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IN-VITRO STUDY OF ANTICONVULSANT ACTIVITY ON THE FRUITS EXTRACTS OF *COCCINIA INDICA* (WIGHT &ARN.) INDUCED MAXIMAL ELECTROSHOCK SEIZURE (MES) MODEL IN RATS

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ABSTRACT:

Aim of the study: *In-vitro* study of Anticonvulsant activity on the fruits extracts of *Coccinia indica* (Wight &Arn.) induced maximal electroshock seizure (MES) model in rats.

Materials & methods: For the assessment of anticonvulsant activity, the animals were divided into five groups of five animals each albino rats. **Group I** received Normal saline, **Group II** received Phenytoin, **Group III** received 200 mg/kg of CIEE suspended in Tween 80, **Group IV** received 400 mg/kg of CIEE suspended in Tween 80, and **Group V** received 600 mg/kg of CIEE suspended in Tween 80. The results are expressed in mean ± S.E.M. (n=5) Statistical analysis was done by one way ANOVA, followed Dunnett multiple comparison test vs. control. P<0.01 was considered as statistically significant.

Results: In the present *In vitro* study, an anticonvulsant effect of ethanolic extracts of fruits *Coccinia indica* Wight & Arn. has been determined duration of HLTE 14.96, 3.49, 9.68, 8.20, 4.90 sec. The CIEE exhibited maximum anticonvulsant activity at 4.90 sec. at 600mg/kg (P<0.01) and it was significant when compared with control. In the present study, CIEE at the dose of 600mg/kg shows potent anticonvulsant activity as compared to 200mg/kg & 400mg/kg. The ethanolic extract of CI possesses potent anticonvulsant effect against MES induced and it is evidenced by its good anticonvulsant activity of 600 mg/kg against control and as positive control groups.

Conclusions: In conclusion these observations suggest that 600 mg/kg doses of HLTE exhibit potent anticonvulsant activity.

INTRODUCTION: The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world.

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Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [1]. Herbal medicine is still the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in photochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines [2].

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [3]. This situation forced to search for new antimicrobial substances. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.

Antimicrobials of plant origin have enormous therapeutic potential [4]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [5]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body [6].

Coccinia indica belongs to the family Cucurbitaceae. It is growing wild throughout India and also cultivated in various parts of India. It is commonly known as kundru [7]. The whole plant is traditionally used for various medicinal purposes. Leaves of this plant are used in Indian folk medicine for treatment of number of ailments including diabetes, wounds, ulcers, inflammation, in eruptions of skin, fever, asthma and cough. Earlier scientific investigation of *Coccinia indica* showed that the crude extract has hepatoprotective [8, 9 10, 11, 12, 13], anti-diabetic hypolipidemic [14, 15, 16, 17], anti-bacterial [18] and anthelmintic activity [19], analgesic and antipyretic activity [20], Wound healing activity [21], anti-inflammatory [22, 23] though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample.

The present investigation was therefore taken up to establish identity of fresh and dried fruits anticonvulsant activity for the standardization of the drug.

MATERIALS AND METHODS:

Collection and Authentication: The fresh fruits of wildly growing plant *Coccinia indica* were collected from the field areas of eastern Uttar Pradesh region during the month of February, 2009. For identification and taxonomic authentication, sample of plant material was given to National Vrakshayurveda Research Institute (NVRI) Jhansi, India. The text report from National Vrakshayurveda research institute, Jhansi, India and confirmed the authenticity of plant material sample was *Coccinia indica* with voucher Specimen no. NVRI -SOP-20932, 01-09-2012. Collected plants were shade-dried and coarsely powdered. Fruits of *Coccinia indica* Wight & Arn. and authentication specimen record card given respectively in **figure 1 & 2**.

FIGURE 1: FRUITS OF *COCCINIA INDICA* WIGHT & ARN.

