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PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL ACTIVITIES WITH RESPECT TO ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *JATROPHA CURCAS* FRUIT

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Keywords:

Jatropha curcas, Analgesic, Anti-inflammatory, Hot plate, MEJC

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ABSTRACT: Objective: To evaluate the analgesic and anti-inflammatory effects of the methanolic extract of the *Jatropha curcas* (MEJC) belonging to the family Euphorbiaceae. **Materials and Methods:** Phytochemical screening was done by standard procedures. Acute toxicity studies were done by using female albino mice weighing 23-35 gm as per OECD 425 guidelines. In the hot plate models for analgesic activity, the oral administration of *J. curcas* extract at the three different doses of MEJC like (100, 200, 400 mg/kg) and the reference drug diclofenac sodium (25 mg/kg) were administered. The control group was taken using normal saline 0.9% NaCl solution. While in the carrageenan-induced paw edema models, the same dosage regimen as per the analgesic activity were followed but the standard drug dose of aspirin is 150 mg/kg. **Results:** Phytochemical screening revealed the presence of flavonoids, steroids, alkaloids, tannins, carbohydrates, cardiac glycosides. The extract was found to be non-toxic up to the dose of 2000 mg/kg after the acute toxicity test. In analgesic all the three doses of MEJC *i.e.* 100mg/kg (9.33 ± 0.33 sec), 200 mg/kg (10.67 ± 0.88 sec) and 400 mg/kg (11.33 ± 0.88) significantly increases in the jumping time in the hot plate as compared to control (4.33 ± 0.33) (p-value <0.0001). The result is also quite satisfying when compared with the standard drug at 25 mg/kg (13.33 ± 0.33). And anti-inflammatory activity it shows inhibition of edema MEJC *i.e.* 100 mg/kg (0.71 ± 0.03), 200 mg/kg (0.7 ± 0.03), 400 mg/kg (0.6 ± 0.03) which is comparable with the standard drug aspirin 150 mg/kg (0.53 ± 0.02). **Conclusion:** This result suggests that the fruits of *Jatropha curcas* has anti-inflammatory and analgesic properties comparable with those of standard drugs and may be useful for the treatment of painful inflammatory conditions.

INTRODUCTION: Plants are the most important sources of medicine. Plant-derived compounds (Phytochemical) have been attracting much interests as natural alternatives to synthetic compounds. Extracts of plants were used for the treatment of various diseases, and this formed the basis for all traditional systems of medicine [1].

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The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries [2].

Jatropha curcas is widely used in traditional medicine in Africa, Asia, and Latin America to cure various ailments such as skin infections, diarrhea, gonorrhea, fever and several other diseases caused by microorganisms. *Jatropha curcas* has also been used as antidote, remedy, medicine, and potential source of herbal drugs in dental complaints and against constipation [3]. It is a tropical plant that can be grown in low to high rainfall areas either in the farm as a commercial

crop or on the boundaries as a hedge to protect fields from grazing animals and to prevent erosion [4]. The leaves are remedy for jaundice, applied by rectal injection. In India the latex of the stem is mixed with salt to clean teeth. The roots are used for treating chest disease or may be cooked with gruel and given to patients suffering from kidney diseases [5].

MATERIALS AND METHODS:

Plant Material: The plant material collected from 'Kampru' region of Assam. The plant was authenticated by the Botanical Department of Guwahati University, Assam, India (Plant authentication number HERB/BOT/GU/2017/17) and the plant was thoroughly washed with water and the fruit was dried in shade for 3 weeks.

Preparation of Extract: Collected fruits were washed thoroughly and kept for drying in shade and were powdered to 40 mesh size with light petroleum ether (40-60 °C), and again the marc was extracted with methanol for 72 h. The solvent was removed under reduced pressure, and semi-solid mass was obtained and was concentrated by vacuum drying to yield a solid residue. This was kept in the refrigerator for phytochemical screening and activity studies.

Drugs and Chemicals: Diazepam 10 mg/kg and normal saline (0.9% NaCl solution) methanol, petroleum ether, carrageenan, aspirin 150 mg/kg CMC, *etc.*

Animal: Swiss albino mice (20- 25g) and Wistar rat (180-225g) were used. They were maintained at 25 ± 2 °C and relative humidity of 45-55% and under standard environmental conditions.

Institutional Animal Ethical Committee approved the protocol (Approval number- GIPS/IAEC/ B.Ph/2017/) all experiments were carried out within 2 days.

