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RESEARCH PAPER

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACT OF RHEUMATIC TEA FORMULA (RTF) IN RATS AND MICE

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ABSTRACT

The analgesic and anti-inflammatory activities of the ethanolic extract of Rheumatic Tea Formula (RTF) a polyherbal tea consisting the leaves of *Eucalyptus globulus*, *Albizia chevalieri* and bark of *Salix alba* were studied in mice and rats using acetic acid induced writhing, hot plate method, formalin induced pain and carrageenan induced rat paw edema. The extract at doses of 50, 100 and 200 mg/kg produced a significant ($P<0.05$) and dose dependent inhibition of writhing induced by acetic acid. In the hot plate method the extract at all doses also produced a significant ($p < 0.05$) dose dependent increase in latency of pain. In formalin induced pain the extract exhibited significant ($P<0.05$) analgesic activities in both first and second phase of the formalin induced pain at doses of 250, 500 and 1000 mg/kg. The ethanolic extract significantly ($P<0.05$) and dose dependently reduced paw edema induced by carrageenan. The present study suggests that RTF contains bioactive constituents that possess analgesic and anti-inflammatory effects, the former being mediated centrally and peripherally justifying its ethnomedicinal use in pain and inflammation associated with rheumatism.

Key words: Rheumatic Tea Formula, Analgesic, Anti-inflammatory, Acetic –acid, Carrageenan

INTRODUCTION

Rheumatic Tea Formula (RTF) is a polyherbal tea remedy consisting three plants product namely the leaves of *Eucalyptus globulus* (*turare in Hausa*) and *Albizia chevalieri* (*katsari in Hausa*) and bark of *Salix alba*. The formula is used for the treatment of pain and inflammation associated with rheumatism by the National Association of Nigerian Traditional Medicine Practioners (NANTMP) in Gwarzo Local Government Area of Kano State, Northern Nigeria. Undoubtedly medicinal plants remain an important source of new chemical substances with potential therapeutic effects on pain (Gupta *et al*., 2006).

Studies on *Salix alba* by Gyawali *et al*., 2013, showed that the bark has both analgesic and inflammatory effects in albino mice, while the phytochemical screening showed the presence of alkaloids, tannins and glycosides. *Eucalyptus globulus* leaves contain mainly essential oils of oxygenated monoterpenes (46.5%) and terpinene-4-ol (23.46%) as its constituents (Akolade *et al.,* 2012). Volatile oil from the leaves of *Eucalyptus globulus* is also used topically to treat sore muscles and rheumatism while combination of eucalyptus and peppermint showed promise as an analgesic (Del *et al*., 2009). Albizia species are widely used for asthma, as anti-septic, anti-dysenteric, anti-tubercular, anti-oxidant and antimicrobial agent (Kokila *et al.*, 2013). Phytochemical screening of aqueous extract of *Albizia chevalieri* revealed the presence of saponins and flavonoids (Andrew *et al*., 2013).

Although there are documented works on the three plants individually, none however, exists for the plants in combination; prompting the need to study the formula to ascertain if the combination is as effective as claimed. To the best of our knowledge, no documented work exists on its efficacy. The present study therefore, evaluates the analgesic and anti-inflammatory potentials of the ethanolic extract of RTF in mice and rats.

MATERIALS AND METHODS

Collection and Extraction of Plant Materials: The Rheumatic Tea Formula (RTF) was collected from the National Association of Traditional Medical Practitioners in Gwarzo, Local Government Area of Kano State, Nigeria. The tea bags were emptied and the powdered content collected. Four hundred and sixty four grams (464 g) of RTF was extracted with seventy percent aqueous ethanol (70/30 v/v) by cold maceration for 7 days to ensure maximum extraction with occasional stirring. The extract was filtered to remove the mac and the filterate evaporated to dryness over a water bath at 50 degree celsius (50˚C). The extract was stored and prepared freshly when required for experiment.

