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Full Length Research Paper

Antinociceptive and antipyretic properties of the pharmaceutical herbal preparation, *Radix Bupleuri* in rats

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***Chai hu* is a Chinese herb growing both in northern and southern China. It has the English names hare's ear and Chinese throwax root. Its botanical name is *Bupleurum Chinense D.C*. The pharmaceutical preparation of the extract is called *Radix Bupleuri* (*R. Bupleuri*). In this study the antinociceptive and antipyretic properties of *R. Bupleuri* were studied in mice. Yeast was used to induce pyrexia while acetic acid induced writhes and tail flick methods were used to investigate the nociceptive properties of the extract. Acute toxicity studies were also done to determine the median lethal dose LD_{50}. The extract significantly ($P < 0.05$) reduced acetic acid induced writhes compared with the control. The extract (50 mg/kg) showed a higher percentage inhibition of the acetic acid induced writhes compared to the positive control, acetyl salicylic acid (ASA). Tail flick model also revealed an increase in latency time for mice to flick their tail out of a warm bath maintained at a temperature of 50°C at the 25 mg/kg dose level compared to the control ($P < 0.05$). The extract also significantly ($P < 0.01$) reduced the temperature of rats after pyrexia induction. The results of this study justify the use of this extract by traditional Chinese herbalist as pain killer and for the treatment of fever.**

Key words: *Radix Bupleuri,* herbal, antipyretic, antinociceptive.

INTRODUCTION

The importance of pain management cannot be overemphasized. Almost every disease that affects man comes with pain as one of the symptoms. There are various methods and strategies used for pain management however; better alternatives that have fewer side effects are still sought. Chinese have long used a variety of herbal concoctions for various diseases and a huge range of these herbs have strong antinociceptive properties. The present study aims to study the antinociceptive properties of the Chinese extract, *R. Bupleuri*, commonly called *Chai Hu*. This herb has been used for various ailments traditionally for the treatment of eye disorders, menstrual cramps and jaundice. It is also commonly used for headaches, fever and diarrhea.

Studies by Shang Hai Yi Ke, 1986 have shown *R. Bupleuri* to stimulate both humoral and cellular immunity in mice. *R.Bupleuri* has also been demonstrated to possess strong bactrostatic and anti - viral effects against β-hemolytic *streptococcus, Vibrio cholera, mycobacterium tuberculosis*, poliomyelitis and the hepatis virus (Zhong, 1998). Previous work (Yao, 1979) has shown the median lethal dose (LD_{50}) to be 1.19 g/kg via intraperitonial (i.p) injection of the essential oil in mice. This study was undertaken to evaluate the scientific basis of the use of *R.Bupleuri* for various ailments which include fevers and menstrual cramps in traditional Chinese medicine.

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MATERIALS AND METHODS

Chemical

Acetic acid was provided from glacial chemicals co. (Searle, England). Chai Hu (*R.Bupleuri*) extract was purchased from GKH natural herbs (Panyu, China). Drugamol was purchased from drug field pharmaceuticals (Nigeria).

Animals

Swiss albino mice (20 - 25 g) and Wistar rats (180 - 250 g) of either sex maintained at the animal facility centre of NIPRD, Abuja, were used. The animals were housed under standard conditions of temperature, (25 ± 2°C) and light, (approximately12/12 h light -dark cycle) and fed on standard diet and given water *ad libitum*. The studies were carried out following the principles of good laboratory practice and animal handling (National Institutes of Health Guide for the care and use for Laboratory animals; Publication No. 85-23, revised 1985).

Acute toxicity study

The oral acute toxicity was evaluated using 18 adult albino rats of either sex weighing between 200 - 250 g with Locke model (1983). Animals were administered extract at different dose levels ranging from 10 - 5000 mg/Kg body weight. The median lethal dose (LD_{50}) was determined as the geometric mean of the highest non lethal dose and the lowest lethal dose of which there is 1/1 and 0/1 survival.

Phytochemical study

Phytochemical screening for various constituents including saponins, alkaloids, tannins, glycosides, flavanoids, anthraquinones and resins was carried out on the crude sample using standard methods (Trease and Evans, 1987). Each of the tests was qualitatively expressed as a positive, +ve or negative, -ve.