Acute Toxicity Test: The acute oral toxicity study was performed as per OECD guideline 425. The animals were fasted overnight prior to the experimental procedure. The animals were divided into six groups and given different doses of plant extract (MEJC) *via* oral route (150, 300, 500, 1000, 2000 mg/kg body weight) for four consecutive days and their mortality, loss of body weight and general behavior was recorded from the first dose up to 72 h after the last administration of plant extract. The procedure described in detail earlier in OECD 425 was followed for the determination of the acute toxicity of the extract.

Phytochemical Screening: [6, 7]

Alkaloids Test: Evaporate the methanolic extracts separately. To the residue, add dilute HCl. Shake well and filter. With Filtrate performing the following tests:

A. Dragendroff's Test: To 2-3 ml filtrate, add few drops Dragendroff's reagent. Orange-brown ppt. is formed.

B. Mayer's Test: 2-3 ml of filtrate with few drops Mayer's reagent gives ppt.

C. Wagner's Test: 2-3 ml of filtrate with few drops Wagner's reagent gives reddish brown ppt.

Detection Steroid:

Liebermann's Test: Mix 2 ml extract with chloroform. Add 2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of the test tube. Fast red, then blue and finally green color appears.

Detection Carbohydrate:

A. Molisch's Test: To 2-3 ml of extract, add few drops of alpha-naphthol solution in alcohol shake and add conc. H_2SO_4 from the sides of the test tube. The violet ring is formed at the junction of two liquids.

B. Benedict's Test: Mix equal volume of Benedict's reagent and test solution in a test tube. Hite in boiling water bath for 5 min solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Detection Tannin:

Gelatin Test: 2-3 ml of methanolic extract, add few drops of gelatin solution. It gives a white ppt.

Detection of Cardiac Glycosides:

A. Keller-Killiani Test: To 2 ml of extract, add glacial acetic acid, 1 drop 5% $FeCl_3$ and conc. H_2SO_4. The reddish-brown color appears at junction of the two liquid layers, and upper layer appears bluish-green.

B. Liebermann's Test: Mix 2 ml extract with chloroform. Add 2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of the test tube. Fast red, then blue and finally, green color appears.

Detection Flavonoids:
Shinoda Test: To extract add 5 ml of 95% ethanol/ t-butyl alcohol, few drops conc. HCl and 0.5 g magnesium turnings. Orange, pink, red to purple color appears.

Screening of Analgesic Activity:
Hot Plate Method: Swiss albino mice weighing between (20-25g) were used for evaluation of analgesic activity; mice of both sexes were randomly divided into 5 groups of 3 mice per group. Each mouse was placed upon the heated metal plate (Hot plate) maintained at the temperature of about 50-55 °C. The pain reaction time (PRT), which is the time taken for the react to the pain stimulus was determined with a stopwatch before drug administration. The cut off time was fixed for 15 sec to avoid damage to the tissues of the foot. This served as the control reaction time.

The mice were then treated as follows: Group 1 mice received 10 ml/kg of normal saline 0.9% NaCl solution and served as a negative control. Group 2 mice received diclofenac sodium (25 mg/kg) (positive control), and groups 3, 4 and 5 received 100, 200, and 400 mg/kg of *J. curcas* extract respectively per OS. 30 min after drug administration the PRT for each mice was determined again. The response to the heat stimulus varied with the animals and consisted of one and another of the following types of behaviors: kicking its hindfoot and jumping about, shaking a foot and licking it or raising one or the other of the hindfoot and holding it tightly against the body. Responses involving the forefoot are not considered since they are difficult to distinguish from the normal grooming behavior of the mice [8].

Anti-inflammatory Activity:
Carrageenan - Induced Paw Edema: Acute inflammation was induced using the carrageenan-induced edema model. The rats were starved for 24 h after which they were divided into 5 groups (Groups I-V) of 3 animals each. Rats in group I (negative control, untreated) were given saline at (10 ml/kg) 1 h before 0.1 ml of 1% freshly prepared suspension of carrageenan was injected into the plantar surface of the right hind paw of each rat. Rats in group II were given aspirin, a standard anti-inflammatory drug at the dose of 150 $mg \cdot kg^{-1}$ orally 1 h before carrageenan injection while Group III, IV and V rats were given MEJC orally at the dose of (100 mg/kg, 200 mg/kg, 400 mg/kg) 1 h before carrageenan injection. The linear circumference of the paw was measured after carrageenan injection (0 h) and 3 h after carrageenan injection using a loop of thread tied around the paw such that it was neither too loose nor too tight. The length of the thread around the paw was then measured on a ruler and rounded off to the nearest centimeter. The percentage inhibition was calculated according to the formula:

Percentage of inhibition = $[C_1\text{-}C_0]$ control - $[C1\text{-}C_0]$ test / $[C_1\text{-}C_0]$ control × 100

Where, C_o= Mean paw size at 0 h after carrageenan injection, C_1= Mean paw size at 3 h after carrageenan injection [9].

Statistical Analysis: Data analysis was carried out using Graph pad Prism software and the data were expressed as mean ± SE. The significance level of treatment effect was determined by one-way analysis of variance (ANOVA); p-values less than 0.05 were considered statistically significant.

RESULTS:
Phytochemical Analysis: Different phytochemical investigations with the methanolic extract were demonstrated the nearness of alkaloids, flavonoids, carbohydrate, cardiac glycosides, steroids, tannin **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *J. CURCAS* FRUITS

S. no.	Phytochemical test	Methanol extract
1	Alkaloid	+
2	Carbohydrate	+
3	Cardiac glycoside	+
4	Steroid	+
5	Flavonoid	+
6	Tannin	+

Acute Toxicity Study: In the acute toxicity study no deaths were observed during the 72 h period. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as diarrhea or increased diuresis. The median lethal dose

(LD_{50}) was determined to be higher than the dose tested, *i.e.* 2.0 g/kg.

Analgesic Activity: The result showed that there was a significant difference in the pain reaction time (PRT) before drug administration in all the mice. 30 min after drug administration, the PRT was significantly (P<0.001) increased by the extract and the reference drug in a dose-dependent manner when compared to the normal saline-treated group. A significant difference between the negative group and the group that received the least dose of the extract (100-400 mg/kg). In the experiment the reference drug (Diclofenac sodium) was more effective than the extract in reducing the PRT. In analgesic all the three doses of MEJC *i.e.* 100 mg/kg (9.33 ± 0.33 sec), 200 mg/kg (10.67 ± 0.88 sec) and 400 mg/kg (11.33 ± 0.88 sec) significantly increases in the jumping time in the hot plate as compared to control (4.33 ± 0.33 sec) (p-value <0.0001). The result is also quite satisfying when compared with the standard drug at 25 mg/kg (13.33 ± 0.33 sec) **Fig. 1**, **Table 2**.

Anti-inflammatory Activity: In the anti-inflammatory activity test, MEJC (100-400 mg/kg) caused statistically significant (P<0.001 ANOVA) inhibition of inflammation induced by carrageenan-induced paw edema. The percentage inhibition of the inflammation caused by the JMEC (100-400 mg/kg) was comparable to that obtained with Aspirin (150 mg/kg) which was used as standard.

TABLE 2: ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *JATROPHA CURCAS* FRUIT USING HOT PLATE METHOD (MEAN ± SEM)

Treatment	Dose (mg/kg)	Reaction time in sec			
		15 sec	30 sec	45 sec	60 sec
Group I (Control) normal saline	10 (ml/kg)	3.67±0.33	3.67±0.33	4.67±0.33	4.33±0.33
Group II (Standard) Diclofenac sodium	25	12±0.57*	11.67±0.67*	12.67±0.33*	13.33±0.33*
Group III (Extract) MEJC	100	7.33±0.33*	7.33±0.33*	8.33±0.33*	9.33±0.33*
Group IV (Extract) MEJC	200	9.33±0.33*	9.33±0.67*	11±0.57*	10.67±0.88*
Group V (Extract) MEJC	400	10.33±0.33*	10.33±0.67*	11.33±0.67*	11.33±0.88*

Values are expressed as Mean ± SEM, (n=3). *P<0.0001, significantly different from control.