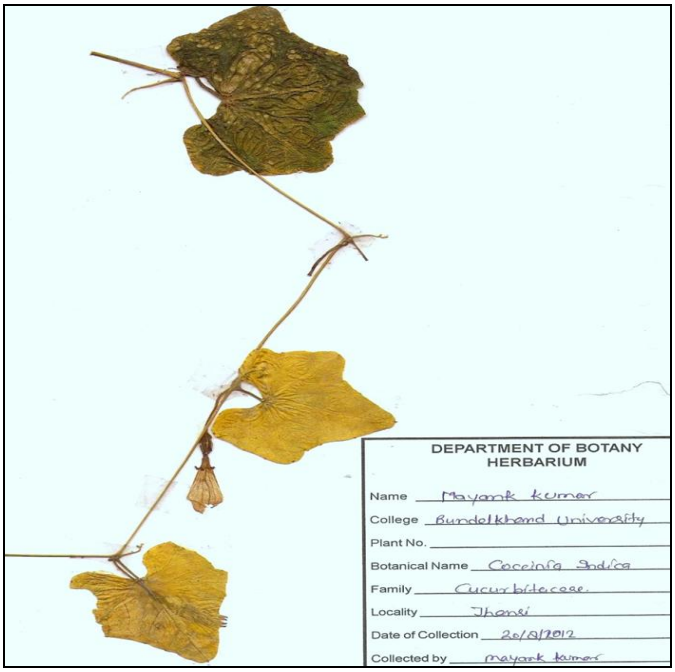

FIGURE 2: AUTHENTICATION OF SPECIMEN RECORD CARD

Extraction of Plant Materials: 250 g coarse powder of air dried fruits of *Coccinia indica* were packed in muslin cloth and subjected to soxhlet extractor for continuous hot extraction with petroleum ether and ethanol for 8 hrs separately. Then the each extracts were filtered and filtrate was evaporated to dryness.

Preliminary phytochemical studies: Preliminary qualitative chemical investigations of ethanolic extract of *Coccinia indica* fruits were performed to know the presence of Carbohydrates, Glycosides, Alkaloids, Tannins, Flavonoids, triterpenoids. Further thin layer chromatography was performed to confirm their presence.

Chemical (Drugs & solutions) and apparatus: The following drugs and chemicals were used. Drugs: Phenytoin (Sain medicaments Pvt. Ltd., Hyderabad) and Chemicals: Tween 80 (Chemical laboratory Pharmacy, BU University Jhansi), Normal Saline (0.9% NaCl solution). The plant extract was suspended in Tween 80 and subjected for anticonvulsant. Phenytoin was also dissolved in Tween 80.

Preparation of Dose: The ethanolic extract of *Coccinia indica* was freshly dissolved in a suitable amount of Tween 80 and was administered at the doses of 200, 400 and 600 mg/kg body wt. p.o. in rats. One hour after p.o. administration, the animals were submitted to the activity. Phenytoin (25mg/kg b. wt.) were dissolved in Tween 80 immediately prior to use and given i.p. Normal saline administered in a volume of 10 ml/kg b.wt.

Animal selection: Male albino rats of wistar strain weighing between 150-200 gm were selected for the anticonvulsant activity. Animals were housed in polypropylene cages with the filter tops and maintained at 25 ± 2 ^{o}C, relative humidity 55 ± 10% under controlled conditions of 12-h light: 12-h dark cycle.

The animals were fed up with commercial rat chow and were given water *ad libitum*. All protocols of the study was approved by the Institutional Animal Ethical Committee with reference number BU/PHARM/IAEC/12/024. The IAEC is approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) with registration number 716/02/a/CPCSEA.

Acute toxicity studies:

1. **Acute oral toxicity:** Acute toxicity study was performed for the extract according to the acute toxic classic method as per OECD guidelines **(OECD Guidelines for the testing of chemicals, 2001).** Swiss albino rats of either sex were used for acute toxicity study. The method of Up and Down was used to determine the dose. The animals were kept fasting for overnight providing only water, after which the extracts were, administered orally 100, 300, 500, 2000, 5000 mg/kg p.o. dose and percent mortality was observed 24 hrs then 72 hrs and thereafter once daily for 14 days.

   The ethanolic extract of fruits of *Coccinia indica* Wight & Arn. did not cause any mortality up to 5000 mg/kg during the observation period of 24 hrs then 72 hrs and thereafter once daily for 14 days and were considered as safe. The oral LD_{50} of the ethanolic extract estimated in mice must be > 5000 mg/kg p.o.

Animal Groups: For the assessment of anticonvulsant activity, the animals were divided into five groups of five animals each albino rats.

Group I received Normal saline.

Group II received Phenytoin.

Group III received 200 mg/kg of CIEE suspended in Tween 80.

Group IV received 400 mg/kg of CIEE suspended in Tween 80.

Group V received 600 mg/kg of CIEE suspended in Tween 80.

Assessment of anticonvulsant activity: Corneal electrodes were used for bilateral delivery of electrical stimulus. Electroconvulsive shock (50 mA for 0.2 sec) was delivered through corneal electrode to induce Hind Limb Tonic Extensor (HLTE) phase in rats. There are five phases observed in mice after giving maximal electroshock.

The five phases are;

(i) Flexor

(ii) Extensor

(iii)Convulsion

(iv)Stupor and

(v) Recovery or Death are noted and also the time spent by mice in each phase.

