonly known as "hatkora" or "shatkora" in English known as Wild Orange [13]. The fruit of *Citrus macroptera* (var annamensis) is edible and popular among the people of Bangladesh and Assam of India as green matured fruits mainly among Sylheti communities used in cooking for flavoring curry mainly meat dishes, pickle preparation and oil is used in perfume production [14]. There are very less literature available for its therapeutic value. It is reported that stem bark of *Citrus macroptera* possess antioxidant activity [15]. Essential oil obtained from leaves possess antimicrobial activity and traditionally fruits as appetite stimulant and treatment of fever [16] and previously we have reported fruit peel extracts for its anti-oxidant and neuroprotective activity [17].

Joha Rice is one of the 40000 varieties of species *Oryza sativa* and popular for its great aroma and equally remarkable taste. It is commonly used to prepare Kheer or Payash or Pulao like traditional recipe [18]. Laokuldilok T *et al* [19] reported that several pigmented rice brans have free radical scavenging and antioxidant activity. Muntana N and Prasong S [20] reported total phenolic contents and their antioxidant activities of Thai white, red and black rice bran extracts. Rao AS *et al.* [21] reported antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. In our previous study we have reported Joha Rice for its in-vitro antioxidant activity [22].

Materials and methods

Drugs, Chemicals and Reagents

The main drugs, chemical and reagents used are Ethanol (Changshu yangyuan Chemical, China), Tween 80 (Accord labs, Hyderabad), Diazepam (Nicholas piramal India Ltd). All the reagents were analytical grade and freshly prepared.

Collection and Authentication of Plant Material

Oryza sativa var. Joha Rice and fruits of *Citrus macroptera* Var. Annamensis were collected from local market (Howraghat, Karbianslong District) of Assam in the month of May 2012 and were authenticated by Prof. Dr. K. Madhava Chetty, Taxonomist, SVU University, Chittoor, Andhra Pradesh (India). The Vouchers specimens were kept for further references.

Preparation of Extracts

Oryza sativa var. Joha Rice and fruits of *Citrus macroptera* Var. Annamensis were extracted using successive solvent extraction method using n-hexane, chloroform and ethanol using standard procedure in Soxhlet apparatus. Finally extracts were concentrated by distilling off the solvent and then evaporated to dryness on the water-bath, until to get the dried extract. The extract obtained with each solvent was

weighed and its percentage in terms of the air-dried weight of the plant material was then calculated and also the consistency of the extract was noted.

Preliminary Phytochemical Analysis

The various extracts of *Citrus macroptera* Var. annamensis and *Oryza sativa* var. Joha Rice were subjected to preliminary phytochemical screening using standard methods [23-24].

Experimental Animals

Swiss albino Mice of either sex (weight 25-30 g body weight) were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25°C for 12 hr light/dark cycle and given standard pellet diet and water. The animals were accustomed to the laboratory conditions for a week prior to the experimentation. The fresh diet and water for the animals has to be supplied daily to the animals. The condition of the animals has to be supervised daily till the completion of the experiment. This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee. IAEC permission was taken as per CPCSEA guideline. IAEC of Anurag Pharmacy College is approved by CPCSEA (Reg. No. (1712/PO/a/13/CPCSEA) and the study is Protocol IAEC number. APC/02/IAEC of 2014-2015.

Vehicles and Preparation of Doses

To prepare the dosage forms Ethanolic extract of *Citrus macroptera* Var. annamensis and *Oryza sativa* var. Joha Rice were made a suspension with 1% Tween 80. The dose in required concentration was administered at 1ml/100g body weight of the animal.

Acute Oral Toxicity Study

The acute oral toxicity procedure was followed by using OECD 423 guidelines [25].

Experimental Design

On the day of the experiment, the Swiss albino mice of both sexes weighing 25-30 gms were divided randomly into six groups of six animals each and treated with Ethanol extract of *Citrus macroptera* (EECM) fruit peel and ethanol extract of *Oryza sativa* Var. Joha Rice. (EEJR) and with other Standard drugs for one week.

Group-I: Control (Vehicle 1% Tween 80, p.o)

Group-II: EECM (250mg/kg in 1% Tween 80, p.o)

Group-III: EECM (500mg/kg in 1% Tween 80, p.o)

Group-IV: EEJR (250mg/kg in 1% Tween 80, p.o)

Group-V: EEJR (500mg/kg in 1% Tween 80, p.o)

Group-VI: Diazepam (10mg/kg, in 1% Tween 80, p.o) [26]

Behavioural evaluation was carried out on the last day (7th Day) at 60 minutes post drug/vehicle administration.

