

## EVALUATION OF THE ANTI -INFLAMMATORY, ANALGESIC, AND ANTIPYRETIC ACTIVITIES OF METHANOL EXTRACT OF *PARMELIA PERLATA* LICHEN

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### ABSTRACT

*Parmelia perlata* is an important lichen that traditionally used to treat inflammation, fever, headache, pain but its medicinal activities have not been proven by researches. In this study, the methanol extract of *Parmelia perlata* (MEPP) lichen (200 and 400mg/kg orally) prepared by cold maceration, was used to study the anti-inflammatory activity by formalin-induced rat paw edema method, to evaluate analgesic activity by Eddy's hot plate method and to study antipyretic activity by yeast-induced pyrexia method. Albino rats (140-200 g) of tow sexes were used for the study. The paw volume and pyrexia in rats were reduced, and the latency period was prolonged significantly ( $P < 0.05$ ) compared to control. This study proved the traditional uses of this lichen and concluded that the methanol extract of *parmelia perlata*

possessed significant analgesic, anti-inflammatory, and antipyretic activity.

**KEYWORDS:** Anti-inflammatory, analgesic, antipyretic, lichens, *Parmelia perlata*.

### INTRODUCTION

Inflammation and pain are some of the most common manifestation of many diseases.<sup>[1]</sup> Chronic inflammatory diseases are still one of the main health problems of the world's population.<sup>[2]</sup> The term inflammation covers a complex series of reparative and protective response to tissue injury, whether caused by infection, burn, toxic chemicals, allergens or

other noxious stimuli.<sup>[3]</sup> The uncontrolled and persistent inflammation may act as an etiologic factor for many of chronic illnesses. Pain has been defined as an unpleasant sensory and emotional experience associated with tissue damage.<sup>[4]</sup> Pyrexia is a fever caused as a secondary impact of infection, malignancy or other disorder states.<sup>[3]</sup> Pain and fever are usually associated with inflammation.<sup>[5]</sup> So anti-inflammatory drugs are used to treat disorders which leads to inflammation, pyrexia, and pain of whatever cause.<sup>[6]</sup>

Non steroidal anti-inflammatory drugs (NSAID) act as analgesic, antipyretic and anti-inflammatory, and opioids are used for management of pain. These synthetic drugs are of least interest nowadays due to their potential side effects.<sup>[7]</sup> such as :gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates.<sup>[8]</sup> In the last few decades, alternative anti-inflammatory and analgesic agents have regained their popularity in the treatment against several human ailments. And search for safe and effective agents have been given priority in scientific research in herbal system of medicine.<sup>[2]</sup>

Lichens were used since ancient times as one of the natural drugs. They are symbiotic association between two entirely different types of microorganisms.<sup>[9]</sup> They produce characteristic secondary metabolites which have considerable biological activities.<sup>[10]</sup> *Parmelia .p* (Huds) Ach (Charila ) is a lichen, a member of the family *Parmeliaceae* which is primarily used for its medicinal properties, especially in Asian countries.<sup>[11]</sup> This lichen grows on old trees, walls, rocks, and irregularly spreading over the substratum giving the appearance of the flower.<sup>[12]</sup> So it is commonly known as (stone flower), and (Hazaz –al-sakhr).<sup>[13]</sup>

*Parmelia perlata* is thallus, foliaceous, membranous, leaf like horizontally spreading lobes. The thallus is dirty white or grayish brown, with bitter or saline taste. It has a folkloric use for the management of diarrhea and dyspepsia, posses the antiemetic, analgesic, and astringent activity, its smoke relief headache.<sup>[14]</sup> It also use as food supplement, treat infections, inflammation, skin diseases, dysentery, cough, fever and renal calculi.<sup>[15]</sup> It reported in unani literature as antidote, cardi tonic, and wound healing agent.<sup>[13]</sup> It also been used as brown dye for wool, bioindicator of air pollution of heavy metals, and as antibiotic in several countries.<sup>[16]</sup> *Parmelia Perlata* contain usinic acid ,atronin, salazinic acid, lecanoric acid, protolichestic acid, tridecyl myrestate, icosan-1-ol.<sup>[14]</sup>

*Parmelia perlata* is very important drug in tradition system, but many of its traditional uses like: analgesic, anti-inflammatory, and antipyretic are needed to be scientifically explored.<sup>[17]</sup> So in this study we focused on these three biological activities and assessed them in methanol extract of Sudanese *Parmelia perlata*.

