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Evaluation of Analgesic Activity of Hydroalcoholic Extract of Curcuma longa Rhizomes in Albino Rats

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ABSTRACT

The present study was aimed to investigate analgesic activity of the hydroalcoholic extract of Curcuma longa (HAECL) rhizomes in albino rats. The HAECL (100, 200 and 400 mg/kg; p.o.) was evaluated for its analgesic activity by employing tail immersion test and hot plate test. In tail immersion test the HAECL at a dose of 100, 200 and 400 mg/kg showed significant ($p<0.05$), ($p<0.01$) and ($p<0.01$) respectively analgesic activity at 30 min time interval but at 60 min time interval all the three doses showed significant ($p<0.01$) analgesic activity as compared to control group. In hot plate test the test drug at 200 mg/kg and 400 mg/kg body weight showed significant ($p<0.01$) analgesic activity. The HAECL rhizome shows analgesic activity in albino rats and was comparable with pentazocin. In addition, the acute toxicity study revealed that HAECL have not shown any toxicity on the animals up to the doses of 1000 mg/kg and the phytochemical investigation of the HAECL shows the presence of carbohydrate, terpinoids, tannins, flavonoids, alkaloids and glycosides.

Keywords: Curcuma longa; Rhizomes; Analgesic activity; Tail Immersion test; Hot plate test

INTRODUCTION

Drug discovery has been driven by a variety of technology plat forms which can also expedite the development of therapeutic agents from herbal medicines. The development of synthetic chemicals for therapeutic use is by and large a random process that might result in serendipitous discovery; many pharmaceutical companies are now focused on the development of plant-derived drugs. Complementary and Alternative Medicine Therapies (CAMTs), which have been gaining popularity throughout the world, are classified into drug-based CAMT and nondrug-based CAMT [1]. Among various drug-based CAMTs, medicines, more than 80% commonly practiced are herbs of plant origin. A wealth of experience on the application of herbal/plant materials used in promoting health has accumulated over centuries, and the information is readily available for modern scientific research on drug discovery. The earliest evidence of human use of plants for healing can date back to the Neanderthal period [2,3].

As a consequence, plant-derived herbs may interact favorably with the human body and hence produce beneficial effects in terms of health promotion. Not surprisingly, the observations of eating behavior and serendipitous events in wild animals have led to the discovery of herbs/plants with therapeutic potential. In this regard, the study on self-medication in animals may offer a novel approach to drug discovery for humans. For instance, novel anti-malarial compounds were isolated from the leaves of Trichilia rubescens based on a behavioral survey of chimpanzees from a natural population in Uganda [4].

Today, approximately 80% of antimicrobial, cardiovascular, immunosuppressive, and anti-cancer drugs are of plant origin. It is widely accepted that more than 80% of drug substances are either directly derived from natural products or developed from a natural compound [5]. Around 50% of pharmaceuticals are derived from compounds first identified

or isolated from herbs/plants, including organisms, animals, and insects, as active ingredients [6]. Presently drugs which are available for the management of pain are narcotics analgesics (e.g., opioids), NSAIDs (e.g. salicylates) and corticosteroids (e.g., hydrocortisone). Theses synthetic drug are expensive and also possess serious side and toxic effects. Non-Steroidal Anti-Inflammatory Drugs (NSAID) causes gastric lesions and opiates induced tolerance and dependence, the use of these drugs as analgesic agents have not been successful in all cases [7,8]. Plant and phytomedicine symbolized safety and are serving several purposes whether health, protection from disease or nutrition. Therefore there is a need to intensify our research with the aim of developing an analgesic drug from plant origin.

MATERIAL AND METHODS

Collection and identification of plant material

The rhizomes of Curcuma longa were collected from the local market of Berhampur, Odisha. Then the rhizomes are cut into small pieces by a sharp knife and dried under shade for fifteen days and converted into course powdered by using hand grinding mill.

