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Antipyretic and antinociceptive potential of *Ricinus communis* L. and *Withania somnifera* L. hydroalcoholic extracts in Wistar rats: A comparative study

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Abstract: The present study was designed to evaluate the antipyretic and antinociceptive activities of *R. communis* leaves and *W. somnifera* roots hydroalcoholic extracts in Wistar rats. To assess the antipyretic activity, Brewer's yeast suspension was used to induce hyperthermia. Antinociceptive activity was observed using acetic acid-induced abdominal writhing, formalin-induced paw licking reflex and heat-induced pain models. *R. communis* and *W. somnifera* extracts were used at 150, 250 and 500mg/kg. Results showed that administration of both plants significantly (p<0.001) lowered rectal temperature (^{o}C) in a dose-dependent manner from 1h to 4h of study. *R. communis* and *W. somnifera* extracts showed a dose-dependent reduction in abdominal writhing induced by acetic acid and decreased the paw licking reflex in formalin-induced nociceptive response. In the heat test, *R. communis* and *W. somnifera* extracts exhibited significant (p<0.001) analgesic effects evidenced as an increase in latency time. However, *R. communis* exhibited prominent antipyretic and antinociceptive activities at 250 and 500mg/kg as compared to *W. somnifera.* Conclusively, *R. communis* and *W. somnifera* could be a potential source of antipyretic and analgesic agents which require further studies.

Keywords: Antipyretic, antinociceptive, *Ricinus communis, Withania somnifera*.

INTRODUCTION

Pain and pyrexia are the most common manifestations of several diseases and have become one of the principal areas of scientific research. Pain can be described as an emotional experience and unpleasant sensation associated with underlying tissue injury and/or damage which can be described in such terms. It is a primary protective response but causes discomfort (Ezenyi *et al*., 2021). Harmful stimuli trigger the nociceptors through various chemical mediators such as vasoactive amines, nitric oxide, leukotrienes, prostaglandins, peptides, excitatory amino acids and cytokines which induce inflammation and pain. Prostaglandins modify the interleukins, bradykinins and TNF-α linked free nerve endings transducing properties and cause hyperalgesia. Inflammation is considered to have a secondary impact on pyrexia. Cytokines are generated in response to pyrogens that interfere with the temperature set point in the hypothalamus and induce fever. Therefore, increased production of prostaglandins is considered to be associate with pyrexia and pain (Alam *et al*., 2020).

Various synthetic drugs are efficient in reducing pain and fever. Although, these agents are associated with adverse effects include blood dyscrasias, cardiovascular instability, respiratory depression, gastric disturbance, hepatotoxicity and nephrotoxicity (Bindu *et al*., 2020). Herbal medicines have been recognized in several reports for their significant therapeutic impact with few side effects in comparison to modern medicines. According to surveys, it is estimated that 80% of the population in 3rd world countries rely on traditional medicines. Plants still provide structurally novel compounds that may lead to drug development. Also, ethnomedicinal studies could be a potential source of developing new and potentially more effective products (Tufail *et al*., 2020).

Ricinus communis L., known as 'Castor oil plant', belongs to the Euphorbiaceae family. Castor is widely spread throughout tropical regions of the world. It has significant medicinal value as it possesses antioxidant, antibacterial, antiasthmatic, antiulcer, anticancer, anti-inflammatory, anti-arthritic and hepatoprotective properties (Javanshir *et al*., 2020; Hussain *et al*., 2021). *Withania somnifera* Dunal, also called 'Ashwagandha', is a perennial herb that belongs to the family Solanaceae. It has been cultivated and used in folklore medicines due to its antioxidant, antifungal, antibacterial, antitumor, antidiabetic, cardioprotective, chondroprotective and immunomodulatory properties (Mukherjee *et al.*, 2020; Hussain *et al*., 2021). Considering the potential use of *R. communis* and *W. somnifera* in traditional medicine, the present study is proposed to ascertain their antipyretic and antinociceptive properties in animal models.

MATERIALS AND METHODS

Plant materials

R. communis leaves and *W. somnifera* roots were collected (March, 2019) from the botanical garden of University of Agriculture, Faisalabad, Pakistan. The specimens were deposited to Department of Botany, University of Agriculture, Faisalabad, Pakistan for future

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reference (*Ricinus* communis: 212-1-19, *Withania* somnifera: 212-2-19).