Acetic acid induced writhes

The method described by Koster et al. 1959 was used. Healthy adult mice were randomized into six groups of five mice each. The mice in Group I which served as the negative control received 5 ml normal saline/kg body weight intraperitoneally. The mice groups II, III, IV and V were pretreated with 6.25, 12.5, 25 and 50 mg extract/kg body weight intraperitoneally (i.p) respectively while group VI received Acetyl salicylic acid (ASA) 150 mg/kg body weight. 1 h after pre - treatment mice in all the groups were given 10 ml/Kg body weight of 0.7% acetic acid i.p. The numbers of writhes in each animal were counted for 10 minutes. Treatment groups were compared to the control group and the percentage inhibition (figure 2) was determined using the formula:

% Inhibition = (Control mean – test mean) ÷ control mean × 100

Tail flick test

Healthy adult mice were randomized into five groups of five mice each. The group I mice served as the negative control and were given 5 ml normal saline /kg body weight. Mice in group I were pretreated with 5 ml normal saline/kg body weight (i.p) while mice in groups II, III and IV were pretreated with 10, 20 and 25 mg extract/kg body weight respectively (i.p). Group V animals received 10 mg/kg morphine sulphate. 30 minutes later, each mouse was restrained in horizontal cylinders leaving the tail hanging freely and then the tail inserted in warm water maintained at 50°C and the time taken for the mouse to flick its tail (tail - flick latency) was evaluated. The tail - flick latency was evaluated at 0, 30, 60 and 90 mins, with 0 min being the first reading. The tail - flick latency was determined before extract administration, recorded as time 0 min. The extract was administered with different dose and tail - flick latency determined at 30, 60 and 90 mins.

Yeast induced pyrexia

Rats were divided into five groups (group I - V) of five rats each. A rectal thermometer was inserted deep into the rectum and the temperature was recorded. The normal body temperature of each rat was measured rectally prior to any fever induction. Fever was induced by a subcutaneous injection of 20 ml/kg body wt. of 20% w/v yeast suspended in methyl cellulose solution (Loux et al., 1972). Rats were then returned to their housing cage. After 24 h of yeast injection, the rectal temperatures of the animals were again taken as described previously.

Then *R. Bupleuri* was administered i.p at doses of 12.5, 25 and 50 mg/kg body weight to animals in group II, III and IV respectively. 5 ml normal saline/Kg body weight was administered i.p to animals in group I. Animals in group V received the standard drug drugamol (20 mg/kg, body wt.) i.p. Rats were restrained for recording of their rectal temperatures at intervals of 30 mins, after the drug administration (Figure 1 and Table 2)

Statistical analysis

Data were expressed as ± SEM and statistically analyzed using student's t - test and 2 -way ANOVA. Results were considered statistically significant when $P < 0.05$.

RESULTS

Acute toxicity study

The extract did not produce any signs of toxicity in treated rats and mortality was not recorded up to a maximum dose of 5000 mg/kg.

Phytochemical study

The photochemical screening showed positive results for terpenes, sterols, flavanoids, resins, balsams and carbo-hydrates. Negative results were obtained for constituents such as alkaloids, anthraquinones and tannins.

Acetic acid

The extract significantly showed a dose dependent inhibitory effect on writhes in mice caused by acetic acid (P value < 0.01) between the doses of 12.5 - 50 mg/kg body weight. At dose of 50 mg/kg, the extract showed the highest inhibitory effect and the inhibitory effect generally