Evaluation of Skeletal Muscle Relaxant Activity

Rota Rod test in Mice

The rotarod test is used to evaluate the activity of drugs interfering with motor coordination [27-28]. The rotarod test was used to determine the effect of drugs on motor coordination. The instrument (a horizontal rotation device, Rota Rod, Edison) was set at a rate of 25 rpm. Each rat was placed on the rod and those animals that remained on the rod for 3 mins were selected for the study. The animals were then evaluated for motor coordination basal reading (the time each animal falls off from the rod/ time spent by animal in rotarod.

After 60 min of administration of the tested drugs the each animal was kept on rotarod and the time each animal falls off from the rod each animal were recorded.

Grip strength test

The mice was allowed to hold with the forepaws a steel wire (2mm in diameter and 35 cm in length), placed at a height of 50 cm over a cushion support. The length of time the rat was able to hold the wire was recorded. This latency to the grip loss is considered as an indirect measure of grip strength [29]. After 90 min of administration of the tested drugs the each animal was kept on wire and the time each animal falls off from the wire of each animal were recorded.

Statistical Analysis

The data were analyzed by using Graph pad Prism software

version-5 by one way analysis of variance (ANOVA). The test was followed by Dunnet’s ‘t’-test, *p* values less than 0.05 were considered as significance.

Result

Results of Solvent Extraction

Oryza sativa var. Joha Rice and fruits peel of *Citrus macroptera* Var. Annamensis were extracted using successive solvent extraction method using n-hexane, chloroform and ethanol using standard procedure in soxhlet apparatus. Ethanolic extract of fruits peel of *Citrus macroptera* Var. Annamensis was Intense green oily semisolid with percentage yield 22.15% and Ethanolic extract of *Oryza sativa* var. Joha Rice was Brownish black oily semisolid with percentage yield 3.6%.

Table 1: Percentage Yield drugs

	Solvent	Texture of Extract and colour	Colour	% Yield
<i>Citrus macroptera</i> Var. Annamesis (Fruit peel extracts)	Ethanol	Oily semisolid	Intense green	22.15
<i>Oryza sativa</i> var. Joha Rice	Ethanol	Oily semisolid	Brownish black	3.6



Fig 1: Ethanolic extract of *Citrus macroptera* Var. Annamensis fruit peel extract (EECM)



Fig 2: Ethanolic extract of *Oryza sativa* var. Joha Rice (EEJR)

Result of Preliminary Phytochemical Analysis

The ethanolic extracts of *Citrus macroptera* fruit peel extracts and ethanol extract of *Oryza sativa* var. Joha Rice were tested for different phytoconstituents using standard procedures for

alkaloids, glycosides, saponinins, tannins, terpinoids, reducing sugars, phenolic compounds, flavanoids, protein, carbohydrates and volatile oils. The results are given below Table-2.

Table 2: Results of Preliminary phytochemical analysis

Phytoconstituents	Ethanolic extract of <i>Citrus macroptera</i> Var. Annamensis fruit peel extract (EECM)	Ethanolic extract of <i>Oryza sativa</i> var. Joha Rice (EEJR)
Alkaloids		
Mayer's test	+ve	-Ve
Hager's test	+ve	-Ve
Dragendorffs test	-ve	-Ve
Wagner test	+ve	-Ve
Protein		
Biuret's Test:	+Ve	+Ve
Millon's test	+Ve	+Ve
Ninhydrin test	+/-Ve	+/-Ve
Xanthoprotein test	+/-Ve	+/-Ve
Carbohydrate		
Molisch 's test	-Ve	+Ve
Barfoed's test	-Ve	+Ve
Benedict's test	-Ve	+Ve
Fehling test	-Ve	+Ve
Reducing sugar	-Ve	-Ve
Tannins		

