

Full Length Research Paper

Anti-pyretic and anti-inflammatory effects of the methanolic extract of the rind of *Citrullus lanatus* on albino Wistar rats

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Received 2 September, 2015; Accepted 13 November, 2015

The present study aims to investigate the anti-pyretic and anti-inflammatory effects of the methanolic extract of the rind of *Citrullus lanatus* on male albino Wistar rats. Two set of rat groups designated groups 1 to 5 and A to E were used respectively for the anti-pyretic and anti-inflammatory studies. For the antipyretic study, fever was induced by subcutaneous injection of 20 ml/kg of a 20% suspension of brewer's yeast in normal saline. Group 1 served as negative control and received 2 ml/kg bw of extract vehicle, groups 2, 3 and 4 received 100, 200 and 500 mg/kg bw of the methanolic extract of the rind *Citrullus lanatus*, respectively and group 5 served as positive control and received 100 mg/kg of aspirin. Rectal temperatures were determined in all rats at hourly intervals for 4 consecutive times. For the anti-inflammatory study, acute inflammation was induced by sub-plantar injection of 0.1 ml of egg albumin into the left hind paw. Groups A to E rats were treated similar as groups 1 to 5 rats. Paw circumference was subsequently determined with the aid of a Vernier caliper at 30, 60, 90 and 120 min post induction of inflammation. Significant reduction in rectal temperatures were observed for both groups 3 and 4 rats ($p < 0.05$): reducing from an initial value of $38.16 \pm 0.67^\circ\text{C}$ 18 h post induction of pyrexia to $36.33 \pm 0.21^\circ\text{C}$ 4 h post treatment amongst group 3 rats. A significant reduction in hind paw circumference was also observed for both groups C and D rats ($p < 0.05$). For instance, amongst group D rats, there was a significant reduction in hind paw circumference from 3.30 ± 0.00 mm at 30 min to 3.04 ± 0.06 mm at 120 min post induction of inflammation. In conclusion, the present study reports the potential anti-pyretic and anti-inflammatory effects of the methanolic extract of the rind of *C. lanatus*.

Key words: Anti-inflammatory, antipyretic, *Citrullus lanatus*, egg albumin, brewer's yeast.

INTRODUCTION

Pyrexia (fever) is an elevation of body temperature above the normal range due to a change in the hypothalamic temperature set-point (Axelrod et al., 2008). Pyrexia may

result from infection, tissue damage, inflammation, graft rejection or other disease states (Annan et al., 2013). It is produced by certain endogenous substances which

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include tumor necrosis factor-alpha (TNF α) and prostaglandins (Kluger, 1991). Overall, a febrile response is the body's natural way of creating an environment where infectious agents cannot survive with ease (Annan et al., 2013). On the other hand, pyrexia enhances disease progression by increasing tissue catabolism, exacerbating dehydration and other existing complaints (Spacer and Breder, 1994). Anti-pyretic drugs are agents used to reduce elevated body temperature. They are known to act either centrally on the temperature regulation centers of the hypothalamus or peripherally by inducing vasodilatation and heat dissipation (Adesokan et al., 2008). They also act by inhibiting the biosynthesis of prostaglandin E2 (Kurokawa et al., 1998); possibly by inhibiting COX-2 expression (Luo et al., 2005; Gege-Adebayo et al., 2013). Long time usage of these antipyretic drugs produces undesirable side effects including gastrointestinal disorders, renal damage and hepatic toxicity (Chaudhary, 2010). Herbal medications continue to be the mainstay of primary health care for 75-80% of the world's population; especially in developing countries (Gege-Adebayo et al., 2013). *Citrullus lanatus* (watermelon) is a prostrate or climbing annual plant with several herbaceous, firm and stout stems up to 3 m long. The leaves are herbaceous but rigid, becoming rough on both sides: 60-200 mm long and 40-150 mm broad, but usually deeply 3-lobed with the segments again lobed or doubly lobed; the central lobe being the largest (Erhirhie and Ekene, 2013). Watermelon is a rich natural source of lycopene, a carotenoid of interest because of its antioxidant properties and potential health benefits (Erhirhie and Ekene, 2013). *Cucurbitaceae* plants are known to contain bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids (Yuan et al., 2006). Every part of the watermelon fruit has nutritional value, including the rind and the seeds. The rind is prescribed in cases of alcoholic poisoning and diabetes (Erhirhie and Ekene, 2013). Further, the rind has been shown to contain alkaloids, saponin, cardiac glycosides, flavonoids, phenol, moisture, lipid, protein, fiber and carbohydrates (Erukainure et al., 2010); also its ameliorative effect in lead toxicity on semen parameters and reproductive hormones of male albino Wistar rats have recently been reported from our center (Kolawole et al., 2014).