Preparation of hydro alcoholic extract

At about 100 g of coarsely powdered rhizome was taken in a beaker and macerated with 50 ml of solvent containing methanol and water at a ratio of 1:1 for 24 hrs. Then in the next day the macerated powder of the rhizome was extracted using soxhlet apparatus with 250 ml of solvent containing equal proportion of methanol and water, maintained at a temperature of 60°C. The extraction was continued for 4-6 hrs until colour faded of the powder and the extract was evaporating to dryness under water bath. The dried exact was stored in a desiccator till further use**.**

Preliminary phytochemical screening

Preliminary phytochemical screening of the extract was performed and shows presence of compounds like carbohydrates, terpenoids, tannins, flavonoids, alkaloids and glycosides. Those compounds are termed as secondary metabolite and are responsible for therapeutic effects.

Animals

Swiss albino rats weighing 120-140 g of either sex were maintained under controlled condition of light and dark (12 hr) and temperature 25 ± 1°C in the animal house of Roland Institute of Pharmaceutical Sciences, Berhampur. The animals were acclimatized for one week prior to actual experiment. All the Pharmacological activities were carried out as per CPCSEA norms, after obtaining the approval from the Institutional Animal Ethical Committee of Roland Institute of Pharmaceutical Sciences, Berhampur.

Acute toxicity test

Acute oral toxicity study was carried out as per OECD guide line 423. Animals of both the sexes were selected by random sampling technique for the study. A single dose of hydroalcoholic extract of Curcuma longa (HAECL) rhizome starting at a dose of 200 mg/kg and progressively moving from 400 mg/kg, 600 mg/kg, and 800 mg/kg and up to 1000 mg/kg body weight was administered. All animals were closely observed for the toxic symptoms like behavioral changes, locomotion, muscle spasm, loss of righting reflex, tremor, convulsion and mortality for 24 hrs and further supervised for a period of 14 days for occurrence of toxic symptoms and mortality [9].

ANALGESIC ACTIVITY STUDY METHODS

Tail immersion test

The experiment was carried out by measuring tail withdrawal time from hot water [10]. Rats were randomly divided into five groups containing six animals each (n=6), food was withdrawn for 12 hrs but not with water. After 12 hrs group-I received distilled water p.o., group-II was given pentazocin 20 mg/kg, i.p., group-III, IV and V was given 100, 200 and 400 mg/kg of HAECL respectively, p.o. by feeding tube. After 30 min of pentazocin administration and 1 hr of extract administration, about 3-5 cm of the tail of each rat was dipped into a water bath containing warm water maintained at the temperature of 50 ± 10°C and the time taken for the rat to flick the tail known as the Pain Reaction Time (PRT) was recorded for all the rats.

Hot plate method

The study was performed using the effect of hot plate induced pain in rat [11,12]. Adult rats of either sex were randomly divided into five groups containing six animals per group (n=6). Food was withdrawn for 12 hrs but not with

drinking water. The latency time (fore paw licking and jumping response) were assessed by placing each rat upon a heated metal plate (Hot plate) maintained at a temperature of about 55 ± 1°C within a restraining cylinder. The latency time for each rat was determined using a stop watch. The cut off time was put at 20 s, this served as control reaction time. After the basal reaction time Group-I received distilled water, p.o., group-II was given pentazocin 20 mg/kg, i.p and group III, IV and V was given 100, 200 and 400 mg/kg of HAECL rhizome respectively by oral route through a feeding tube. The latency time for each rat was again determined using the same method as above.

Statistical analysis

The data were expressed as mean ± SEM of 6 animals. Results were analysed stastistically by one-way ANOVA followed by Dunnet's multiple comparison tests using prism software 0.5 version. The difference were considered significant if $p<0.05$.

RESULTS

Toxicity evaluation

The HAECL rhizome did not show any mortality up to 1000 mg/kg body weight dose. There was no change in behavior, locomotion, muscle spasm, loss of righting reflex, tremor and convulsion was observed in 24 hrs. Further no mortality was observed after 14 days.

Preliminary phytochemical evaluation

The extract was yellowish dry powder and had a fragrant in odour. The yield was 13.4% w/w dry matter. The phytochemical test of HAECL rhizome contains various phytoconstituents is shown in Table 1.