Lead acetate test	+Ve	-Ve
Ferric chloride test	+/-Ve	-Ve
Alkaline reagent test	-Ve	-Ve
Saponins		
Frothing test	+Ve	+Ve
Terpenoids		
Salkowski test	+Ve	+Ve
Glycosides		
General test	-Ve	-Ve
Bromine water test	-Ve	-Ve
Flavanoids		
General test	+Ve	+Ve
Shinoda test	+Ve	+Ve
Steroids		
Liebermann-Burchard's test	+/-Ve	+/-ve
Phenols		
General test (Ferric Chloride test)	+Ve	+Ve
General test (Sodium Chloride test)	+Ve	+Ve
Fat and fixed oils		
Stain test	+Ve	+Ve
Saponification test	+Ve	+Ve
Volatile oil		
General test	+Ve	+Ve

Note: + ve indicates positive result, - ve indicates negative result, whereas +/-ve indicates not clearly given result

Results of Acute Oral Toxicity Studies

Acute oral toxicity studies of Ethanolic extract of *Citrus macroptera* (EECM) fruit peels and ethanol extract of *Oryza sativa* Var. Joha Rice. (EEJR) was carried out according to OECD guideline 423 in mice using starting dose of 2000 mg/kg, p.o. The animals exhibited normal behaviour, without any signs of passivity, stereotypy and vocalization. Both EECM and EEJR showed no toxicity though repeated. LD 50 cut off value was obtained from the Flow Chart of OECD-423 and dose for Administration were taken as 1/20th and 1/10th of LD₅₀ as high and low dose. Under the category of class-VI or Unclassified, LD₅₀ was calculated and LD₅₀ is 5000 or more.

$$ED_{low} = 1/20 * 5000 = 250$$

$$ED_{high} = 1/10 * 5000 = 500$$

Skeletal muscle relaxant activity in Rota Rod Test in mice

The effect of pretreatment with EECM and EEJR with (250 mg/kg, p.o and 500 mg/kg,p.o) for 7 days shows effect of skeletal muscle activity on rota rod and results are given in Table-3 and presented in Figure-3. The effect on time spent rota rod in mice were compared with Standard (Diazepam; 10mg/kg, p.o) which showed significant decrease in time spent in rota rod.

Table 3: Effect of Ethanol extract of *Citrus macroptera* fruit peel and ethanol extract of *Oryza sativa* var. Joha Rice on skeletal muscle relaxant activity in Rota Rod Test

Groups	Treatment	Time spent in Rota Rod (Sec) Mean±SEM
Group I	Control (Vehicle 1% Tween 80,p.o)	324.71±10.68
Group II	EECM (250mg/kg in 1% Tween)	238.34±13.94*
Group III	EECM (500mg/kg in 1% Tween)	178.82±7.88**
Group IV	EEJR (250mg/kg in 1% Tween 80,p.o)	480.01±15.45#*
Group V	EEJR (500mg/kg in 1% Tween 80,p.o)	487.71±17.30#*
Group VI	Diazepam (5mg/kg, in 1% Tween 80, p.o)	25.15± 3.03***

(Values are in Mean ± S.E.M (n=6); ^{ns} -Non Significant, **p*<0.05, ***p*<0.01, ****p*<0.001 when compared with Control using One way ANOVA followed by Dunnett's "t" test.).

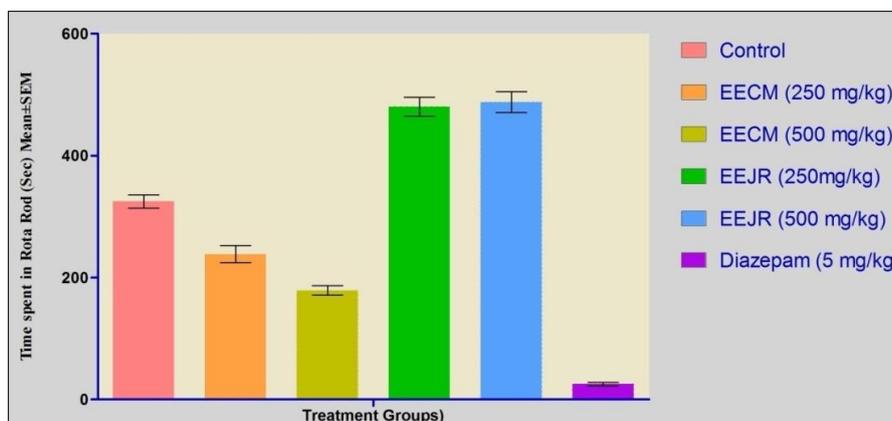


Fig 3: Effect of Ethanol extract of *Citrus macroptera* fruit peel and ethanol extract of *Oryza sativa* var. Joha Rice on skeletal muscle relaxant activity in Rota Rod Test in mice

Skeletal muscle relaxant activity in Grip strength Test in mice

The effect of pretreatment with EECM and EEJR with (250 mg/kg, p.o and 500 mg/kg,p.o) for 7 days were studied for

Grip strength test in thread in mice, and they were compared with standard drug diazepam. The results were given in Table-4 and presented in Figure-4.