Preparation of extracts

Shade dried *R. communis* leaves and *W. somnifera* roots were powdered. Each powdered plant (200g) was macerated in 2L of solvent (ethanol: water, 70:30 v/v) for 3 days with manual shaking at regular intervals. Extracts were filtered (Whatman filter paper No. 1) and concentrated by using a rotary evaporator. The obtained extraction yields of *R. communis* leaves extract (RCLE) and *W. somnifera* roots extract (WSRE) were 19.3% (w/w) and 13.8% (w/w), respectively.

Experimental animals

Adult healthy female Wistar rats (weight range: 150-220 g) were purchased and housed at the animal house facility of Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan. All experimental animals were acclimatized for one week. Standard laboratory conditions i.e. 12h light/dark cycle, 25±2^{o}C room temperature, 40-60% humidity and proper ventilation were maintained throughout the study. Standard pellets chow diet twice a day and fresh water *ad-libitum* were provided.

Ethical approval

The study protocols were approved by the Ethical standards of animal care and Institutional Biosafety Committee, University of Agriculture, Faisalabad, Pakistan (D. No. 3498/ORIC). All animals were treated and cared for according to guidelines of the International Association for Study of Pain (Zimmermann, 1983).

Evaluation of antipyretic activity

Brewer's yeast-induced pyrexia model was used to assess the antipyretic activity of RCLE and WSRE (Lino *et al*., 2017). For this purpose, the rectal temperature of rats was recorded over 1h period and the average temperature of the individual rat was calculated. Subcutaneous injection of 10 ml/kg of yeast suspension (15% w/v) was given to induce hyperthermia. Rats were selected who showed an increase in rectal temperature (0.5^{o}C or above) after 18h of Brewer's yeast injection. The selected rats were grouped (n=6) and treatments were administered orally as Group I: received normal saline (3ml/kg), Group II: was given paracetamol (150mg/kg), Group III to V: administered with RCLE (150, 250 and 500mg/kg) and Group VI to VIII: treated with WSRE (150, 250 and 500 mg/kg). Then post-treatment rectal temperatures were measured at 1h interval up to 4h of study.

Antinociceptive activity by using acetic acid-induced abdominal writhing test

Antinociceptive effect of RCLE and WSRE was observed in the acetic acid-induced abdominal writhing test (Bhowmick *et al*., 2014). Acetic acid causes severe abdominal pain and writhing when injected intraperitoneally. The analgesic activity was assessed as a decrease in writhing numbers in comparison with control group. For this study, rats were allotted to eight groups (n=6) and treatments were given orally as Group I: received normal saline (3ml/kg), Group II: received aspirin (200mg/kg), Group III-V: Administered with RCLE (150, 250 and 500mg/kg) and Group VI-VIII: treated with WSRE (150, 250 and 500mg/kg). After 1h of oral treatments, rats were intraperitoneally injected with 10ml/kg of 0.6% acetic acid solution. Writhing was counted for 30min and % inhibitions were calculated (Alam *et al*., 2020).

$$\%\,\text{inhibition} = \frac{[(\text{Writhing}_{\text{Control}} - \text{Writhing}_{\text{Treated}})]}{\text{Writhing}_{\text{Control}}} \times 100$$

Antinociceptive activity by using formalin-induced paw reflex test

In this study, formalin was used to induce pain, and analgesic activity of RCLE and WSRE was observed against paw licking reflex of rats as described previously (Cidade *et al*., 2016). Rats were grouped (n=6) as Group I was administered with normal saline (3 ml/kg) and aspirin (200 mg/kg) was given to Group II. Six groups were treated with RCLE and WSRE at 150, 250 and 500 mg/kg, respectively. Treatments were administered *via* oral route. Each rat was subcutaneously injected with 20 µl of 1% formalin solution in the left hind paw after 1h of treatments. Pain response as paw licking reflex was recorded after injecting formalin in early (0-5 min) and late (15-30 min) phases and % inhibition was calculated (Alam *et al*., 2020).

