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Evaluation of Pharmacological Activities from the Combination of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica

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ABSTRACT
The objective of our study was to investigate the analgesic, anti-inflammatory and CNS depressant activities from the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves. Analgesic effects of combined aqueous and methanolic extract were assessed by acetic acid induced writhing method. At a dose of 50 mg/kg and 100 mg/kg body weight the combined aqueous extracts were showed 29.41% and 58.82% where as combined methanolic extract showed 70.58% and 98.52% writhing inhibition respectively. The anti-inflammatory activities of the combined extracts were estimated by measuring the mean increase in hind paws volume in carrageenan-induced method. In this method the aqueous extract exhibits an inhibition of paw volume of 66.66% and 83.33% where methanolic extract exhibits 95% and 98% inhibition respectively at the same dosing manner. In CNS depressant activity, the test animals showed significant decreased in number of movement at a dose of 100 mg/kg body weight for both the extracts. This study recommends that combined methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves has significant activities and can be used as a new and potential source of analgesic, anti-inflammatory and CNS depressant agent of natural origin.

Key Words: Combined extract, analgesic, anti-inflammatory, CNS depression.

I. INTRODUCTION

From the beginning of civilization, plants have been useful for managing different types of infectious diseases around the world [1]. Nowadays the phytochemists are investigating plants to treat various infectious diseases [2]. The World Health Organization, 2003, was reported that approximately 80% of the population of developing countries relies on plant-based traditional medicines to fulfill their primary health care needs [3]. All the medicinal plants are compatible with human body and are an important component of the health care system [4]. Several phytochemicals have been isolated from various plant species which received a considerable attention due to their analgesic, anti-inflammatory and CNS depressant activities [5].

Inflammation is the complex reaction between blood vessel and leukocytes and response to injury of cells and body tissues through different factors such as infectious agent, chemicals, thermal injury, and mechanical injuries [6]. Majority of anti-inflammatory agents are capable to inhibit the cyclooxygenase pathway which is responsible for prostaglandins synthesis [7]. Pain is an unpleasant sensory and emotional experience. Different types of fracture or infection are responsible for acute pain whereas chronic pain is usually triggered by psychosocial stress [8].Current antidepressant drugs have proven to be effective but having adverse side effects the researcher are trying to develop new antidepressants agent from natural origin[9, 10]. The antidepressant effect of herbs has been paid more and more attention now a day.

It is concern that the herbal medicines have the advantage in combining their active components to obtain synergistic or additive effects which give to the plants an efficiency superior to some of their isolated components [11]. The main aim of the study is to provide insight about the combined effects of the leaf extracts from the selected medicinal plants and their effectiveness against of pain, inflammation and in CNS depression. To our best knowledge, there are no evident of combined pharmacological study on Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves have been done. This is

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the first time report of combined pharmacological study on these medicinal plants in Bangladesh.

II. METHOD AND MATERIALS

Plant collection and identification

The leaves of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica were collected from Rajshahi University campus, Bangladesh. The plant was authenticated by Botany Department, University of Rajshahi.

Preparation of plant Extract

The fresh leaves were thoroughly washed with water and dried in shade. Then leaves were cut into small pieces to make it suitable for grinding purpose and finally dried in an oven at 40-45^{o}C for 36 hrs. The materials were grinded into coarse powder with the help of a grinder and extracted by the cold extraction process in which the plant materials were treated with water and methanol respectively. Powder of each plant materials having a weight of about 1.5 kg were taken in an amber colored reagent bottle separately and soaked in 2.5 litre distilled water and methanol respectively. The bottles with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated by using rotary evaporator.

Experimental animal

Swiss albino mice of both sex and the weight between 20-20gm were used in the experiment. The animals were adopted to the new environment at the room temperature (25°±2°c) with a relative humidity 55±5 % in a standard wire meshed plastic cages for 4 to 5 days prior to performance of the experiment.

Determination of analgesic activity

Acetic acid induced writhing method

The analgesic activity of the tests sample were determined by acetic acid induced writhing method as described by Sharma et. al., 2010 [12]. Control animals were received distilled water and Diclofenac sodium was used as a standard. The number of abdominal constrictions was counted over a period of 20 min which is known as writhing. The percentage of inhibition of analgesic activity was calculated by the following equation-

$$\% \text{ inhibition} = \left[\frac{A-B}{A}\right] \times 100$$

Where, A= Average number of writhing of control group

B= Average number of writhing of test group

Study of anti-inflammatory activity

Carrageenan induced paw edema method

The anti-inflammatory activities of investigated crude extracts were determined by method namely-Carrageenan induced paw edema in Swiss albino mice illustrate by Elisabesky et al., 1995 [13].