The present study was undertaken to evaluate the possible antipyretic and anti-inflammatory effects of the methanolic extract of the rind of *C. lanatus* using albino Wistar rats as model. This is to further determine the possible medicinal uses of *C. lanatus*.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh plant and fruits of watermelon were obtained from a local market in Rivers State, Nigeria. The plant materials were identified and authenticated by Dr. C Ekeke of the Department of Plant

Science and Biotechnology, University of Port Harcourt, Nigeria. Voucher specimens were also deposited with the herbarium number: UPH/V/1214.

The rinds were peeled off from the whole fruit washed thoroughly, sun-dried and milled into a fine powder. The method of extraction employed was percolation (Adesanya et al., 2011). 24 g of the powdered sample was soaked in a beaker containing 100 ml of 98% methanol for a period of 48 h and then filtered with a Whatman No. 1 filter paper size. The volume of filtrate obtained was 150 ml before concentration; the filtrate was subsequently concentrated using a rotary evaporator. The weight of residue obtained was 8.5 g.

Determination of median lethal dose (LD₅₀)

Acute toxicity study (LD₅₀) was determined using the method described by Lorke (1983). The (LD₅₀) of the extract was found to be greater than 2000 mg/kg body weight.

Experimental design

Two sets of 25 male albino Wistar rats were used each for the antipyretic and anti-inflammatory studies designated: groups 1 to 5 and groups A to E, respectively. There were a total of 5 rats in each of the groups. The rats were aged 8 and 10 weeks and weighed between 170 and 200 g. Each rat was placed in separate cages in the Animal House of Madonna University, Nigeria under natural day and night cycles. The rats had free access to normal rat chow and tap water *ad libitum*. They were allowed two weeks of acclimatization prior to commencement of the study. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, NRC revised 1985).

Brewer's yeast induced pyrexia

Fever was induced in groups 1 to 5 rats by subcutaneous injection of 20 ml/kg of a 20% suspension of brewer's yeast in normal saline below the nape of the neck as previously described by Someze et al. (2009). The temperature of each rat was measured 18 h post induction with a rectal thermometer inserted 3-4 cm into the rectum and only rats that showed an increase of at least 0.5°C in temperature were used for the antipyretic study. The rats were subsequently treated as follows:

Group 1: Negative control group. Rats in this group were given 2 ml/kg bw of extract vehicle.

Group 2: Low dose extract group. Rats in this group were treated with 100 mg/kg bw of the extract of the rind of *C. lanatus*.

Group 3: Medium dose extract group. Rats in this group were treated with 200 mg/kg bw of the extract of the rind of *C. lanatus*.

Group 4: High dose extract group. Rats in this group were treated with 500 mg/kg bw of the extract of the rind of *C. lanatus*.

Group 5: Positive control group. Rats in this group were given 100 mg/kg bw of aspirin.

The extract of the rind of *C. lanatus*, the extract vehicle and aspirin were administered to each rat using an oral cannula. After administration of the extract, rectal temperatures were further determined in all rats at hourly intervals for 4 consecutive times.

Induction of paw edema

The method described by Rathesh and Helen (2007) was used with minor modifications. Briefly, the second set of 25 rats was also divided into 5 groups of five rats each. Group A served as a

Table 1. Effect of the methanolic extract of the rind of *C. lanatus* on rectal temperature following brewer's yeast induced pyrexia in albino Wistar rats.