Table 4: Effect of Ethanol extract of *Citrus macroptera* fruit peel and ethanol extract of *Oryza sativa* var. Joha Rice on skeletal muscle relaxant activity on Grip Strength Test in mice.

Group	Treatment	Time spent in thread (Sec)
I	Control (Vehicle 1% Tween 80,p.o)	68.00±6.59
II	EECM (250mg/kg in 1% Tween)	56.12±3.59 ^{ns}
III	EECM (500mg/kg in 1% Tween)	51.33±4.10*
IV	EEJR (250mg/kg in 1% Tween 80,p.o)	73.33±3.40# ^{ns}
V	EEJR (500mg/kg in 1% Tween 80,p.o)	78.83±4.50# ^{ns}
VI	(Diazepam; 5 mg/kg, in 1% Tween 80, p.o)	45.50±4.73*

(Values are in Mean ± S.E.M (n=6); ^{ns} -Non Significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, # means increases compared when compared with Control using One way ANOVA followed by Dunnet's "t" test.)

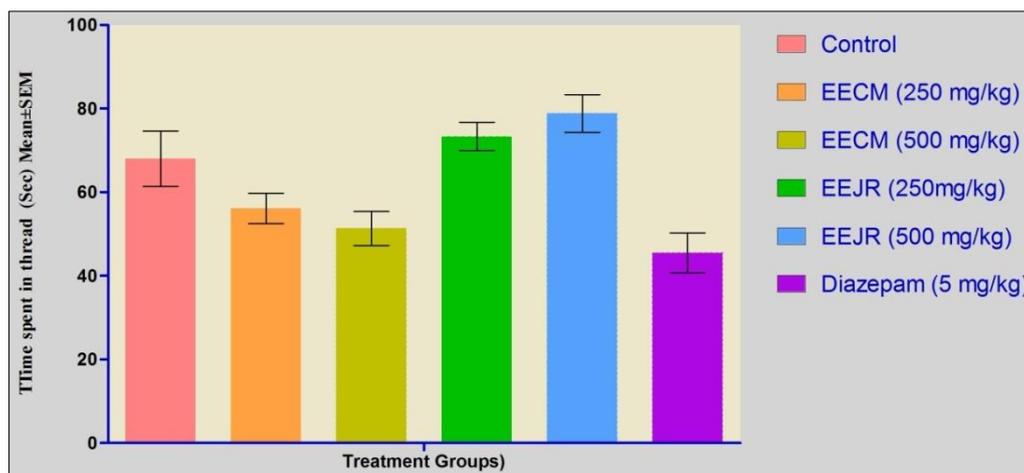


Fig 4: Effect of Ethanol extract of *Citrus macroptera* fruit peel and ethanol extract of *Oryza sativa* var. Joha Rice on skeletal muscle relaxant activity on Grip Strength Test in mice

Discussion

In skeletal muscle relaxant activity, the pretreatment of ethanolic extract of *Citrus macroptera* (EECM) fruit peels showed significant activity in Rota rod as well as Grip strength test. Moreover, it was found to increase slight performance of the mice in both test models. The slight increase in performance is may be due to physical activity causing muscle fatigue due to release of ROS during activity, and anti-oxidants present in *Citrus macroptera* scavenge those free radical. The pretreatment of ethanolic extract of *Oryza sativa* var. Joha rice (EEJR) showed non significant activity in Rota rod as well as Grip strength test. Moreover, it was found to increase slight performance of the mice in both test models. As some previous studies already suggested that exercise and activity increases oxidative stress in cells^[30] and antioxidant like alpha tocopherol increases swimming endurance of trained swimmers^[31].

Conclusion

Finally, from this study it can conclude that ethanolic extract of *Citrus macroptera* (EECM) fruit peels showed significant activity in Rota rod as well as Grip strength test but of ethanolic extract of *Oryza sativa* var. Joha rice (EEJR) showed non significant activity in Rota rod as well as Grip strength test instead it increases performance in both dose level.

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