Animals: Adult Wistar rats (110-250 g) and Albino mice (16-30 g) of both sexes were used during this work. The rats were obtained from animal houses of Ahmadu Bello University, Zaria and University of Jos, Nigeria. The animals were kept at the animal house of the Department of Pharmacology, Bayero University Kano. They were kept under normal conditions with free access to food and water. All animals were allowed at least twenty four hours (24hrs.) to acclimatize before commencement of all experiments. Rats for carrageenan paw edema experiment were denied water overnight prior to the experiment to avoid false results.

Drugs/Chemicals: Chemicals of analytical grade were used in the experiments; Ethanol, carrageenan (Sigma Aldrich), Ketoprofen (Hovid), Pentazocine, Acetic acid, Formalin, Normal saline, Distilled water

Phytochemical Screening: The preliminary screenings for phytochemical constituents were carried as reported by Sisidharan *et al.,* (2011).

Median Lethal Dose (LD_{50}) Determination: The median lethal dose (LD_{50}) was determined in both rats and mice using the method described by Lorke (1983).

ANALGESIC STUDIES

Acetic-Acid Induced Writhing in Mice: The method employed was as described by Gaertner *et al*., (1999). Thirty (30) mice were divided into 5 groups of 6 mice each, group 1 served as control and was administered normal saline (1ml/kg *p.o*), Group 2 was given standard drug, Ketoprofen (10mg/kg *i.p*) while groups 3, 4 and 5 were given the extract at doses of 50, 100 and 200mg/kg respectively. After 30 min, 1% acetic acid solution (0.1ml *i.p*) was administered to all the five groups and the number of writhes was counted 5 mins after the administration of acetic acid for a period of 10 mins. Writhing is characterised by wave of contraction of the abdominal muscles followed by extension of the hind limbs. Percentage of writhing inhibition of the extract was calculated in relation to the control as follows:

$$\text{Percentage inhibition} = \frac{\text{inhibition of control} - \text{inhibition of extract}}{\text{Inhibition of extract}} \times 100$$

Hot Plate Test in Mice: Hot plate test in mice described by Wilson *et al.,* (2003), as modified was adopted. The mice were selected based on their pain threshold, all the mice were placed singly on the hot plate kept at temperature of 45 ± 1^{o}C and mice that responded within 2 sec were selected. Thirty mice were divided into 5 groups of 6 mice each. Group 1 served as control and received normal saline (1ml/kg *p.o*). Group 2 was treated with pentazocine (20 mg/kg *i.p*), while groups 3, 4 and 5 received 50, 100 and 200 mg of RTF extract respectively. After 30 mins, the mice from all the groups were placed singly on hot plate kept at 45 ± 1^{o}C. The reaction time was noted (time taken for a mouse to lick its paw or jumped away from the hot plate). The experiment was repeated at 30, 90, 150 and 210 mins respectively and the reaction time noted. The percentage thermal pain was calculated as follows:

$$\% \text{ protection against thermal pain} = \frac{(\text{test mean} - \text{control mean})}{\text{Control mean}} \times 100$$

Formalin Induced pain in Rats: The method by Hunskaar *et al.*, (1985) was adopted in this experiment. Thirty six rats were divided into 6 groups of 6 rats each. Group 1 was given normal saline (1 ml/kg *p.o*), while groups 2 and 3 were administered pentazocine (20 mg/kg *i.p*) and ketoprofen (10 mg/kg *i.p*) respectively. Groups 4, 5 and 6 were administered 250, 500 and 1000 mg/kg doses of RTF extract. Thirty (30) minutes after the administrations, 50µL of freshly prepared 2.5 % formalin was injected subcutaneously into the sub-plantar surface of the left hind paw of each rat. The rats were put individually into an observation chamber and monitored after 5 mins and then at the end of 45 mins. The severity of pain was monitored based on the following scale:

i. Rat stands firmly or walk on the injected paw.....0
ii. The injected paw partially elevated or favored…….1
iii. The injected paw is clearly lifted off the floor……...2
iv. The rat licks, chews or shakes the injected paw…….3

ANTI-INFLAMMATORY STUDIES

Carrageenan Induced Rat Paw Edema: Thirty rats were divided into 5 groups of six rats each; the first group was administered normal saline at dose of (1 ml/kg) which served as control. The second group was given ketoprofen (10 mg/kg *i.p*) dose, while groups 3, 4 and 5 were administered RTF extract at doses of 125, 250 and 500 mg/kg *i.p*. Acute inflammation of the right hind paw was induced by administering 0.1 ml of 1% w/v suspension of carrageenan *i.p* into the sub-plantar surface of hind limb paw. The diameter of the injected paw was measured using digital vernier calliper at time 30, 60, 120, 180 and 240 minutes. Paw edema is characterized by increase in the diameter of the injected paw (Winter *et al*., 1963).