Edema that was induce by the sub plantar injection of carrageenan (0.1ml 1% solution in 0.9% saline solution) in left paw a half hour after oral administration of standard and test drug. The degree of inflammation was indicated by the difference between initial and after treatment paw volume.

The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula-

$$\% \text{ Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_c and V_t represent average paw volume of control and treated animal respectively.

Central nervous system (CNS) depressant activity

Open Field Test

The CNS depressant activity of the tests sample were determine by the method described by Walsh and Cummins, 1976 [14].The animal allowed to move on the floor of an open field of half square meter was separated into a series of alternatively black and white color squares and then count the total number of squares by mice for 5 min on 30, 60, 90 and 120 min after oral administration of test samples.

Hole Cross Test

The study method was described by Takagi et al., 1971[15].A wooden cage having a size of 30×20×14 cm with a partition fixed at the middle and a hole of 3 cm diameter was used in this method. The mice was placed on one side of the chamber and the number of passages of mice through the hole from one chamber to other was recorded for 3 min on 30, 60, 90 and 120 min after oral administration of tested samples.

III. STATISTICAL ANALYSIS

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The analysis was performed using Microsoft Excel software 2007. The results were finally expressed graphically as percentages.

IV. RESULT

Analgesic activity

Table 1: Analgesic activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves at two different dose.

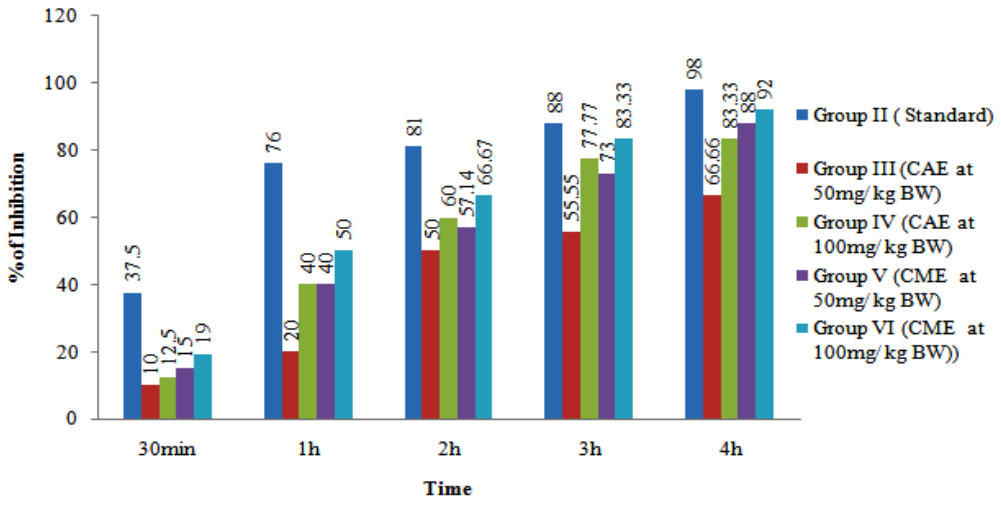
Sample	Dose	%Inhibition of Writhing
Group II (Standard)	10mg/kg	88.23
Group III (CAE)	50mg/kg	29.41
Group IV (CAE)	100mg/kg	58.82
Group V (CME)	50mg/kg	70.58
Group VI (CME)	100mg/kg	98.52

Anti-inflammatory activity

Table 2: Anti-inflammatory activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves in Carrageenan induced paw edema method.

Sample	Dose	% of Inhibition				
		30min	1h	2h	3h	4h
Group II(Standard)	10mg/kg	37.5	76	81	88	98
Group III(CAE at 50mg/ kg BW)	50mg/kg	10	20	50	55.55	66.66
Group IV(CAE at 100mg/ kg BW)	100mg/kg	12.5	40	60	77.77	83.33
Group V(CME at 50mg/ kg BW)	50mg/kg	15	40	57.14	73	88
Group VI(CME at 100mg/ kg BW)	100mg/kg	19	50	66.67	83.33	92

Fig 2: Anti-inflammatory activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves in Carrageenan induced paw edema method.