Antinociceptive activity by using heat test

A modified method of Akindele *et al*., (2012) was adopted to evaluate the antinociceptive activity of RCLE and WSRE using the hot plate method. A sensitivity test was done for the selection of test animals. Rats were exposed to 55±1^{o}C for few seconds (s). Paw licking and jumping within 15s was considered a pain response. Then selected rats were divided into eight groups (n=6) and orally treated as Group I: given normal saline (3ml/kg), Group II: received diclofenac (50mg/kg), RCLE (150, 250 and 500mg/kg) given to Groups III-V and WSRE (150, 250 and 500mg/kg) was administered to Groups VI-VIII. The reaction time of all groups was again recorded after 30, 60, 90 and 120min of treatments, using 30s as cut-off time.

STATISTICAL ANALYSIS

The obtained data were analyzed by applying one-way and two-way ANOVA and Tukey's multiple comparison test using Graph Pad Prism® software (version 6.01). The level of significance (p<0.05) between control and treated groups was determined and results were presented as Mean ± SD.

Table 1: Antipyretic activity of RCLE and WSRE on brewer's yeast-induced pyrexia in rats

Groups	Rectal temperature (°C)					
	Pre-treated		Post-treated			
	Initial	18h	1h	2h	3h	4h
Control	37.56±0.14	39.69±0.11	39.67±0.19	39.58±0.18	39.57±0.25	39.44±0.27
Paracetamol (150 mg/kg)	37.90±0.28	39.38±0.22	38.12±0.29**	38.06±0.40**	37.83±0.33***	37.81±0.38***
RCLE (150 mg/kg)	37.71±0.27	39.47±0.19	38.96±0.20	38.88±0.12	38.34±0.11*	37.94±0.22***
RCLE (250 mg/kg)	37.43±0.19	39.28±0.14	38.79±0.41	38.67±0.35	37.92±0.51***	37.86±0.56***
RCLE (500 mg/kg)	37.24±0.17	39.44±0.29	38.16±0.46**	38.02±0.31**	37.89±0.16***	37.71±0.53***
WSRE (150 mg/kg)	37.61±0.22	39.41±0.18	39.15±0.19	38.94±0.12	38.57±0.12*	38.11±0.19**
WSRE (250 mg/kg)	37.34±0.16	39.59±0.18	38.84±0.50	38.73±0.35	38.11±0.33**	37.90±0.59***
WSRE (500 mg/kg)	37.58±0.12	39.53±0.17	38.42±0.53*	38.31±0.32*	37.92±0.28***	37.77±0.28***

Table 2: Antinociceptive activity of RCLE and WSRE on acetic acid-induced abdominal writhing in rats

Groups	Number of Writhing	% inhibition
Control	34.00±2.36	--
Aspirin (200 mg/kg)	12.66±1.21***	62.77
RCLE (150 mg/kg)	30.00±2.36	11.76
RCLE (250 mg/kg)	21.16±4.49**	37.76
RCLE (500 mg/kg)	14.26±1.55***	58.06
WSRE (150 mg/kg)	33.16±3.31	2.47
WSRE (250 mg/kg)	25.16±3.18**	26.00
WSRE (500 mg/kg)	16.00±2.52***	52.94

Table 3: Antinociceptive activity of RCLE and WSRE on formalin-induced paw licking response in rats

Groups	Early Phase (0-5 min)		Late Phase (15-30 min)	
	Licking response (s)	% inhibition	Licking response (s)	% inhibition
Control	65.33±6.65	--	67.00±8.96	--
Aspirin (200 mg/kg)	48.50±6.80***	25.76	20.66±3.07***	69.16
RCLE (150 mg/kg)	63.16±2.04	3.32	62.00±4.19	7.46
RCLE (250 mg/kg)	60.66±2.50	7.15	40.66±6.28***	39.31
RCLE (500 mg/kg)	54.00±3.74**	17.34	33.00±2.44***	50.75
WSRE (150 mg/kg)	66.00±4.33	1.03	62.50±6.59	6.72
WSRE (250 mg/kg)	59.50±3.01	8.92	48.00±3.57***	28.36
WSRE (500 mg/kg)	56.50±3.20*	13.52	38.83±2.86***	42.04