## MATERIALS AND METHODS

### Plant material

The lichen material was collected from the Airquait mountain in Red sea State, Eastern Sudan, and dried under shade. The botanical identification was made and authenticated by Dr. Yahia Suleiman at the herbarium of Medicinal and Aromatic plants & Traditional Medicine Research Institute, National Center For research, Khartoum, Sudan and voucher specimens were deposited there for further reference.

### Chemicals

Diclofenac sodium (Azal pharmaceutical industries Co. ltd, Khartoum, Sudan), Aspirin (CIMA – Sudan), Paracetamol (city Pharma Co, Khartoum, Sudan), , Brewer's yeast (Merck Germany), formalin(Sigma Lambda, USA), Tween80(Acros organics . Newjersy. U. S. A).

### Animals

Albino rats of either sex were used in all experiments. Animals were purchased from the animal Section of the Technological center, University of Alahfad , Khartoum, Sudan . The animals were maintained in standard laboratory conditions (25°C and light/dark cycles i.e. 12/12h) and were fed with standard food and water ad libitum. The animals were acclimatized for 10days under laboratory conditions before carrying out the experiments. The Institutional Animals Ethics Committee approved all the experimental protochol.

### Plant extraction

Hundred gm of the dried *parmelia perlata* lichen were extracted by cold maceration method as it prepared traditionally , with one liter of methanol (95% %) for 48h at room temperature, with shaking. The extract was filtered and the methanol evaporated by rotary evaporator for concentration of extract. Then it was air dried and subjected to biological activities assay.

### Drug administration

The test extract was administered by dissolving in distal water for anti-inflammatory test, and suspending in Tween 80 solution for both analgesic and antipyretic test. All standard drugs

and *P.P.M.E* were administered orally using gastric gavages. Tween 80 was used in analgesic and antipyretic experiments as control and vehicle for the methanol extract, but for anti-inflammatory test the distal water was used as control and vehicle for lichen extract.

#### **Evaluation of anti-inflammatory activity by formalin induced paw edema:**

The animal were divided into four groups. Each group contain 6 rats. The first group was treated orally with distal water. The second group was served as standard and treated with Diclofenac sodium(10mg/kg).p.o . Group 3 and Group 4 were treated orally with PPME 200mg/kg and 400mg/kg respectively. After 30 min , the inflammation was induced by intraplantar injection of 0.2ml of 1% w/v solution of formalin into the sub-plantar region of left paw. The paw was marked with ink at the level of lateral malleolus . The paw volume was measured at 0,1,2,3 hr after formalin injection by simple displacement apparatus. The anti-inflammatory activity in animals that receive methanolic extract of *Parmelia perlata* and Diclofenac (10mg/kg) was compared with that of control. The percentage inhibition of edema was calculated as follow.

$$\text{Percentage inhibition of edema} = 1 - V_t / V_c * 100$$

Where  $V_t$  is the inflammatory increase in paw volume in drug-treated rats, and  $V_c$  is the inflammatory increase in paw volume in control group of rats. Percentage inhibition of edema is proportional to anti-inflammatory activity.<sup>[3]</sup>

#### **Analgesic activity by Hot plate test**

The animals were divided into five groups of five animals in each, group (1): served as control and received Tween 80(5%), group (2): received PPME (200mg/kg body weight), group (3): received PPME (400mg/kg body weight), group (4):received standard drug Aspirin (30mg/kg body weight). The animals are kept in Eddy's hot plate maintain at constant temperature (55+- 0.5C), and the reaction of animals such as paw licking, or jump response was taken as the end point.