Statistical Analysis: The data were expressed as Mean $\pm$ Standard Error of Mean. The results were analyzed by student's t-test. Results were regarded as significant at $P<0.05$.

RESULTS

Phytochemical Screening: The phytochemical screening of the ethanolic extract of the RTF showed the presence of glycosides, saponins, alkaloids, reducing sugars and tannins.

Median Lethal Dose (LD_{50}): The intraperitoneal median LD_{50} value of RTF extract in rats was found to be 3,808 mg/kg body weight and the oral LD_{50} was greater than 5000 mg/kg body weight. The intraperitoneal LD_{50} value for mice was 775 mg/kg body weight.

Acetic-Acid Induced Writhing in Mice: The ethanolic extract of RTF significantly ($P < 0.001$) reduced the acetic acid induced abdominal writhes in mice in a dose dependent manner. The highest percentage inhibition of 82.5 % was shown in 200 mg/kg dose (Fig.1a). Ketoprofen10 mg/kg gave 89.4% inhibition (Fig1b).

Hot Plate Test in Mice: RTF extract produced significant dose dependent increase in reaction time ($P < 0.001$) with the highest activity shown at 0.5h. The highest protection by the extract was observed at 200 mg/kg dose. There was also significant ($P < 0.05$) increase in reaction time up to 2.5h at doses of 100 and 200 mg/kg. The effect of the extract started to decline from 2.5h while the effect of pentazocine was maintained till 3.5 h (Table.1).

Formalin Test in Rats: During the first phase (5 min.) of formalin test, RTF extract showed a significant ($P < 0.001$) decrease in the pain scale in a non-dose dependent manner. At the second phase (45 min.) the extract produced a dose dependent significant ($P < 0.05$) decrease in the pain scale of formalin test. Pentazocine also showed significant ($P < 0.005$) reduction of pain scale in both phases but with decline in activity at the second phase, while ketoprofen significantly ($P < 0.001$) reduced the pain scale in both phases (Fig.2).

Carrageenan Induced Rat Paw Edema: The sub-plantar injection of 1% carrageenan suspension produced a local edema with its peak at 3h in the control rats. RTF extract showed significant ($P < 0.001$) inhibition of carrageenan-induced paw edema over a span of 4h dose dependently. The extract showed more inhibition of carrageenan-induced paw edema compared to ketoprofen at all doses tested. The highest percentage inhibition of 91.1% was observed at the dose of 250 mg/kg at 4h of carrageenan injection (Table 2).