Table 4: Antinociceptive activity of RCLE and WSRE on heat-induced pain in rats

Groups	Latency time (s)				
	Pre-treated	Post-treated			
		30 min	60 min	90 min	120 min
Control	8.36±0.14	8.62±0.58	8.47±0.25	8.69±0.53	8.71±0.47
Diclofenac (50 mg/kg)	8.23±0.09	14.94±0.54***	19.00±0.39***	17.88±0.71***	17.21±0.97***
RCLE (150 mg/kg)	8.51±0.27	10.02±0.47*	15.74±0.91***	13.70±0.95***	9.73±0.66*
RCLE (250 mg/kg)	8.31±0.09	10.49±0.36*	17.41±0.74***	15.52±0.76***	12.57±0.47**
RCLE (500 mg/kg)	8.20±0.07	11.08±0.47***	19.44±0.34***	18.82±0.37***	15.40±0.31***
WSRE (150 mg/kg)	8.39±0.22	9.56±0.69	14.98±0.83***	12.95±0.64***	9.18±0.64
WSRE (250 mg/kg)	8.35±0.06	9.71±0.52*	16.81±0.53***	13.85±0.71***	12.23±0.55***
WSRE (500 mg/kg)	8.28±0.12	9.90±0.83*	17.47±0.84***	17.53±0.99***	14.60±1.43***

Results were analyzed by applying two-way ANOVA and Tukey's tests and presented as mean±SD (n=6). Values are presented as mean±SD. *p<0.05, **p<0.01, ***p<0.001, significant difference from Control group.

RESULTS

Effect of RCLE and WSRE on Brewer's yeast-induced pyrexia

Results mentioned in table 1 are showing the antipyretic effect of RCLE and WSRE on brewer's yeast-induced pyrexia in rats. Elevation in rectal temperature was observed after 18h of injection. Rats administered with RCLE and WSRE demonstrated a progressive reduction in rectal temperature started as early as 1h and the antipyretic effect was maintained up to 4h of study. Both extracts exhibited a dose-dependent as well as time-dependent reduction in rectal temperature in comparison to control group.

Effect of RCLE and WSRE on acetic acid-induced abdominal writhing

Antinociceptive activity evaluated through abdominal writhing induction using acetic acid in rats. The aspirin (reference drug) treated rats showed a significant (p<0.001) reduction in writhing as compared to the Control group. RCLE and WSRE administered at 150, 250 and 500 mg/kg exhibited a dose-dependent analgesic effect (table 2). A significant (p<0.001) antinociceptive effect of RCLE (58.06%) and WSRE (52.94%) was observed at 500 mg/kg as compared to aspirin (62.77%).

Effect of RCLE and WSRE on formalin-induced paw licking reflex

Paw licking reflex using formalin injection was induced in rats to observe the antinociceptive effect of RCLE and WSRE (table 3). Aspirin used as a reference drug significantly (p<0.001) reduced paw licking reflex in both phases. In the neurogenic phase that lasted for 0-5 min, RCLE and WSRE produced less significant analgesic effects as compared to aspirin. In the late phase (15-30 min), which was indicative of inflammatory pain, RCLE and WSRE showed a dose-dependent analgesic effect.

Effect of RCLE and WSRE on heat-induced pain response

The antinociceptive activity of RCLE and WSRE was evaluated using the hot plate method in rats (table 4). Administration of diclofenac (50mg/kg) showed a significant (p<0.001) increase in latency time from 30 min to 120 min in comparison to control group. RCLE and WSRE exhibited a dose-dependent increase in latency time as compared to control and diclofenac-treated groups. A higher antinociceptive effect was observed on all doses of RCLE and WSRE at 60 min of the experiment.

DISCUSSION

R. communis and *W. somnifera* are valuable medicinal plants that are commonly used in folklore medicines. Several studies reported their pharmacological effects such as antimicrobial, antiulcer, antiasthmatic, anti-diabetic, hepatoprotective, cardioprotective, immuno-modulatory and anticancer potential (Mukherjee *et al*., 2020; Javanshir *et al*., 2020; Hussain *et al*., 2021). We previously evaluated the chemical composition, antioxidant and anti-inflammatory activities (under publication) and ant-arthritic activities of RCLE and WSRE (Hussain *et al*., 2021). Our previous findings showed that RCLE and WSRE could considerably alleviate the inflammation in animal models and demonstrated a significant attenuation of pro-/anti-inflammatory cytokines in the adjuvant-injected rats led to an anti-arthritic effect.