The animals were placed individually in hot plate regulated at temperature (55+- 0.5C), before the treatment and its reaction time was determined. After noting the initial reaction time, the treatment should be given to each rat. Then each animal placed in Eddy's hot plate under regulated temperature to obtain animal response, licking of forepaw or jump of the hot plate surface was recorded as the hot plate latency. Mice with baseline latencies of <5s or >30s were eliminated from the study. The reaction time was noted by stop watch and then the

reaction time were redetermined after 30,60,90min, after oral administration of standard and test drugs.<sup>[19]</sup>

% protection against thermal stimulus = Test mean-control mean / control mean \*100

### Antipyretic test by Brewer's induce pyrexia method

Animals were selected for experiment after confirmation of approximate constant rectal temperature for 3days. The antipyretic activity of methanol extract was evaluated based on Brewer's yeast-induced pyrexia in rats.<sup>[20]</sup> All groups were fasted overnight but allowed free accesses to drinking water and after 24h rectal temperature of each mouse was recorded. Pyrexia was induced by sub-cutaneous injection of (10ml/kg) of 15%w/v Brewer's yeast suspension bellow thenape of the neck. The induction of pyrexia was confirmed by rise in temperature more than 0.5°C, while animals showed rise in temperature less than 0.5°C were excluded from experiment.<sup>[21]</sup> The rectal temperature of each rat was measured at time 0h using thermalert, and before injection of the yeast .At 18h after injection of yeast the different groups were treated with: PPME (200 and 400mg/kg), and standard drug paracetamol (150mg/kg), Tween 80 (5%v/v) was used as suspending agent. The control group received the vehicle only. the rectal temperature was then recorded after 1,3,5h after administration of treatment.<sup>[21]</sup>

Percent reduction of pyrexia =  $B - C_n / B - A \times 100$

Where, B represents temperature after pyrexia induction; C<sub>n</sub> temperature after 1, 3, and 5 h and A, normal body temperature.

### Statistical analysis

The results obtained were expressed as mean ± SEM (Standard error of mean) of six animals for anti-inflammatory test and five animals of analgesic and antipyretic test. For statistical analysis, ANOVA was followed by post hoc Dennett's test for multiple comparisons. Effects were considered to be significant at the  $P < 0.05$  level.

## RESULTS AND DISCUSSION

### Anti-inflammatory activity by formalin induced paw edema

The anti-inflammatory activity of PPME at test doses (200 and 400mg/kg) orally administered, is presented in Table(1).The percent protection of inflammation is presented in Figure(1). The extract (200 and 400mg/kg) induced significant ( $P < 0.05$ ) anti-inflammatory

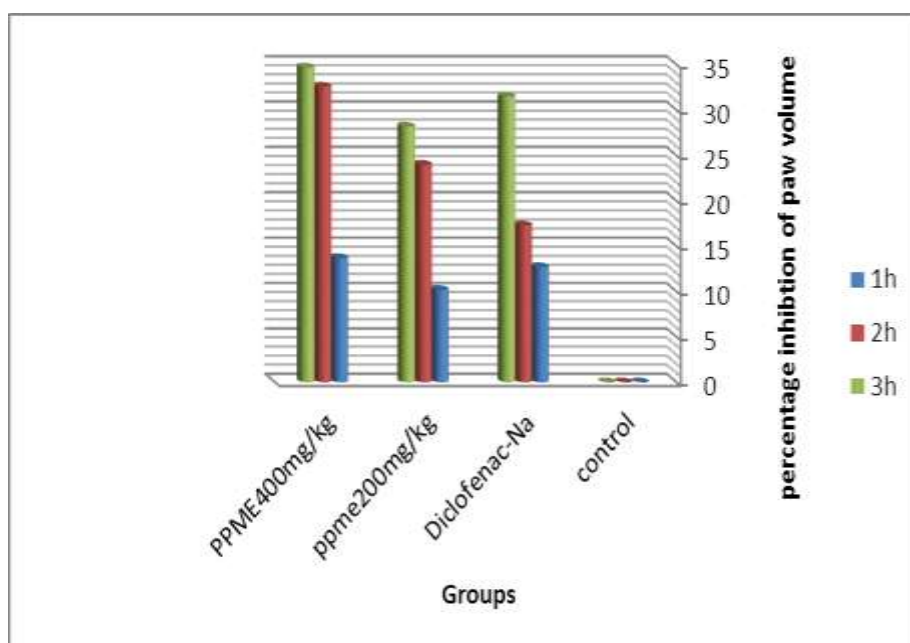
effect, and the anti-inflammatory effect of Diclofenac sodium (10mg/kg) was greater than that of the extract(200mg/kg) , but less than that of extract(400mg/kg) as presented in Figure(1). The PPME at the dose (400mg/kg) exhibited an anti-inflammatory activity that became significant ( $P < 0.05$ ) 3 h after the injection of formalin with a maximum effect of 34.7 %.