Prior to delivery, the current output was checked by using multimeter. The electrical stimulus was applied using a stimulator apparatus (Biocraft Scientific System Pvt. Ltd., Agra, India) for five groups of five animals each. The orientation for the anticonvulsant affect was abolition of HLTE within 10 sec after delivery of the electroshock (**Figure 3**). Delivery of MES using electroconvulsiometer to mice with the help of corneal electrode (**Figure 4**) Phases occur after giving electroshock (**Figure 5**) Graphical representation of anticonvulsant activity of *Coccinia indica* on maximal electroshock (MES) induced seizures in rats.

FIGURE 3: DELIVERY OF MES USING ELECTROCONVULSIOMETER TO MICE WITH THE HELP OF CORNEAL ELECTROD

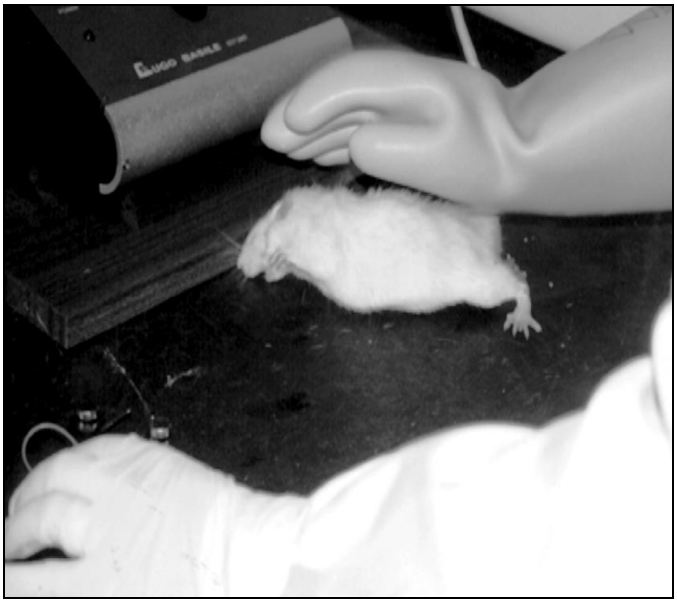

FIGURE 4: PHASES OCCUR AFTER GIVING ELECTROSHOCK

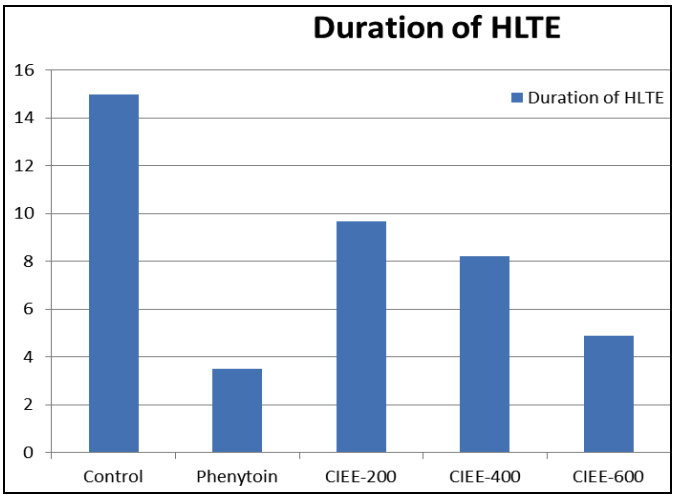

FIGURE 5: GRAPHICAL REPRESENTATION OF ANTICONVULSANT ACTIVITY OF *COCCINIA INDICA* ON MAXIMAL ELECTROSHOCK (MES) INDUCED SEIZURES IN RATS

Assessments for anticonvulsant activity Control, Standard drug (Phenytoin), *Coccinia indica* ethanolic extract by MES model are given in **table 1, 2, 3** respectively. Effect of *Coccinia indica* extract on MES induced seizures in rats are also given in **table 4** respectively.

TABLE 1: ASSESSMENT FOR ANTICONVULSANT ACTIVITY OF CONTROL BY MES MODEL

Treatment	Body Wt. (gm)	Dose	Duration (Sec.) in various phases of Convulsion				
			Flexor	HLTE	Convulsion	Stupper	R/D
Control	158	Normal Saline (10ml/kg)	4.17	14.60	21.05	A	D
	135		4.85	14.80	20.16	A	D
	168		4.65	14.66	24.54	32.05	R
	138		4.17	16.88	A	A	D
	156		4.40	15.86	24.67	31.45	R