Table 1: Preliminary phytochemical evaluation

Phytoconstituents	Results
Carbohydrates	+
Proteins	-
Terpenoids	+
Tannins	+
Saponins	-
Flavonoids	+
Alkaloids	+
Glycosides	+
Steroids	-
Present: +, Absent: -	

ANALGESIC EVALUATION

Tail immersion response in Albino rats

Analgesic activity was investigated by tail immersion method. The tail withdrawal time was taken as the parameter for the evaluation of analgesic activity. There was no significant difference has been observed in reaction time of the control group at different time interval. Further there was no significant difference has been observed when treated groups are compared with control at basal point. The standard drug (pentazocin) at 20 mg/kg showed the significant difference ($P<0.01$) against control at 15 min, 30 min, 60 min respectively (Table 2). The test drug (HAECL) at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg showed significant difference ($P<0.05$) ($p<0.01$) and ($P<0.01$) respectively at 30 min time interval but all the three doses showed significant difference ($P<0.01$) compared to control group (Table 2). Hence we find that HAECL rhizomes have analgesic activity which is shown in Table 2.

Hot plate response in Albino rats

Analgesic activity was investigated by Hot plate method. The reaction time was taken as the parameter for the evaluation of analgesic activity. There was no significant difference has been observed in reaction time of the control group at different time interval. Further there was no significant difference has been observed when treated groups are compared with control at basal point. The standard drug (pentazocin) at 20 mg/kg showed a significant difference ($P<0.01$) against control at 15 min, 30 min, 60 min respectively (Table 3). The test drug (HAECL) at a dose of 200 and

Table 2: Reaction time of HAECL and pentazocin using tail immersion test

Groups	Treatment	Dose (mg/kg)	Pain Reaction Time (s) Mean ± SEM			
			Basal	15 min	30 min	60 min
1	Distill water	-	4 ± 0.33	4.3 ± 0.1	4.6 ± 0.19	4.6 ± 0.30
2	Pentazocine	20	5 ± 0.36	7.3 ± 0.33**	10 ± 0.36**	10.6 ± 0.42**
3	HAECL	100	5 ± 0.36	5.2 ± 0.36	6.3 ± 0.33*	7.3 ± 0.33**
4	HAECL	200	4.6 ± 0.33	5.5 ± 0.33	7.6 ± 0.49**	9 ± 0.36**
5	HAECL	400	5 ± 0.33	6 ± 0.23	7.6 ± 0.33**	10 ± 0.23**
Values are expressed as Mean ± SEM (n=6), *p<0.5,**p<0.01 as compared to control group						

Table 3: Reaction time of HAECL and pentazocin using hot plate test

Groups	Treatment	Dose (mg/kg)	Reaction Time (s) Mean ± SEM			
			Basal	15 min	30 min	60 min
1	Distill water	-	4.6 ± 0.49	5.66 ± 0.49	5 ± 0.36	4.66 ± 0.66
2	Pentazocin	20	4.83 ± 0.30	7.66 ± 0.21**	10.6 ± 0.33**	10.6 ± 0.33**
3	HAECL	100	4.6 ± 0.33	5 ± 0.36	5.6 ± 0.23	5.6 ± 0.33
4	HAECL	200	4 ± 0.36	4.6 ± 0.33	7.3 ± 0.33**	7.6 ± 0.33**
5	HAECL	400	4 ± 0.25	6 ± 0.51	10 ± 0.51**	10.3 ± 0.42**
Each values are expressed as Mean ± S.E.M.(n=6), *p<0.05, **p<0.01 as compared to control group						

400 mg/kg showed significant (P<0.01) difference compared to control group at 30 and 60 min time interval. Hence we find that HAECL rhizomes have analgesic activity which was shown in Table 3.

DISCUSSION AND CONCLUSION

The result from the tail immersion test and hot plate test showed the evidence for the analgesic activity of the extract. Herbal drugs having analgesic activity have the chemical constituents like glycosides, alkaloids, flavonoids, saponins, tannins, terpinoids. From the phytochemical screening, we observed that the HAECL contains various phyto-constituents like glycosides, alkaloids, flavonoids and other bioactive compounds. So it may be considered that the following phyto-constituent of the rhizome may be a drug candidate for its analgesic activity. Further studies are required to know the mechanism of action and actual chemical constituents that are responsible for analgesic activity. Here the models like tail immersion and hot plate model are help to know the analgesic activity. Another model like acetic acid induced writhing test and grid shock test can be used to study the analgesic effect of extract. It is concluded from the above study that the HAECL rhizomes contain various phyto-constituents may be responsible for the analgesic activity and was also comparable with standard drug like pentajocine.

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