In the current study, the antipyretic and analgesic effects of RCLE and WSRE were assessed. Antipyretic activity is the characteristic property of compounds that possess a prostaglandins synthesis inhibitory action. Brewer's yeast suspension was used to induce pyrexia in rats to evaluate the antipyretic potential of RCLE and WSRE as it is considered to be a useful method for the screening of plants or synthetic formulation for their antipyretic activity (Zhang *et al*., 2019). In our study, RCLE and WSRE exhibited a dose-dependent as well as time-dependent antipyretic effect (table 1). Results depicted that RCLE and WSRE have significant (p<0.001) antipyretic properties and may have some influence on prostaglandin synthesis. In addition, RCLE at 250 and 500 mg/kg showed better effects in contrast to WSRE.

Previous studies have shown that analgesic activity involves various mechanisms (Feng *et al*., 2003). Antinociceptive activity of RCLE and WSRE was observed in three different animal models. Acetic acid injection in rats produces abdominal writhing through increasing the endogenous substances such as prostaglandins (PGE_2, $PGE_{2\alpha}$), serotonin and histamine release in peritoneal fluids. The efficacy of peripheral analgesics is commonly assessed by using the acetic acid-induced writhing model (Dai *et al*., 2021). Current study results showed the significant antinociceptive effect of RCLE and WSRE at 250 and 500 mg/kg by reducing acetic acid-induced abdominal writhing while non-significant (p<0.05) analgesic effects were observed at 150 mg/kg dose of both plants (table 2). It indicates the peripheral analgesic potential of bioactive compounds of plants, since any compound that decreases the number of writhing possesses peripheral pain inhibition potential by inhibiting the synthesis of prostaglandins (Feng *et al*., 2003).

Formalin test was used in this study to gain more specific evidence on the possible mechanism of analgesic effect of RCLE and WSRE at graded doses in rats. The biphasic nociceptive behavior was examined by injecting formalin in the rat paw. Formalin causes activation of peripheral nociceptors in the early neurogenic phase that lasts for 0

to 5 min. A secondary inflammatory response triggered by formalin occurs after 15 to 30 min, which is a combination of central sensations and swelling of peripheral tissue (Kumar and Vinayak, 2020). In the current study, RCLE and WSRE at 500mg/kg exhibited significant (p<0.05) analgesic effect in the early phase while 250 and 500 mg/kg doses of both plants significantly (p<0.001) decreased the paw licking reflex in the late phase (table 3). Although RCLE and WSRE showed a more pronounced effect in the late phase. Pre-treatment with RCLE and WSRE showed reduction in paw licking during formalin-induced early neurogenic and late inflammatory phases, hence, it can be concluded that extracts may possess both central and peripheral effects.

The central analgesic effects of RCLE and WSRE were evaluated using the thermal test. Thermal stimulus triggers the spinal reflex which can be measured concerning the reaction time of rats. A thermal method is employed to assess the central mechanism mediated analgesic effect while compounds that act through peripheral route are ineffective on this kind of painful stimulus (Mazzitelli *et al*., 2018). The present study demonstrated that RCLE and WSRE at 250 and 500 mg/kg exhibited significant (p<0.001) antinociceptive effects from 60 to 120 min which indicates inhibition of central neurotransmission associated thermal stimulation (table 4). Therefore, the findings of chemically and thermally induced nociceptive stimulus tested in the current study depicted that RCLE and WSRE may contain bioactive compounds which are associated with central and peripheral antinociceptive effects.

CONCLUSION

This study showed that RCLE and WSRE at 250 and 500 mg/kg possess marked antipyretic and antinociceptive activities while less significant effects were found at 150 mg/kg dose of these plants. The findings of current study suggest the use of RCLE and WSRE in pyrexia and pain and confirmed their traditional uses. However, isolation of phytoconstituents with possible antipyretic and antinociceptive properties is required to understand the clear mechanism of action in further studies.

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