**Table(1): Effect of orally administration of P.P.M.E (200,400g/kg ), and Diclofenac-Na in formalin induced paw edema test.**

Groups numbers	Dose (mg/kg)	MPV(ml)				
		NPS	0h	1h	2h	3h
(1)	10ml/kg	$0.73 \pm 0.02$	$1.07 \pm 0.02$	$1.08 \pm 0.02$	$1.05 \pm 0.03$	$0.93 \pm 0.01$
(2)	10mg/kg	$0.73 \pm 0.04$	$1.03 \pm 0.01$	$0.83 \pm 0.04$	$0.83 \pm 0.05$	$0.72 \pm 0.03$
(3)	200mg/kg	$0.78 \pm 0.3$	$1.12 \pm 0.04$	$0.90 \pm 0.04$	$0.75 \pm 0.03$	$0.75 \pm 0.03$
	400mg/kg	$0.67 \pm 0.05$	$1.14 \pm 0.04$	$0.87 \pm 0.02$	$0.68 \pm 0.03$	$0.68 \pm 0.04$

(P.P.M.E: *parmelia perlata* methanol extract, MPV: mean paw volume ml, NPS: normal paw size, (1): control group, (2): Diclofenac-Na, (3) : PPME).

Values are represented as mean  $\pm$  S.E.M. (n=6) .The data was analyzed by ANOVA followed by Dennett's test. ( $P < 0.05$ ) indicate statistically significant different compared with control.



**Figure 1: Mean percentage inhibition of edema after oral administration of P.P.M.E (200,400mg/kg), and Diclofenac-Na after 1,2,3 h of treatment.**

(The data was analyzed by ANOVA followed by Dennett's test. ( $P < 0.05$ ) statistically significant different compared with control).

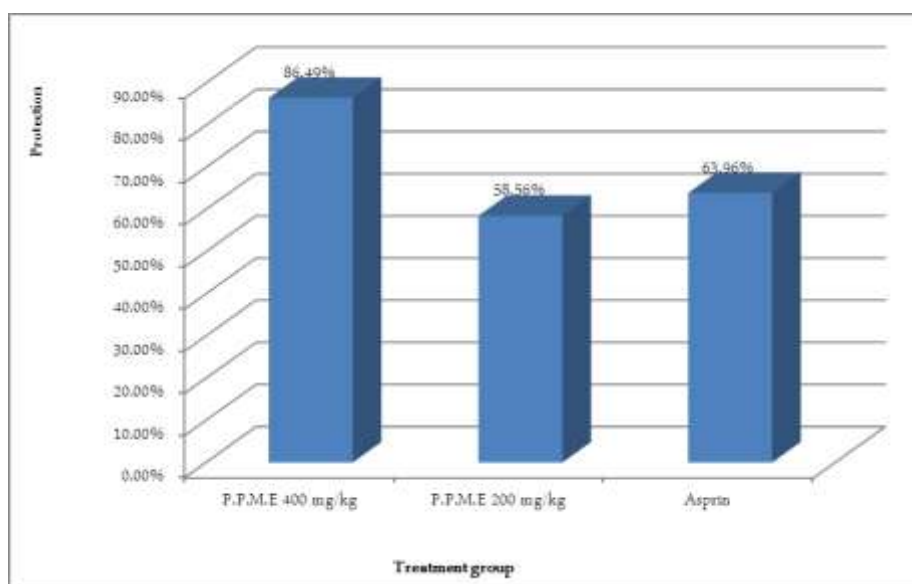
### Analgesic activity by Hot plate test