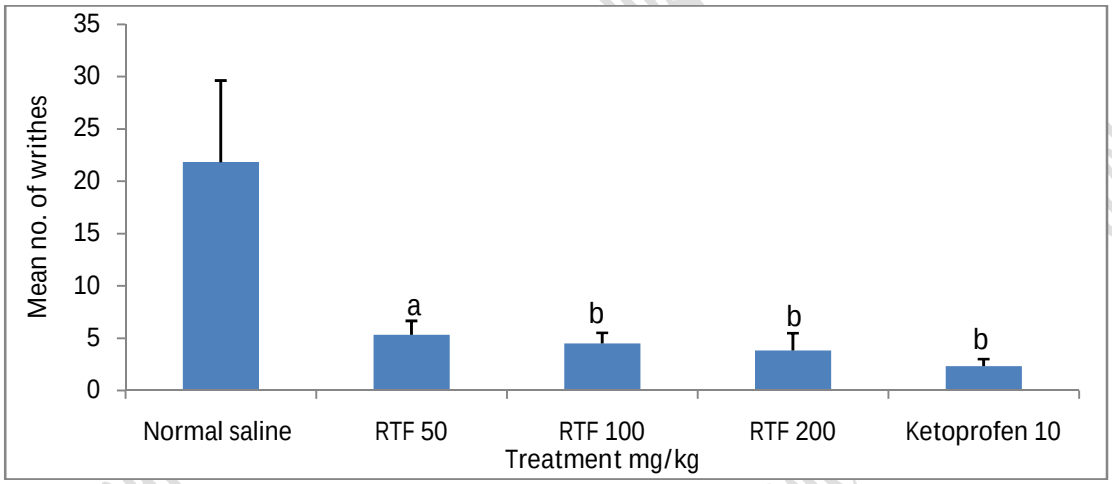

Fig1a; Effect of ethanolic extract of RTF on acetic-acid induced writhing test in mice: n = 6, a = P ≤ 0.05, b = P ≤ 0.001; compared to control, Student t-test for all values. RTF (Rheumatic Tea Formula)

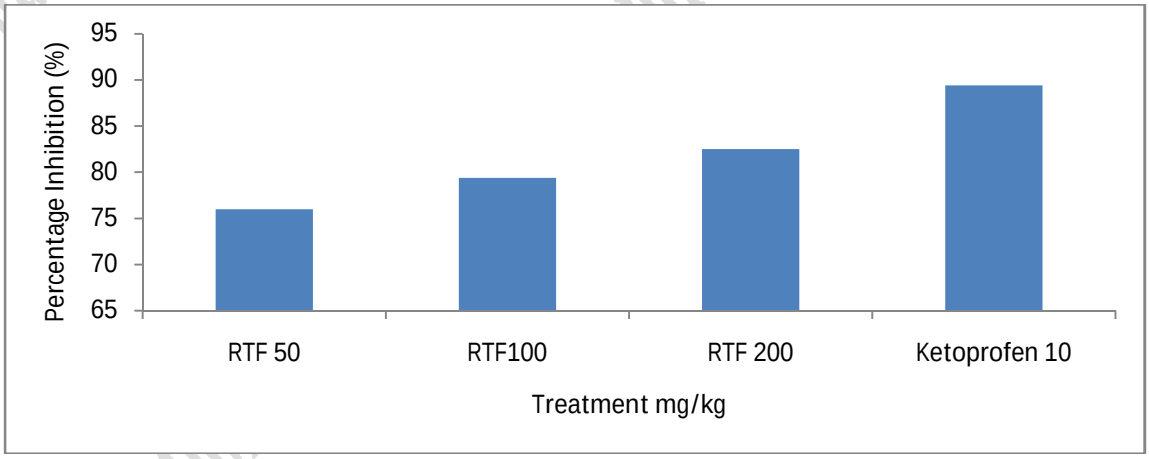

Fig.1b; Percentage inhibition of writhes in acetic acid induced writhing in mice RTF (Rheumatic Tea Formula)

Table 1: Effect of ethanolic extract of RTF on hot plate reaction time test in mice

Treatment mg/kg	Reaction Time (Sec)			
	0.5h	1.5h	2.5h	3.5h
N/S	1.23 ± 0.10	1.51 ± 0.28	1.68 ± 0.27	1.63 ± 0.27
RTF 50	3.33 ± 0.49^{c}	2.00 ± 0.26	1.83 ± 0.48	1.68 ± 0.17
RTF100	3.50 ± 0.22^{c}	3.17 ± 0.40^{b}	2.67 ± 0.33^{a}	1.83 ± 0.31
RTF 200	4.50 ± 0.72^{c}	3.17 ± 0.65^{a}	2.50 ±0.43	2.17 ± 0.31
Pentazocine 20	6.17 ± 1.11^{c}	5.00 ± 1.73^{a}	4.50 ± 0.62^{c}	2.33 ± 0.21^{a}

Values are mean ± SEM (n = 6). (a = P < 0.05, b = P < 0.005 and c = P <0.001) (t-test)