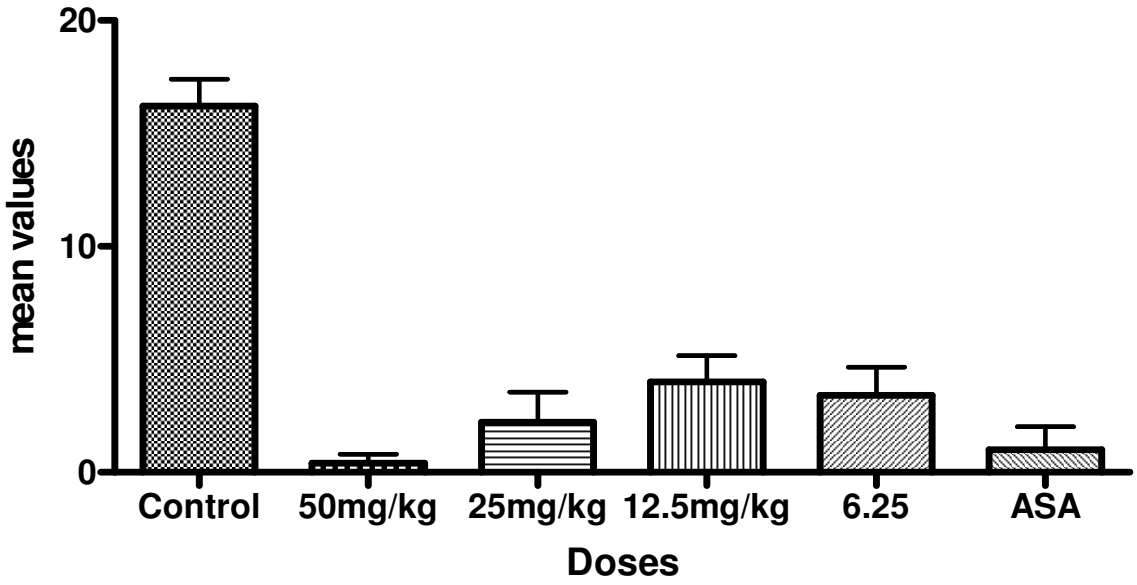

Figure 1. Graph showing effects of oral administration of *R.Bupleuri* extract at 50 mg/kg, 25 mg/kg,12.5 mg/kg and 6.25 mg/kg dose levels and Acetyl salicylic acid (ASA) on acetic acid induced writhes in mice.

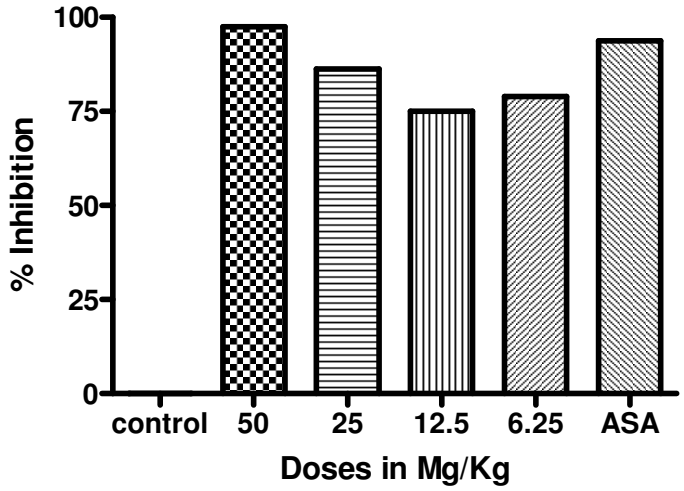

Figure 2. Percentage inhibition of acetic acid induced writhes produced by oral administration of *R.Bupleuri* to mice.

decreased as the dose was lowered. The extract at 50 mg/kg showed a higher inhibitory effect compared with acetyl salicylic acid, this was however not statistically significant ($P > 0.05$).

Tail flick

The extract showed no significant increase in tail flick latency compared with control 30 min after extract administration. At T2, 60 min after extract administration, the extract only showed a significant increase in tail flick latency at the 20mg/kg dose level. At 90 mins after extract administration however, a significant increase was recorded at the 25 mg/kg dose level and all other dose levels showed an insignificant increase. (Table 1)

DISCUSSION

This study has shown that *R. Bupleuri* has dose dependently showed antinociception in both the acetic acid