The results of the hot plate test revealed that the latency time was significantly ( $P < 0.05$ ) increased from 58.56% to 86.49% at the dose of 200 to 400 mg/kg. The effect was dose dependent and the maximum effect was observed after 60min as shown in Table(2). The percent inhibition of pain in the standard analgesic (Aspirin) 63.96% was less than that of (PPME200mg/kg) group, and more than that of (PPME 200mg/kg) group as shown in Figure Figure(2).

**Table (2): Effect of P.P.M.E (200 -400mg/kg) and Aspirin orally administered in hot plat test.**

Groups	Dose (mg/kg)	Mean protection inhibition of pain			
		0min	30min	60min	90min
(1)	10ml/kg	6.2±0.2	7.2±0.58	5±0.3	3.8±0.37
(2)	30mg/kg	7.6±0.68	12±0.55	11±1.76	5.8±0.58
(3)	200mg/kg	6.8±0.49	12±1.58	8±2.07	8.5±3.08
	400mg/kg	7±0.45	12±4.05	12.2±2.22	8.2±1.71

(PPME: *parmelia perlata* methanolic extract, (1): Control group, (2): Aspirin group, (3): PPME group). Values are reported as mean ± S.E.M.(n=5). The data was analyzed by ANOVA followed by Dennett's test. ( $P < 0.05$ ) indicated statistically significant values from control.



**Figure (2): Mean percent of pain protection inhibition of P.P.M.E (200 and 400mg/kg) and Aspirin (30mg/kg) on hot plate pain in mice.**

Each column represents the mean ± SEM of 5 animals, The data was analyzed by ANOVA followed by Dennett's test. ( $P < 0.05$ ) indicated statistically significant values from control.



### Antipyretic test

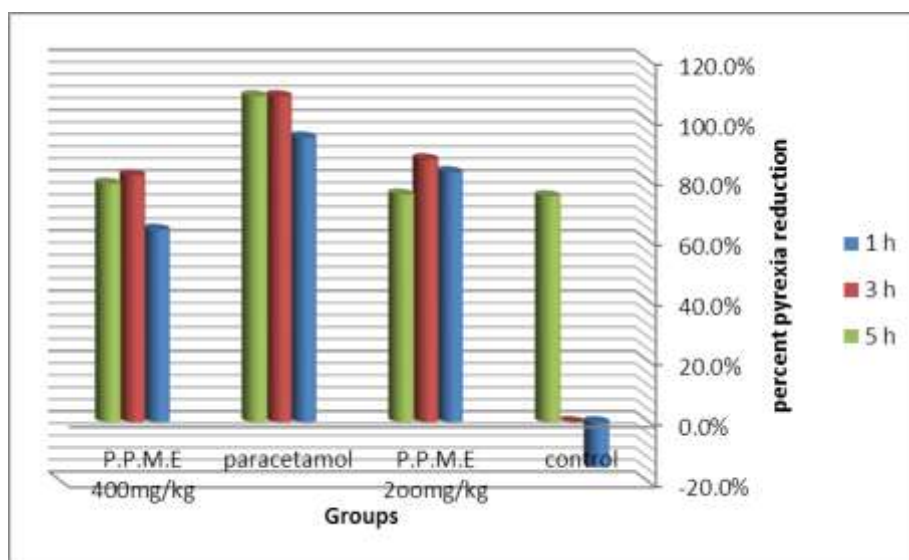
Methanol extract of *Parmelia perlata* was significantly decreased the body temperature induced by yeast in rats up to 3h as shown in Table(3).