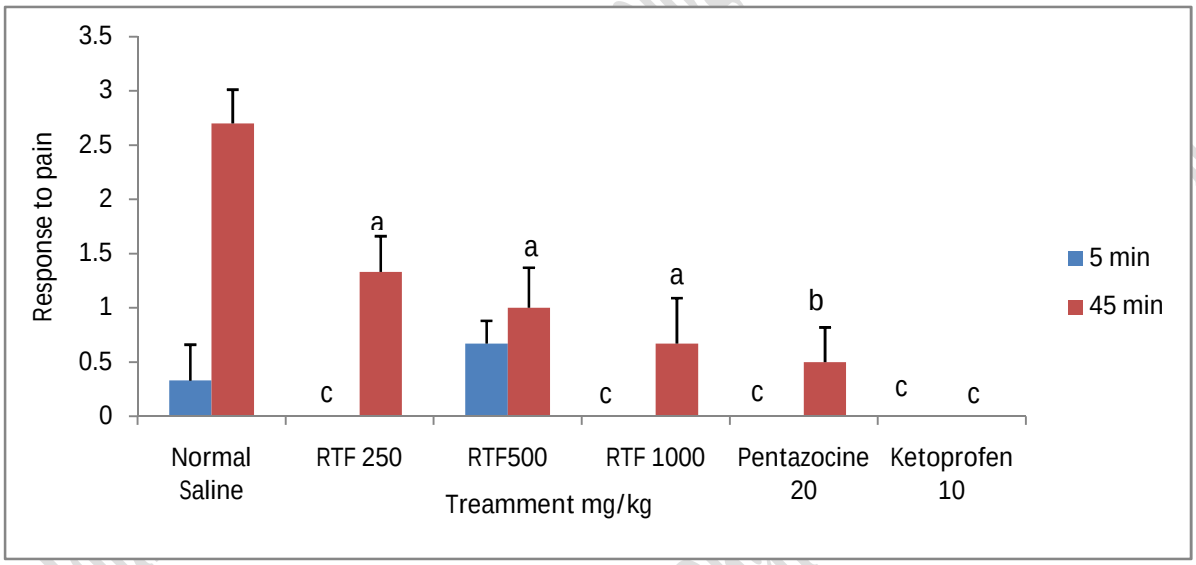

Fig.2; Effects of ethanolic extract of RTF on formalin induced pain test in rats; n=6, a = P ≤ 0.05, b = P ≤ 0.005, c = P ≤ 0.001; (t-test). RTF (Rheumatic Tea Formula

Table 2: Effect of ethanolic extract of RTF on carrageenan-induced paw edema in rats

Treatment (*i.p.*) mg/kg	0.5h	Edema 1h	Diameter 2h	(mm) 3h	4h
N/S (1ml)	1.45±0.23	1.67±0.60	1.76±0.23	1.78±0.28	1.52±0.34
RTF (125)	1.03±0.13	0.96±0.19[a] (42.5)	0.80±0.16[b] (54.4)	0.64±0.18[b] (64.0)	0.22±0.18[b] (85.6)
RTF (250)	1.06±0.04	0.93±0.10[b] (44.3)	0.78±0.07[b] (55.7)	0.44±0.17[b] (75.2)	0.12±0.07[c] (91.9)
RTF (500)	1.13±0.19	0.83±0.15[b] (50.2)	0.75±0.17[b] (57.4)	0.36±0.14[c] (79.8)	0.20±0.07[b] (86.5)
Ketoprofen (10)	1.53±0.02	1.35±0.23 (19.2)	1.17±0.21[a] (33.5)	1.06±0.20[a] (40.4)	0.60±0.20[a] (60.5)

Values are mean; ±SEM n= 5, a=P<0.05, b=P<0. 005, c=P<0.001. Values in brackets ( ), are the % inhibition of edema. RTF (Rheumatic Tea Formula)

DISCUSSION:

The preliminary phytochemical screening of Rheumatic Tea Formula (RTF) showed the presence of secondary metabolites like glycosides, saponins, alkaloids, reducing sugar and tannins. These phytochemical constituents possess various pharmacological activities; saponins possess anti-inflammatory and anti-allergic effects (Yassin *et al*., 2013) and antibacterial activity (Soetan *et al.,* 2006). Saponins and glycosides are also known to possess anti-inflammatory and anti-nociceptive activities (Akkol *et al*., 2007).