TABLE 3: ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *JATROPHA CURCAS* FRUIT BY CARRAGEENAN INDUCED PAW EDEMA IN RAT

Treatment	Dose (mg/kg)	Carrageenan induced paw edema (%)			
		0 h	1 h	2 h	3 h
Group I (Control) normal saline	10 (ml/kg)	0.57±0.03	0.65±0.03	0.78±0.02	0.88±0.04
Group II (Standard) Aspirin	150	0.41±0.02	0.48±0.02	0.53±0.02	0.53±0.02
Group III (Extract) MEJC	100	0.42±0.02	0.58±0.02	0.67±0.03	0.71± 0.03
Group IV (Extract) MEJC	200	0.41±0.02	0.51±0.02	0.65±0.03	0.7±0.03
Group V (Extract) MEJC	400	0.41±0.02	0.53±0.03	0.53±0.02	0.6±0.03

Values are expressed as Mean ± SEM, (n=3). *P<001, significantly different from control.

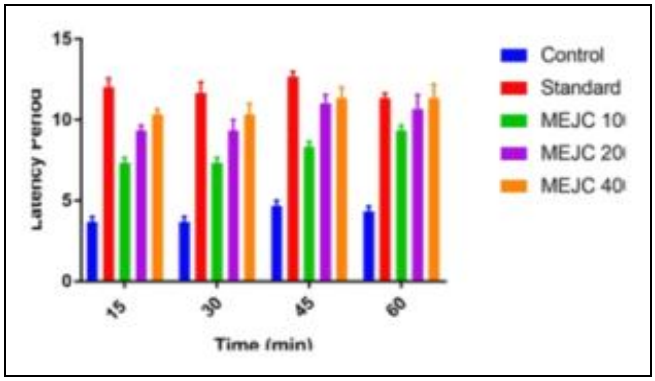

FIG. 1: GRAPH SHOWING THE ANALGESIC TEST RESULTS BY HOT PLATE METHOD

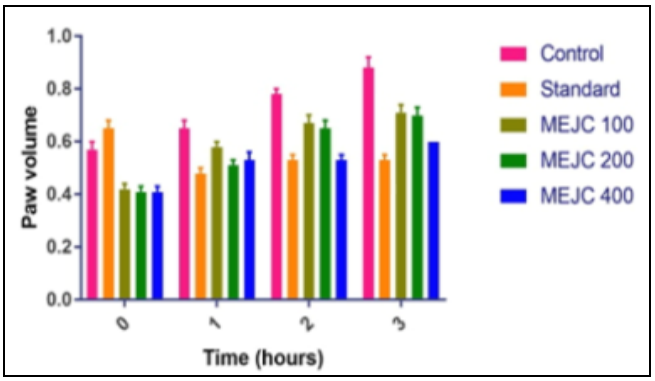

FIG. 2: GRAPH SHOWING ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN INDUCED PAW EDEMA

These were also dose dependent. Anti-inflammatory activity it shows values are expressed as Mean ± SEM, (n=3) MEJC *i.e.* 100 mg/kg (0.71± 0.03), 200 mg/kg (0.7 ± 0.03), 400 mg/kg (0.6 ± 0.03) which is comparable with the standard drug 150 mg/kg (0.53 ± 0.02) **Table 3**, **Fig. 2**.

DISCUSSION AND CONCLUSION: The analgesic and anti-inflammatory activity of fruits of plant have been assessed. Phyto-constituents exhibit in plants to be specific alkaloids, flavonoids, tannins and carbohydrates, cardiac glycosides. In the present study, Wister albino rats (180-200 gm) were chosen for screening anti-inflammatory impact of the extract to see anti-inflammatory activity. On the other hand albino mice (20-30 gm) were chosen for screening analgesic activity. The consequences of this study showed that the methanolic extract of the fruit of plant *Jatropha curcas* was exceptionally successful. Hence, scientific work will necessary for development of herbal drugs, which have low toxic effects and high therapeutic effects.

The result was satisfying when compared with the standard drug at 150 mg/kg anti-inflammatory activity shows inhibition of edema MEJC *i.e.* 100 mg/kg (0.71 ± 0.03), 200 mg/kg (0.7±0.03), 400 mg/kg (0.6 ± 0.03) which is comparable with the standard drug aspirin 150 mg/kg (0.53 ± 0.02). In analgesic all the three doses of MEJC *i.e.* 100 mg/kg (9.33 ± 0.33 sec), 200 mg/kg (10.67 ± 0.88 sec) and 400 mg/kg (11.33 ± 0.88). This result suggests that fruits of *Jatropha curcas* has anti-inflammatory and analgesic properties comparable with those of standard drugs and may be useful for the treatment of painful inflammatory conditions.

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CONFLICT OF INTEREST: There is no conflict of interest related to this work.

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