Table 1. Tail flick results

	Group I (Saline 5 ml/kg)	Group II (*R.bupleuri* 10 mg/kg)	Group III (*R.bupleuri* 20 mg/kg)	Group IV (*R.bupleuri* 25 mg/kg)	Group V (10 mg/kg Morphine sulphate)
T1	7.8±037	8.4±1.25	7.6±0.60	7.6±024	5.0±1.10
T2	9.4±1.25	7.7±0.80	18.60±3.60*	11.2±2.40	12.2±2.35
T3	9.8±0.97	9.6±0.87	10.8±1.85	14.8±3.68*	9.8±3.87
T4	11.2±1.83	8.8±0.73	9.0±1.18	6.8±1.24*	9.2±0.58

T1= Tail flick latency before drug administration
T2= Tail flick latency 30min after drug administration
T3= Tail flick latency 60 min after drug administration
T4-= tail flick latency 90 min after drug administration
*significantly different from control at $p < 0.05$.

Table 2. Yeast induced pyrexia: The effect of *R. Bupleuri* and drugamol on yeast - induced pyrexia in rats.

Treatment	Rectal temperature (°C)					
	After yeast injection at			After drug administration		
	0 h	24 h	30 min	60 min	90 min	120 min
Control Vehicle 5ml/kg	36.15±0.24	37.27±0.20	37.70±0.33	38.35±0.21	38.95±0.156	39.90±0.16
drugamol 20mg/kg	36.24±0.27	37.06±0.43	36.50±0.14	35.91±0.14	34.91±0.26	34.54±0.18
R.Buplueri 50mg/kg	35.42±0.26	36.6±0.35*	36.48±0.26 *	36.57±0.22*	36.59±0.19*	36.43±0.27*
**R.Buplueri* 25 mg/kg	34.91±0.22	35.82±0.29 *	35.16±0.31*	34.90±0.30 *	34.73±0.20*	34.64±0.27 *
R.Bupleuri 12.5 mg/kg	34.78±0.23	35.84±0.23 *	35.17±0.26*	34.89±0.27 *	35.18±0.28*	34.90±0.24 *

*$P < 0.001$

induced writhes and the tail flick method. The extract has produced no signs of toxicity in rats and can be considered safe to use as indexed by the lethal median dose of > 5000 mg/kg. The acetic acid method has been shown to detect antinociception of both peripheral and central analgesics (Collier et al., 1968). It is thus widely used as a screening method for analgesia. It is also capable of detecting antinociception at dose levels that may appear inactive with other methods (Collier et al., 1968). It is generally considered that compounds with less than 70% inhibition have minimal activity. In this study, the extract has shown higher than 70% inhibition even at the least dose level used. This thus suggests strong analgesic property of the extract.

The tail flick method was used in order to investigate the possible mode of action of the extract. The tail flick method has been shown to be selective for morphine – like drugs. It differentiates between centrally acting and peripherally acting analgesics.

These results however suggest the possibility of some central activity as the extract showed activity at the 25 mg/kg dose level in the tail flick test. The onset can however be said to be delayed as the tail flick latency was only significantly increased 60 and 90 minutes after extract administration. Studies by Tsjolsen and co workers, 1992 have shown that weak analgesics such as salicylic acid and paracetamol have very little or no effect on responses to test with phasic stimuli such as the tail flick and hot plate tests. Tail flick tests are particularly sensitive to central analgesics (Carlisson and Jurna, 1987). Fever may be the result of infection or as a result of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic drugs are used to reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and a drug like drugamol does not influence body temperature when it is elevated by other factors such as exercise or increases in ambient temperature Goodman and Gilman, 1996. The present result show that the *R. Bupleuri* possesses a significant antipyretic effect in yeast - provoked elevation of body temperature in rats and its effect is comparable to that of drugamol.

The phytochemical analysis revealed the presence of several components including terpenes. These compounds have been found to produce analgesia in certain compounds such as cannabis (Ross and Elsohly, 1996). Certain terpenes have also being shown to possess potent analgesic activity acting at central receptors and antagonized by naloxone (Rao et al., 1990). This might

explain the slight central activity revealed by the tail flick test. Based on the results of the present study it can be concluded that *R. Bupleuri* has both analgesic and antipyretic properties hence its use by traditional Chinese herbalist. More detailed studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

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