The maximum antipyretic effect was observed at 200mg/kg i.e.: 87%, while the antipyretic effect of paracetamol was 108%. The percent inhibition of pyrexia is shown in figure (3)

**Table(4): Effect of oral administration of PPME (200, and 400mg/kg) , and paracetamol in yeast induced pyrexia.**

Treatment	Rectal temperature in C of various time					
	Dose mg/kg	-18 h	Zero h	1 h	3 h	5 h
(1)	-	37.7±0.13	38.9±0.17	39.0±0.20	38.9±0.20	38.0±0.30
(2)	150	37.8±0.15	39.2±0.29	37.8±0.16	37.6±0.17	37.6±0.12
(3)	200	37.5±0.15	39.4±0.14	37.8±0.18	37.7±0.15	37.9±0.28
	400	37.7±0.06	39.2±0.08	38.2±0.25	38.0±0.13	38.0±0.22

(PPME :*parmelia perlata* methanol extract , (1): Control group, (2): Paracetamol group, (3): P.P.M.E). Values are expressed as mean ± S.E.M(n=5) .The data was analyzed by ANOVA followed by Dennett's test. (P <0.05) indicated statistically significant values compared with 0h of the same group.



**Figure (3): The percent reduction of pyrexia after 1,3 and 5h of the treatment with paracetamol (150mg/kg) and P.P.M.E(200–400mg/kg)**

(PPME: *parmelia perlata* methanolic extract). Values are expressed as mean ± S.E.M(n=5). The data was analyzed by ANOVA followed by Dennett's test.. (P <0.05 ) indicated statistically significant values compared with 0h of the same group.



## DISCUSSION

inflammation, algesia, and Pyrexia are associated with several pathological conditions. Synthetic drugs available for the treatment of these conditions cause multiple unwanted effects.<sup>[20]</sup> So in this study the anti-inflammatory, analgesic and antipyretic of PPME was investigated. The anti-inflammatory effect extract was investigated using formaldehyde induced edema test. Which had been simple commonly used as experimental animal model for sub-chronic inflammation. Formalin induced paw edema is mediated by an early release of substances bradykinin, followed by release of histamine, serotonin, and prostaglandin.<sup>[17]</sup> The results from this study indicate that *Parmelia perlata* showed significant inhibitory effect on rat paw edema development and this suggestion that reduction of paw volume may be due to the inhibition of these substances, and this may lead to potential usefulness of extract in the treatment of sub-chronic inflammation.

The extracts of the plants also shown a longer latency time than the control group in the hot plate test in a dose related manner. The hot plat test measures the complex response to a non-inflammatory acute nociceptive input and is one of the models normally used for studying central nociceptive activity . It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally.<sup>[8]</sup>

Therefore, the methanolic extracts of the lichen may have a central activity. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action which can be achieved by blocking the cyclo-oxygenase enzyme activity.<sup>[22]</sup> The extract was significantly reduced the rectal temperature of yeast induced pyrexia in rats. Thus it can be postulated that this lichen contained pharmacologically active principle(s) that interfere with the release of prostaglandins. Plants poly phenols(specially flavonoids) have been reported to inhibit inflammation process by regulating the production of pro inflammatory molecules.<sup>[23]</sup> so the phenolic and flavonoids which present in this lichen may be responsible of the observed anti-inflammatory effect in this study. Flavonoids are also known to target prostaglandins which are involved in pyrexia, and from the previous study *Parmelia perlata* as other lichens contain Usinic acids which possess significant anti-inflammatory , analgesic effect , and antipyretic effect in rats.

## CONCLUSION

The data obtained in this study demonstrated that the methanolic extract of whole lichen *Parmelia perlata* possess anti-inflammatory activity, analgesic, and antipyretic effect, and also

scientifically validated the traditional uses of this lichen for treating inflammatory, fever, and pain disorder in the folk medicine. It was concluded that this effect is caused by active secondary principles in the lichen extract. Further studies will be done to isolate the active compound(s), identify and characterize them, as well as identify the possible mechanism of action.

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