Groups	Pre-induction rectal temperature (°C)	Rectal temperature 18 h post induction (°C)	Rectal temperature 1 h post treatment (°C)	Rectal temperature 2 h post treatment (°C)	Rectal temperature 3 h post treatment (°C)	Rectal temperature 4 h post treatment (°C)
Group 1: Negative control (extract vehicle) group.	36.40±0.24	37.83±0.15	38.20±0.12	38.20±0.32	38.40±0.17	38.50±0.33
Group 2: Low dose extract group.	36.42±0.20	37.85±0.22	38.42±0.11	37.53±0.15	36.18±0.40	37.46±0.28
Group 3: Medium dose extract group	36.44±0.23	38.16±0.67*	38.40±0.10	37.41±0.43	36.58±0.37	36.33±0.21*
Group 4: High dose extract group	36.40±0.24	38.16±0.87*	38.05±0.10	36.86±0.35	36.95±0.27	36.64±0.26*
Group 5: Positive Control (aspirin) group	36.43±0.20	38.24±0.09*	37.95±0.04*	36.04±0.31*	36.04±0.36*	36.18±0.45*

Values=Mean±SEM; *: p<0.05 compared with Group 1 rats

negative control and received the extract vehicle (normal saline); while group E served as positive control receiving aspirin at a dose of 100 mg/kg bw. The remaining 3 groups (groups B, C and D) received 100, 200 and 500 mg/kg bw, respectively of the extract of the rind of *C. lanatus*. The extract, extract vehicle and aspirin were administered with the aid of an oral cannula. The rats were then left alone for 30 min, following which acute inflammation was induced by sub-plantar injection of 0.1 ml of egg albumin into the left hind paw. Paw circumference was subsequently determined with the aid of a Vernier caliper 30, 60, 90 and 120 min post induction of inflammation.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Version15.0). The results were analyzed using the One-Way Analysis of Variance (ANOVA) followed by the LSD post hoc tests. A p value <0.05 was considered statistically significant. The results are presented in Tables 1 and 2. All data were expressed as mean± standard error of mean (SEM).

RESULTS

Table 1 shows the effect of the methanolic extract

of the rind of *C. lanatus* on rectal temperature following brewer's yeast induced pyrexia in albino Wistar rats. Group 1 (negative control) rats remained consistently pyretic throughout the duration of the study. However, in contrast, there was a progressive and significant reduction in rectal temperatures following aspirin administration amongst group 5 (positive control) rats (p<0.05): reducing from an initial value of 38.24±0.09°C 18 h post induction of pyrexia with brewer's yeast to 36.18±0.45°C at 4 h post treatment with aspirin. Amongst the extract treated groups, a significant reduction in rectal temperatures were observed for both groups 3 and group 4 rats as compared to group 1 (negative control) rats (p<0.05). For instance, amongst group 3 rats treated with the medium dose of the extract of the rind of *C. lanatus*, the initial value of 38.16±0.67°C at 18 h post induction of pyrexia with brewer's yeast decreased to 36.33±0.21°C at 4 h post treatment: this was found to be significantly lower than the value obtained for group 1 (negative control) rats (p<0.05).

Table 2 shows the effect of the methanolic extract of the rind of *C. lanatus* on the hind paw circumference following egg albumin injection. In a pattern similar to the results of the antipyretic study, there were no significant changes in the hind paw circumference of group A (negative control) rats all through the duration of the study. However, there was a significant reduction in the hind paw circumference amongst group E (positive control group) rats (p<0.05): reducing from an initial value of 3.38±0.16 mm at 30 min to 3.04±0.06 mm at 120 min post induction of inflammation. Furthermore, amongst the extract treated groups a significant reduction in hind paw circumference was also observed for both groups C and D rats only (p<0.05). There was a significant reduction in hind paw circumference from 3.30±0.00 mm at 30 min post induction of inflammation to 3.04±0.06 mm 120 min post induction of inflammation amongst group D rats who were treated with the high dose of methanolic extract of *C. lanatus*.