R = Recovery, D = Death, A = Absent

TABLE 2: ASSESSMENT FOR ANTICONVULSANT ACTIVITY OF STANDARD DRUG (PHENYTOIN) BY MES MODEL

Treatment	Body Wt. (gm)	Dose	Duration (Sec.) in various phases of Convulsion				
			Flexor	HLTE	Convulsion	Stupper	R/D
Phenytoin	130	25 mg/kg	2.15	3.96	10.86	33.40	R
	128		2.46	3.46	10.26	34.86	R
	158		2.86	3.40	10.66	37.80	R
	167		2.32	3.52	10.88	39.19	R
	146		2.46	3.12	11.40	25.19	R

R = Recovery, D = Death, A = Absent

TABLE 3: ASSESSMENT FOR ANTICONVULSANT ACTIVITY OF *COCCINIA INDICA* ETHANOLIC EXTRACT BY MES MODEL

Treatment	Body Wt. (gm)	Duration (Sec.) in various phases of Convulsion				
		Flexor	HLTE	Convulsion	Stupper	R/D
CIEE (200 mg/kg)	168	1.55	10.48	A	A	D
	157	2.77	8.97	18.44	A	D
	139	2.22	7.8	35.18	37.10	R
	130	3.27	11.07	20.39	36.40	R
	145	2.39	10.11	19.40	36.11	R
DFEE (400 mg/kg)	128	2.26	8.93	19.23	33.90	R
	128	2.56	8.76	22.95	31.30	R
	148	2.29	9.36	25.56	35.49	R
	130	2.86	7.22	21.35	28.40	R
	136	1.58	6.75	A	A	D
	177	1.59	4.86	34.60	21.40	R
DFEE (600 mg/kg)	166	2.66	3.38	32.30	22.56	R
	148	2.32	3.66	25.56	24.56	R
	168	3.29	5.86	22.86	A	R
	138	2.36	6.76	A	A	R

R = Recovery, D = Death, A = Absent

TABLE 4: EFFECT OF *COCCINIA INDICA* EXTRACT ON MES INDUCED SEIZURES IN RATS

S. No.	Treatment	Duration of HLTE	Mortality (%)	Recovery (%)
1.	Control	14.96 ± 0.27	80	20
2.	Phenytoin	3.49 ± 0.14	0	100***
3.	CIEE-200	9.68 ± 0.45	40	60**
4.	CIEE-400	8.20 ± 0.34	20	80**
5.	CIEE-600	4.90 ± 0.32	0	100***

Statistical analysis: The results are expressed in mean ± S.E.M. (n=5) Statistical analysis was done by one way ANOVA, followed by Dunnett multiple comparison test vs. control. $P<0.01$ was considered as statistically significant.

RESULTS: In the present *in vitro* study, an anticonvulsant effect of ethanolic extracts of fruits *Coccinia indica* Wight & Arn. has been determined Duration of HLTE 14.96, 3.49, 9.68, 8.20, 4.90 sec. The CIEE exhibited maximum anticonvulsant activity at 4.90 sec. at 600mg/kg ($P<0.01$) and it was significant when compared with control. In the present study, CIEE at the dose of 600mg/kg shows potent anticonvulsant activity as compared to 200mg/kg & 400mg/kg.

Anticonvulsant can act on corneal electrodes were used for bilateral delivery of electrical stimulus. Electroconvulsive shock (50 mA for 0.2 sec) was delivered through corneal electrode to induce Hind Limb Tonic Extensor (HLTE) phase in rats. The ethanolic extract of CI possesses potent anticonvulsant effect against MES induced and it is evidenced by its good analgesic activity of 600mg/kg against control and as positive control group.

DISCUSSION: The present work was to carry out with the objective of ethanolic extract of fruits *Coccinia indica* Wight & Arn., Phytochemical investigation, evaluation of the anticonvulsant activity (*in-vitro*) against induced maximal

electroshock seizure (MES) model in rats. The ethanolic extract of CI possesses potent anticonvulsant effect against MES induced and it is evidenced by its good analgesic activity of 600mg/kg against control and as positive control group.

The preliminary phytochemical screening of fruits *Coccinia indica* Wight & Arn. shows the presence of steroids and flavanoids compounds as major active principle constituents in ethanolic extract of fruits *Coccinia indica* Wight & Arn.. Many of flavonoids and steroids compounds have been reported for hepatoprotective activity. Therefore, there is possibility that ethanolic extract of *Coccinia indica* Wight & Arn. fruits may possess the anticonvulsant activity.

CONCLUSION: It can be concluded that the present study on *C. indica* fruits can serve as an important source of information to the reduction in oxalate excretion was observed on CIEE treatment. This decreased excretion of oxalate may be due to the inhibition of formation of oxalate by the plant extract. The ethanolic extract of CI possesses potent anticonvulsant effect against MES induced and it is evidenced by its good analgesic activity of 600mg/kg against control and as positive control group.

Conflict of interest statement: We declare that we have no conflict of interest.

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