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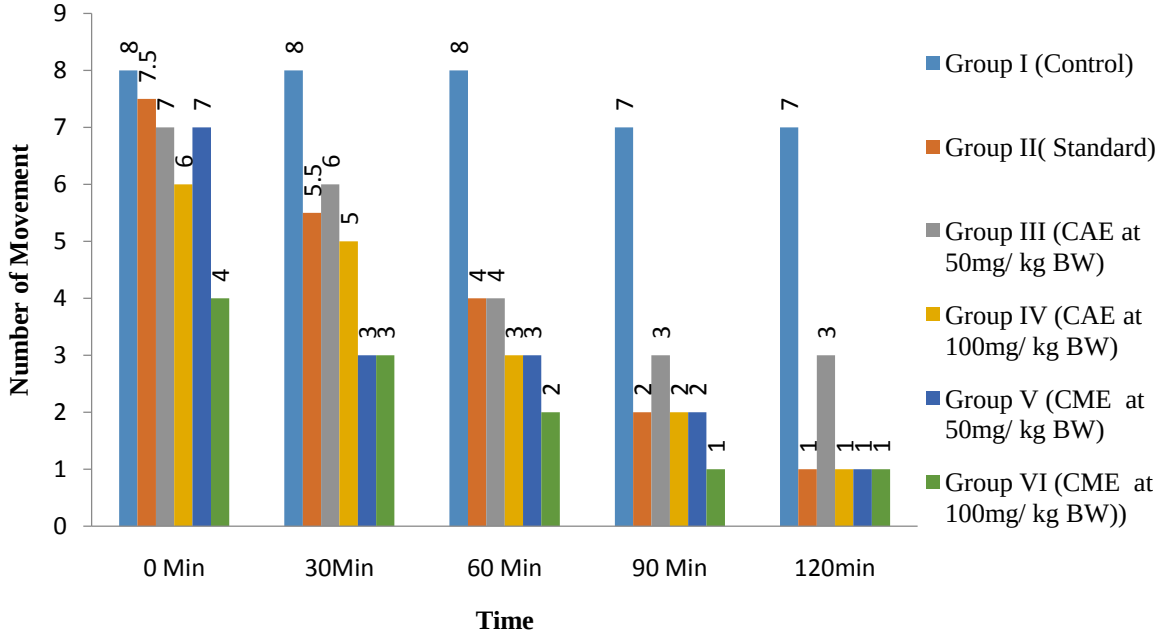
Here,
Group II – Indomethacin,
Group III – Combined aqueous extract of Citrus aurantifolia , Phyllanthus emblica and Tamarindus indica at 50mg/kg body weight.
Group IV - – Combined aqueous extract of Citrus aurantifolia , Phyllanthus emblica and Tamarindus indica at 100mg/kg body weight.
Group V - – Combined methanolic extract of Citrus aurantifolia , Phyllanthus emblica and Tamarindus indica at 50mg/kg body weight.
Group VI - Combined methanolic extract of Citrus aurantifolia , Phyllanthus emblica and Tamarindus indica at 100mg/kg body weight.

Central nervous system (CNS) depressant activity
Hole cross test

Table 3: CNS depressant activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves by hole cross test.

Sample	**Movement**					
	Dose mg/kg	0 Min	30Min	60 Min	90 Min	120min
Group I	-	8	8	8	7	7
Group II	10mg/kg	7.5	5.5	4	2	1
Group III	50mg/kg	7	6	4	3	3
Group IV	100mg/kg	6	5	3	2	1
Group V	50mg/kg	7	3	3	2	1
Group VI	100mg/kg	4	3	2	1	1

Fig 3: CNS depressant activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthu semblica and Tamarindus indica leaves by hole cross test.