Alkaloids have also been shown to exhibit anti-inflammatory, antioxidant, antidepressant, anticancer, anti-diarrheal, cholagouge, hepatoprotective and antimicrobial activities (Singh *et al.,* 2010). Ethanolic extract of RTF significantly ($P < 0.05$) reduced the acetic acid induced writhing in mice in dose dependent manner at the doses tested. The highest percentage inhibition (82.5 %) was observed at the highest dose of 200 mg/kg compared to 89.4 % inhibition

by Ketoprofen. This shows that RTF has peripheral analgesic effect on mice. The acetic acid induced writhing is a visceral pain model which is generally used for screening plants and new agents for analgesic properties (Gene *et al.,* 1998).

It has also been shown that the acetic acid test is a non-specific nociceptive model (Bighetti *et al*., 1999). The intra-abdominal injection of acetic acid leads to the release of pain mediators such as prostaglandin and cytokines which may be responsible for the induced pain (Ikeda *et al*., 2001). Ethanolic extract of RTF investigated in mice using hot plate model showed a significant ($P < 0.05$) analgesic activity, with the highest activity at 0.5h in all doses tested and pentazocine, however the effect of the extract started to decline from 2.5 h with only pentazocine showing significance ($P < 0.05$) at 3.5 h. The highest protection was shown at dose of 200 mg/kg at 0.5h.

The hot plate method was used to elucidate central analgesic effects and is also used to differentiate between peripheral and central acting anti-nociceptive agents (Ramabadran *et al*., 1989). The result revealed that the ethanolic extract of RTF possesses a centrally mediated analgesic effect on mice. In the formalin test in rats, the first phase induced pain is due to direct chemical stimulation of the nociceptors which are transmitted via C fibres and can be suppressed by opioids such as pentazocine (Sayyah *et al*., 2004), while the late phase is due to inflammation and thus sensitive to NSAIDs such as ketoprofen (Hunskaar and Hole, 1987). The extract at the neurogenic phase (first phase), significantly ($P < 0.05$) inhibited formalin induced pain in rats in a non-dose dependent manner with 500 mg/kg dose showing no significance. In the second phase, the extract showed significant ($P < 0.05$) dose dependent inhibition of formalin induced pain in rats.

This investigation revealed that, the ethanolic extract of RTF has both peripheral and central mediated analgesic effect in rats and mice. The anti-nociceptive effects of RTF may be probably due to the presence of saponins and glycosides (Akkol *et al*., 2007). Carrageenan induced inflammation is biphasic, the first phase is due to rapid production of mediators such as serotonin, histamine, and bradykinin while the second phase is from the release of prostaglandin and nitric oxide with peak at 3h, produced by inducible isoforms of cyclo-oxygenase (COX) and nitric oxide synthase (NOS) respectively (Siebert *et al*., 1994). Carrageenan is the phlogistic agent of choice for evaluating anti-inflammatory agents as it is not known to be antigenic and is devoid of apparent systemic effect (Chakraborty *et al*., 2006).

In the carrageenan induced hind paw edema model, the sub-plantar injection of 1% carrageenan suspension produced a local edema with its peak at 3h in the control. The extract showed significant ($P < 0.05$) inhibition of carrageenan induced paw edema over a period of 4h dose dependently, with the extract showing more inhibition compared to ketoprofen at all doses tested. The maximum inhibition of edema (91.1%) was observed at dose of 250 mg/kg at 4h. This result showed that RTF has protective activity against inflammation. This probably may be due to the presence of saponins (Yassin *et al*., 2013), alkaloids (Singh *et al.,* 2010) or glycosides (Akkol *et al*., 2007). From the results obtained in this study, RTF contains bioactive secondary metabolites with significant analgesic and anti-inflammatory properties which justify its use in pain and inflammation associated with rheumatism.

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AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author AM designed the study, performed the experiments and contributed to manuscript review. Author SSC wrote the protocol, performed the experiments and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.