Both anti-pyretic and anti-inflammatory effects of the extract did not exhibit much dose

Table 2. Effect of the methanolic extract of the rind of *C. lanatus* on the hind paw circumference following egg albumin injection in albino Wistar rats.

Groups	Hind paw circumference	Hind paw circumference	Hind paw circumference	Hind paw circumference
	at 30 min (mm)	at 60 min (mm)	at 90 min (mm)	at 120 min (mm)
Group A: Negative Control (Extract vehicle) group	3.32±0.20	3.70±0.12	3.54±0.02	3.26±0.02
Group B: Low Dose Extract Group.	3.60±0.16	3.40±0.00	3.10±0.05	3.12±0.07
Group C: Medium dose extract group	3.40±0.14	3.35±0.15	3.12±0.06	3.00±0.02*
Group D: High dose extract group	3.30±0.00	3.30±0.00	3.14±0.06*	3.04±0.04*
Group E: Positive Control (Aspirin) Group	3.38±0.16	3.52±0.22	3.62±0.07	3.04±0.06*

Values=Mean ± SEM; *: p<0.05 compared with Group A rats.

dependency: no significant group differences were found to exist in the values obtained for the parameters investigated amongst rats in the extract treated groups.

DISCUSSION

The present study attempts to determine the possible antipyretic and anti-inflammatory effects of the methanolic extract of the rind of *C. lanatus* using albino Wistar rats as models. Brewer's yeast has been shown to induce both TNF- α and prostaglandin synthesis (Gege Adebayo et al., 2013). The mechanism of action of some antipyretics such as aspirin (acetylsalicylic acid) and other non-steroidal anti-inflammatory drugs (NSAIDs) in reducing fever is in their ability to inhibit the enzyme cyclooxygenase (COX) and interrupt the synthesis of inflammatory prostaglandins (Annan et al., 2013).

Apparently from the results of our study, the methanolic extract of the rind of *C. lanatus* ameliorated brewer's yeast induced elevation of body temperature in albino Wistar rats. This effect may probably be attributed to its phytochemical constituents which include alkaloids and flavonoids (Erukainure et al., 2010). Alkaloids have been reported to inhibit the synthesis of

prostaglandinE2 (Backhouse et al., 1994), which could eventually reduce elevations of body temperature. Similarly, flavonoids have been shown to exert an antipyretic effect by suppressing TNF- α (Chang et al., 2007). Although, the antipyretic activities of the methanolic extract of the rind of *C. lanatus* were less than those of aspirin, the effects were apparently also higher at higher doses as compared to the effects at lower doses. Apparently, the methanolic extract of the rind of *C. lanatus* exhibited a delayed antipyretic effect.

The anti-inflammatory effects of the methanolic extract of the rind of *C. lanatus* compared fairly with those of the reference drug aspirin. The anti-inflammatory effects of the extract were also most pronounced with maximum suppression of hind paw edema at higher dose. The anti-inflammatory effect of the extract was also fairly sustained, being persistent for 120 min post induction of inflammation. Just et al. (1998) had shown that flavonoids, saponin and steroids possess antioxidant, analgesic and anti-inflammatory properties.

Flavonoids belong to the polyphenol family and are found in most plant material. The most important dietary sources are fruits, tea and soybean. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer,

antioxidant, anti-inflammatory and anti-viral (Cushnie and Lamb, 2011). A variety of flavonoids have also been found to inhibit prostaglandin synthase (COX-2) transcription and production (O'Leary et al., 2004; Hämäläinen et al., 2011). Therefore, the possible anti-pyretic and anti-inflammatory activities of these ethanolic extract of the rind of *C. lanatus* may be probably be most associated with the flavonoids and/or the alkaloidal components of the extract.

In conclusion, the present study reports the potential antipyretic and anti-inflammatory effects of the methanolic extract of the rind of *C. lanatus* in albino Wistar rats. Further studies to elucidate these medicinal effects of *C. lanatus* are recommended.

Conflict of interests

The authors have not declared any conflict of interest.

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