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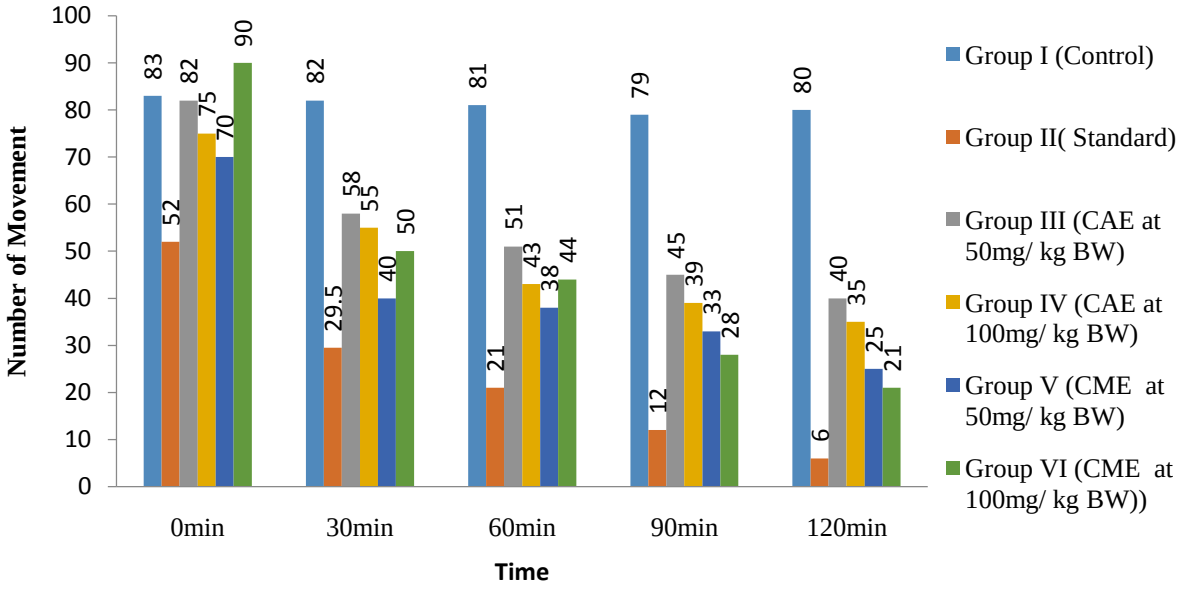
Open field test

Table 4: CNS depressant activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves by open field test.

Sample	Dose	Movement				
		0min	30min	60min	90min	120min
Group I	-	83	82	81	79	80
Group II	10mg/kg	52	29.5	21	12	6
Group III	50mg/kg	82	58	51	45	40
Group IV	100mg/kg	75	55	43	39	35
Group V	50mg/kg	70	40	38	33	25
Group VI	100mg/kg	90	50	44	28	21

Fig 4: CNS depressant activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves by open field test.



Here,

Group II – Diazepam as standard drug

Group III – Combined aqueous extract of *Citrus aurantifolia* , *Phyllanthus emblica* and *Tamarindus indica.*

Group IV - – Combined aqueous extract of *Citrus aurantifolia* , *Phyllanthus emblica* and *Tamarindus indica.*

Group V - – Combined methanolic extract of *Citrus aurantifolia* , *Phyllanthus emblica* and *Tamarindus indica.*

Group VI - Combined methanolic extract of *Citrus aurantifolia* , *Phyllanthus emblica* and *Tamarindus indica.*

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V. DISCUSSION

In the present study analgesic activity of the combined aqueous and methanolic extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves was analyzed by writhing method and anti-inflammatory activity was determined by carrageenan induced paw edema while CNS depressant activity measured by hole cross and open field method.

Acetic acid induces writhing model was used to studied the analgesic activity in which pain is sensed by the release of free arachidonic acid [16]. Prostaglandin [17] acts as a mediator of peripheral analgesic action which can be evaluated by writhing method. At two different doses the combined methanolic extract showed more significant analgesic action of 70.58%and 98.52% as compared to aqueous extract which showed writhing inhibition of 29.41% and 58.82%. Therefore the significant analgesic actions of combined aqueous and methanolic extract were based on the inhibition of prostaglandin pathway.

Carageenan induced paw edema method is most widely used method for testing of anti-inflammatory agents. Anti-inflammatory agents initially inhibit the cycloxygenase enzyme which is involved in prostaglandin synthesis and this mechanism correlates with the standard drug Indomethacin [18]. In this study the aqueous combined extracts reduce edema 83.33% where as methanolic extracts reduce 98% paw edema at a dose of 100 mg/kg body weight. It indicates that the test extracts mediated anti-inflammatory effect by inhibition of prostaglandin synthesis and this is more prominent in methanol extract than that of aqueous extract.

Locomotor activity determines the level of excitability of the CNS [19] and the sedative effect is produce when this activity is decreased [20, 21]. From the result it is reveal that the combined methanolic extract showed significant decrease in locomotor activity. This indicates that the combined methanolic extract of the Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves is more prone to CNS depression as compared to combined aqueous extract.

VI. CONCLUSION

Our findings confirmed the analgesic, anti-inflammatory and CNS depressant activity of the combined extract of Citrus aurantifolia, Phyllanthus emblica and Tamarindus indica leaves. From the study it may be concluded that the test extracts can be replaced as an alternative agent in preventing and treating pain, inflammation and depression. However, further studies are needed to evaluate the safety profile of these plants to be used in combination as safe and natural therapeutic agents.

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COMPETING INTERESTS
The Authors declared that no competing interest exists.

ETHICS APPROVAL
This study was approved by University Ethics Committee at Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh.