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Analgesic and anti-inflammatory activities of the ethanol extract of the leaves of *Helianthus Annus* in Wistar rats

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ABSTRACT

Objective: To investigate the anti-inflammatory and analgesic effects of the ethanol extract of leaves of *Helianthus annus* L. (*H. annus*) in acclimatized Wistar rats. **Methods:** It was undertaken using the albumin induced paw edema model of inflammation as well as both the hotplate and tail immersion analgesic test methods. Doses of the extract tested in experimental rats were 0.5 g/kg, 2 g/kg and 4 g/kg while negative and positive control rats received distilled water and indomethacin respectively. **Results:** It was shown that treatment with the tested doses of the extract effectively inhibited paw edema induced by egg albumin. This effect was comparable if not better than the observations made in rats treated with 10 mg/kg of indomethacin orally. Treatment with the extract was also observed to have significantly increased the mean tolerance time of rats to thermal noxious stimuli compared to control animals that had distilled water and appeared to be more effective than 10 mg/kg of indomethacin treatment. **Conclusions:** These observations confirmed the presence of a strong anti-inflammatory and anti-noiciceptive activity in the ethanol extract of the leaves of *H. annus* and therefore validated the folkloric use of the leaves of this plant in treatment of pro-inflammatory, post traumatic situations.

1. Introduction

Inflammation has been defined as the reaction of vascular and supporting elements of tissues to injury[1]. Injurious stimuli capable of inducing inflammation have been reported to include mechanical, radiations, extremes of temperature, ischemia, infectious and immunological agents[2]. At the macroscopic level, redness, swelling, heat, pain and local loss of function are the cardinal signs of acute inflammation[1]. Although this response initiates the healing and regenerative process in injured tissue[3], it also forms the basis of several pathological conditions[4-6]. Damage of tissues has been reported to be mediated by lytic enzymes released from infiltrating cells and free oxygen radicals generated at the site of inflammation[7,8]. Inflammatory symptoms can be intense enough to reduce the quality of life of the subject in a short term, necessitating therapeutic relief or suppression. Excessive suppression of this response or its symptoms theoretically could be counter productive during the early phases before the healing process is completed even after the injurious stimuli have been successfully neutralized by the response[9]. Pain have been defined for scientific purpose as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage[10]. It was also described as an unpleasant unique physical and psychological experience[11]. Back pain, headache and joint pain caused by osteoarthritis have been reported as the most common chronic pain syndromes.

Anti-inflammatory drugs used in orthodox medicine, many of which also serve as analgesics have been broadly sub-grouped into nonsteroidal anti-inflammatory drugs(NSAIDS) and disease-modifying anti-rheumatic drugs or DMARDS[12]. Pharmacological approach to pain have been summarized into three broad categories of analgesic drugs; non-opiod (paracetamol and NSAIDS), opiod and adjuvant analgesics[13]. Other recognized complementary and alternative methods of relieving pain are acupuncture[14-16], different forms of psychological/mind body therapies[17-21],

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relaxation therapies[17,22,23]. Also several herbal medicines are used as anti-inflammatory and/or analgesic therapy in different traditional medical systems, some of which have had their efficacies validated in animal and/or clinical studies. A few of such reported plants are the secondary storage tubers of *Harpagophytum procubens*[24,25], the bark of Amblygonocarpus andongenesis (*Mimosaceae*)[26], *Cassia auriculata*[27,28], as well as *Capsaicin annum* (*Chili pepper*)[29,30].

The anti-inflammatory and analgesic potentials of some parts of the *Helianthus annus* (*H. annus*) plant could be inferred from some of their folkloric medicinal uses including the use of the crushed leaves as a poultice on wounds, spider bites, snake bites and swellings[31], the use of a tea made from the leaves in the treatment of febrile illness[32]. The use of the decoction of the roots as warm wash on rheumatic aches and pains is another possible indication of the possible presence of anti-inflammatory and analgesic potentials[32]. Adverse effects limiting the use of the available pharmacological anti-inflammatory and analgesic agents in orthodox medicine have been well documented. Hence we need to go on looking for newer drugs with improved efficacies and safety profiles.

This study seeks to investigate the analgesic and anti-inflammatory effects of the ethanol extract of the leaves of *H. annus* as a first step to scientifically validate it's folkloric uses.

2. Material and methods

2.1. Preparation of plant material

The wild variety of *H. annus* was identified with the assistance of Dr Ejebe and authenticated by Mr. Ebigwa of the Botany Department, Delta State University, Abraka.Healthy leaves were harvested, oven dried at 40 ℃ to a constant weight of 120 g and blended (NIPL) into a smooth powder. The pulverized leaves were then soaked in absolute ethanol in Winchester bottles for 24 hours. The filtrate was then evaporated to dryness using a rotor evaporator. 40 g of the powdery residue obtained was then dissolved in 250 mL of distilled water to obtain a uniform stock solution of concentration 0.16 g/mL from which measured doses were used in the animal experiment.

2.2. Preparation of experimental animals

For the test of anti-inflammatory effect, a set of 25 adult male Wistar rats weighing between 200~280 g, while for the analgesic test another set of 20 adult male Wistar rats weighing between 209~260 g were procured from the breeding colony of the College of Health Sciences, Delta State University Abraka and housed in plastic cages in the laboratory animal house of the college. They were acclimatized for 2 weeks before commencement of the experiments. Throughout the study period they had clean drinking water and pelletized rat feeds (Vital Feeds, Lagos).They were maintained under hygienic conditions at room temperature(26~30 ℃) with 12 hours light/darkness exposure. They were also acclimated to the hotplate by having their first feeds on the hotplate for at least 2 min daily. For the anti-inflammatory test the animals were divided into 5 groups with 5 animals per group that received 5 mL/kg distilled water, 10 mg/kg of indomethacin, 0.5 g/kg extract, 2 g/kg extract and 4 g/kg extract respectively.

While in the analgesic test the animals were divided into 4 groups (with five animals per group) that received 0.5 g/kg of extract, 2 g/kg of extract, 10 mg/kg indomethacine to serve as positive control and 5 mL/kg of distilled water as negative control respectively.

2.3. Investigation of anti-inflammatory effects

The weight of animals in each group were determined with a beam balance, each was distinctly labelled using permanent colour markers. Their weights were used to determine the quantity (g) of the extract, indomethacine and distilled water required to meet the different dose specifications. This was subsequently used to determine the volume of the different test solutions (distilled water, 0.16 g/mL uniform stock solution of the ethanol extract and 10 mg/mL indomethacine suspension in water) that was administered to the different groups of rat.

To test the effect of the ethanol extract on acute inflammation, the method described by Winter *et al*[33] was used with a slight modification in the assessment of albumin-induced changes in the volume of the rat paws: Pre treatment and post treatment paw volumes were determined indirectly by immersing it, up to a reference point at the ankle joint marked with a permanent colour marker, into a 20 mL glass beaker filled up to the brim with water. The volume of water displaced was assumed to be equal to the up thrust on the immersed paw according to Achimedes principle[34]. The displaced water was received in a previously weighed glass Petri dish using Electronic Balance (JT201N). The weight of the displaced water and dish were determined and the weight of the dish subtracted from it to determine the weight of displaced water. And since the density of water is known to be unity, the weight and volume of displaced water were assumed to be equivalent.

Male rats used for the investigation were fasted overnight. They were deprived of water during the experiment to ensure uniform hydration and to minimize variability in edematous response[35]. After determination of the initial right paw volume in rats in each group, they were treated with 0.5 g/kg extract, 2 g/kg extract, 10 mg/kg indomethacin and 5 mL/kg distilled water respectively administered orally with the aid of the medicut intravenous cannula[36]. Half an hour later inflammatory edema was induced by injecting 0.1 mL of egg albumin into the sub-plantar surface of the right fore paw of the rats[37]. The paw volumes were determined as explained above at thirty minutes interval after the injection of the egg albumin for a period of 150 minutes. The weights of the displaced water by the paw were recorded in grams with which the percentage changes in paw volume with time were determined in extract, indomethacine, and distilled water treated rats. The level of inhibition of edema for the extract and indomethacine was calculated using the relation[38]:

Inhibition (%) =100[1-(a-x/b-y)].

Where a =Mean paw volume (weight) of treated animals

after egg albumin injection.

x= mean paw volume of treated animals before egg albumin injection

b= mean paw volume of control animals after egg albumin injection

y= mean paw volume of control animals before egg albumin injection

2.4. Investigation of analgesic effects

The evaluation of the analgesic effect of the ethanol extract of the leaves of *H. annus* was carried out by using two different methods that used thermal noxious stimuli; namely the hotplate and the tail immersion analgesic test methods.

2.5. Hotplate analgesic test method

The hotplate (thermal) analgesic test method was used with some modifications[39-41]. The temperature of the hotplate was set as 40 ℃ and each rat was placed on the hotplate to assess the animals' tolerance to heat induced pain. The time taken for each rat to jump out of the hotplate was recorded as a measure of their tolerance to thermal noxious stimulus. Each rat was orally administered its respective dose of the specified treatment group 30 min before the first assessment for effect on response to noxious thermal stimulus was done. Subsequently similar assessments were repeated at half hourly intervals for a total duration of 2 hours. For this method the group of animals that received distilled water served as control to the *H. annus* extract and indomethacin treated groups.

2.6. Tail immersion analgesic test method

The rats in each treatment group had their tail immersed in hot water maintained at (50±1) ℃ (in a 1 litre water-bath) and the time in seconds before each animal flicked its tail off the water was recorded as baseline reaction times and used as a measure of tolerance to noxious thermal stimulus[42]. The animals in each group subsequently had the appropriate doses of their respective test substances administered orally. Half hourly re-immersion of the animal tails into the hot water bath were done for a duration of 2 hours in order to observe for any effect of the different test substances on the animals pain threshold. Here each animal served as its own control. Percentage protection against pain was calculated for both methods using the expression (Ta − Tb/Tb) ×100[43].

2.7. Statistical analysis

The results of the anti-inflammatory test were expressed as Mean ± SEM of weight of displaced water by rat paw, mean percentage change in paw volume and mean percentage inhibition of paw edema; while the results of the analgesic tests were expressed as Mean±SEM of the reaction times (seconds) to thermal noxious stimuli and the mean percentage protection against thermal pain for five rats. Statistical analysis for significant difference between extract treatment, distilled water treatment and indomethacin treatment was done using student *t*-test and single factor ANOVA test of Microsoft Excel 2003 computerized software. *P* values less than 0.05 were statistically significant.

3. Results

3.1. Anti-inflammatory test

The mean weight of the displaced water ranged from 0.4~0.75 g in the 0.5 g/kg extract treated group, 0.55~0.85 g in the 2 g/kg extract treated group, 0.45~1.65 g in the indomethacin treated group and 0.40~2.50 g in the distilled water treated group (Table 1).

These findings suggest that both indomethacin and ethanol extract of *H. annus* leaves have some inhibitory effect on the inflammation mediated paw edema as the indirectly determined paw volumes were lower than that of distilled water treated rats. Also the smaller range and lower peak values of extract treated animals compared to those of indomethacine treated rats also suggest that the extract at these doses resulted in greater attenuation of the inflammatory paw edema.

Within each reference time group, the percentage change in paw volume in the indomethacin and extract treated rats were lower than those of distilled water treatment. Extract treated rats had lower percentage change in paw edema than indomethacin treated rats. The effect of the higher dose (2 g/kg) of extract was also slightly higher than that of the lower dose (0.5 g/kg) of extract treatment (Figure 1).

Table 1

Effects of leaf extract of *H. annus*, and indomethacine on pre treatment and post treatment weight of water (g) displaced by paws of Wistar rats following albumen induced paw edema.

Treatment received	W_0	W_{30}	W_{60}	W_{90}	W_{120}	W_{150}
Indomethacine (10 mg/kg)	0.45±0.01	^{bx}1.15±0.05	^{bx}1.65±0.03	^{bx}1.15±0.02	^{bx}1.15±0.03	^{bx}0.75±0.02
Extract of *H. annus* leaves (0.5 g/kg)	0.40±0.01	^{bc}0.60±0.02	^{bc}0.75±0.05	^{abc}0.65±0.03	^{abc} 0.65±0.02	^{abc}0.50±0.02
Extract of *H.annus* leaves(2 g/kg)	0.55±0.01	^{yz}0.70±0.05	^{yz}0.85±0.01	^{ayz}0.83±0.02	^{ayz} 0.83±0.03	^{ayz}0.60±0.02
Distilled H_2O (5 mL/kg)	0.40±0.02	^{cxy}1.80±0.02	^{cxy}2.50±0.05	^{cxy}2.50±0.03	^{cxy}2.30±0.01	^{cxy}2.20±0.02

Weight of water displaced by paws of rats immersed in a brimful 20 mL beaker, Mean ± 0.05, n=5,W_0 −W_{150} (Time[minutes] post injection of rat paw with albumen when fluid displacement was assessed). ^{a}P= (0.5 g/kg extract vs 2g/kg extract, student *t*-test)<0.05; ^{b}P = (0.5 g/kg extract vs indomethacin, student *t*-test)<0.05; ^{c}P = (0.5 g/kg extract vs control, student *t*-test)<0.05; ^{x}P= (Indomethacin vs distilled water, student *t*-test)<0.05; ^{y}P = (2 g/kg extract vs distilled water, student *t*-test)<0.05; ^{z}P = (2 g/kg extract vs indomethacin, student *t*-test)<0.05.

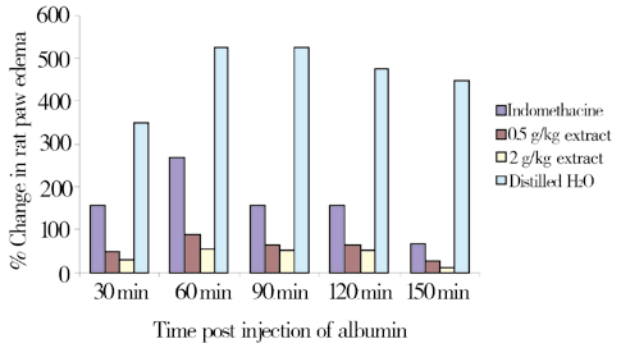

Figure 1. Percentage change in paw volume following albumen induced paw edema in Wistar rats treated with indomethacine, ethanol extract of *H. annus* leaves and distilled water(n=5).

The percentage inhibition of albumin induced paw edema calculated using the formula proposed by Perez[38] revealed that both doses of the extract produced higher percentage inhibition of inflammatory edema than indomethacin at any reference time group post albumin injection.

There were no statistically significant differences between the paw volumes of rats in different groups before induction of paw edema (P>0.05). The mean paw volumes for indomethacin treated and both doses of extract treatment groups showed statistically significant difference from those of their matched time controls (^{cxy}P< 0.05). The effect of the two treatment doses of the extract on changes in paw volume (edema) were also significantly different from those of indomethacin treatment (^{bz}P<0.05) across the various time groups. Comparing the mean paw volumes of the 0.5 g/kg and 2 g/kg extract treatment group with each other (Table1), significant statistical differences(^{a}P< 0.05) were observed at 90 min, 120 min and 150 min post albumin induction of paw oedema volumes suggesting a late onset dose dependent effect.

The percentage inhibition of albumin induced paw edema for both doses of the ethanol extract of *H. annus* leaves were higher than those of indomethacin treated rats for any given reference time group (Figure 2). On comparing the mean percentage inhibition of albumin induced paw edema in extract treated rats and indomethacin treated rats using the single factor ANOVA test, statistically significant differences were observed for both doses of the extract. This suggested that the mean percentage inhibition of both doses of extract treatment were significant higher than that of indomethacin treatment (Figure 2).

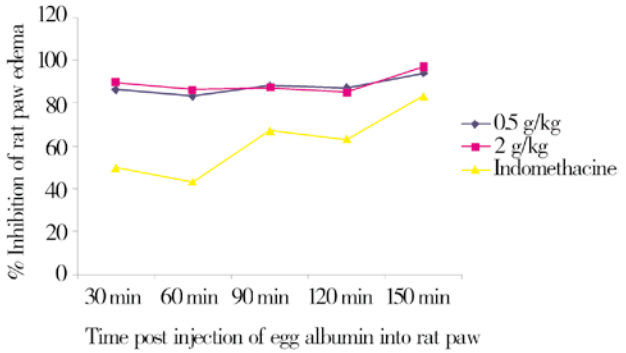

Figure 2. Percentage inhibition of egg albumen induced paw edema in Wistar rats treated with ethanol extract of leaves of *H. annus* and indomethacin(n=5).

When the percentage inhibition of paw edema of both doses of extract were compared with each other, there were however no significant difference between the treatment outcome.

Table 2

Effects of the ethanol extract of leaves of *H. annus* on tolerance for thermal noxious stimulus (hot plate analgesic test method) in Wistar rats.

Treatment received	T_{30}	T_{60}	T_{90}	T_{120}
Indomethacin 10 mg/kg	^{1,2}28.44±2.11	^{1,2,3}23.25±0.72	1.98±0.12	1.35±0.12
H. annus extract 0.5 g/kg	^{4}5.95±1.22	^{4}4.10±0.29	2.06±0.09	1.58±0.06
H. annus extract 2 g/kg	^{4}16.15±0.33	^{4}11.25±0.64	2.10±0.37	1.60±0.27
H. annus extract 4 g/kg	^{4}25.95±0.53	^{4}11.89±0.84	2.85±0.53	2.13±0.44
Distilled H_20 5 mL/kg	^{5,6,7,8}1.15±0.10	^{5,6,7,8}1.35±0.07	^{5,6,8}1.35±0.09	^{6}1.05±0.05

Duration (seconds) spent on hotplate assessed at half hourly intervals post administration of test substances. Mean ±SEM, n=5; ^{1}P<0.05 (10 mg/kg indomethacin tx vs 0.5 g/kg extract tx; student t-test); ^{2}P<0.05(10 mg/kg indomethacin tx vs 2 g/kg extract tx;student t-test); ^{3}P<0.05(10 mg/kg indomethacin tx vs 4 g/kg extract tx;student t-test); ^{4}P<0.05(0.5 g/kg vs 2 g/kg vs 4 g/kg extract tx within specified interval;single factor ANOVA test); ^{5}P<0.05(10 mg/kg indomethacintx vs distilled water:student t-test); ^{6}P<0.05(0.5 g/kg extract tx vs distilled water tx:student t-test); ^{7}P<0.05(2 g/kg extract tx vs distilled water tx :student t-test); ^{8}P< 0.05(4 g/kg extract tx vs distilled water tx :student t-test).

Table 3

Effects of the ethanol extract of *H. annus* leaves on tolerance for thermal noxious stimuli using the tail immersion analgesic test method in Wistar rats.

Treatment received	T_0	T_{30}	T_{60}	T_{90}	T_{120}
Indomethacin 10 mg/kg	3.83±0.31	4.15±0.38	*24.80±0.45	*21.95±0.60	4.96±1.15
H. annus extract 0.5 g/kg	4.05±0.62	6.75±0.52	*7.85±0.12	*7.55±0.45	4.10±0.25
H. annus extract 2 g/kg	6.10±1.07	*25.50±2.12	*16.91±1.10	*11.75±1.37	6.30±0.47
H. annus extract 4 g/kg	4.25±0.52	*28.20±1.89	*31.50±0.92	* 15.00±2.61	4.50±0.15
Distilled H_20 5 mL/kg	5.05±0.34	5.05±0.70	5.30±0.41	4.00±0.43	3.69±0.44

Duration (seconds) which tail remained immersed in water at 50 ℃ before a flick was observed; assessed before and at half hourly intervals post administration of test substance.Mean±SEM, n= 5, *P<0.05(T_i vs T_0 using student t-test).

3.2. Analgesic test

The result showed that both indomethacin and *H. annuus* leaf extract treatments improved the tolerance of rats to noxious thermal stimuli induced by the hotplate method. This effect was particularly obvious at the 30 and 60 minutes post treatment mean tolerance time observations which were significantly different from those of their respective control rats treated with distilled water (P<0.05)(Table 2).

There also appeared to be a dose dependent enhancement of tolerance for thermal noxious stimulus in the *H. annus* extract treated rats that peaked at 30 min but was still significantly high by the 60th minute post administration. Also when the effect of the different doses of the extract were individually compared with that of indomethacin at any specified post treatment interval, statistically significant differences were noted for both 0.5 g/kg and 2 g/kg treatment doses of the extract at both 30 and 60 min (Table 2).

The mean duration of the 4 g/kg *H. annus* extract treated rats on the hotplate assessed by the 30th minute post treatment was not statistically different from that of indomethacin treated rats even though both were significantly different from the control group treated with distilled water (P<0.05/0.01) (Table 2). The 60th minute post treatment mean of the former was however lower than the later with significant difference. These observations suggest that the peak analgesic effect of 4 g/kg of extract was as effective as 10 mg/kg of indomethacin treatment in the first 30 minutes post treatment but also showed a more rapid decline in conferred analgesic effect than the later (Table 2). An absence of statistically significant difference between the mean tolerance times of indomethacin, the three different doses of extract and distilled water treatment groups by the 90th and 120th minutes post treatment suggest that any enhanced tolerance to noxious painful stimulus or analgesic effect at these doses were short-lived in rats.

Also the percentage protection against thermal pain showed a dose dependent rise in the extract treated rats for any given post treatment time (Figure 3). Although indomethacin treatment at a dose of 10 mg/kg showed higher percentage protection against pain than the three doses of extract tested, the protection was also short lived and begins to decline after 30 minutes. However, protection against pain declined slower than for all three doses of ethanol extract of *H. annus* leaves up to the 90th minutes beyond which all the test doses of extract and indomethacin showed similar level of protection against pain (almost non existent).

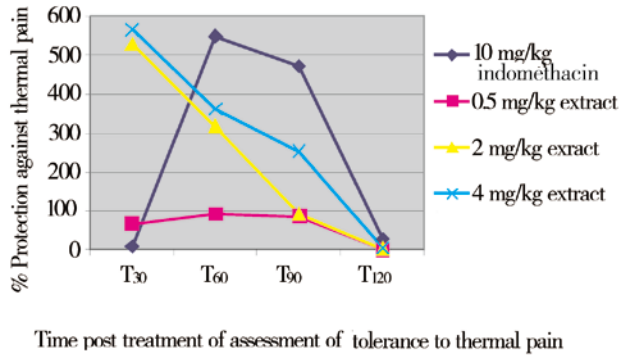

Figure 3. Percentage protection against thermal pain induced by the hotplate analgesic test method in Wistar rats treated with ethanol extract of leaves of *H. annus* and indomethacin, n=5, Mean. T_{30}–T_{120}: tolerance times assessed at indicated minutes after administration of test substance.

The result from the tail immersion analgesic test method corroborated the presence of analgesic potential in the ethanol extract of leaves of *H. annus*, indicated by an increase in the duration of time expended before the immersed tails of the animals were flicked out of the hot water in the water bath. When the mean post-treatment tolerance time were compared with the pre-treatment times and those of distilled water treated rats statistically significant differences were observed for both extract and indomethacin treated groups. However it was noted that indomethacin treatment showed a slower peak effect at 60 minutes which was sustained through the 90th minute and completely lost by the 120th minute post treatment. The dose dependent effect of the extract treated animals was observed to have peaked by the 30th minute before progressive decline through the other assessment intervals for both 4 g/kg and 2 g/kg extract dose levels. At 0.5 g/kg of the extract treatment, significant increases in mean post treatment tolerance times were observed at the 30th, 60th and 90th minutes but the peak effect was somewhat delayed compared to the higher doses.

The percentage protection against pain computed with the data obtained from the tail immersion analgesic test method also suggested a slower onset but more prolonged peak protection against pain by indomethacin compared to extract treatment (Figure 4). Protection against thermal pain by indomethacin increased from baseline to peak by 60 minutes and remained sustained by the 90th minute before effectively disappearing by the 120th minute post treatment. Dose dependent protection against pain was observed for extract treatment and this peaked at 30 minutes before steeply declining for 2 g/kg and 4 g/kg of extract treatment .While 0.5 g/kg of extract treatment showed the least level of pain protection though having an earlier onset time with significant effect already present by the 30th minute, peaking by the 60th minute before showing a steep decline to baseline by the 120th minute.

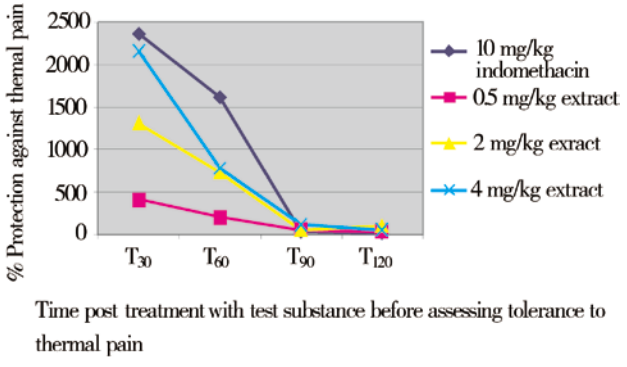

Figure 4: Percentage protection against thermal pain induced by the tail immersion analgesic test method in Wistar rats treated with ethanol extract of leaves of *H. annus* and indomethacin, n=5, Mean. T_{30}– T_{120}: Tolerance times assessed at indicated minutes after administration of test substance

4. Discussion

Edema is one of the cardinal signs of acute inflammation and albumin have been reported to induce inflammatory edema in paw of rodents[44]. The capacity to inhibit and

attenuate the degree of evolution of the cardinal features of inflammation strongly indicates existence of some anti-inflammatory potentials in a drug. Non-steroidal anti-inflammatory drugs (NSAIDs) are known to have anti-inflammatory, analgesic and anti-pyretic potentials[45] and have been approved for clinical use. Their use has however been limited by side effects such as gastric erosion or peptic ulcers, fluid retention in renal impaired persons and many more[46].

The observation of a higher percentage inhibition of albumin induced paw edema by the ethanol extract of leaves of *H. annus* is strongly suggestive of residing anti-inflammatory potential in the extract. And the fact that this effect was significantly higher ($^{bz}P<0.05$) than that of the reference standard (indomethacin) possibly suggest its possession of a higher anti-inflammatory efficacy. Since the leaves of H. annus have been reportedly used as demulscent[47], its use for anti-inflammatory purposes is not likely to be associated with peptic ulcerations unlike indomethacin and other Cox non-specific NSAIDs. Important properties of the saponins have been reported to include expectorant, diuretic, analgesic and promotion of healing[48] while tannins have been reported to possess astringent properties; hasten the healing of wounds and inflamed mucous membranes[49]. These phytochemical constituents may underlie the anti-inflammatory action of the ethanol extract of *H. annus* leaves observed in this study.

Pain is one of the cardinal signs of inflammatory response to tissue injury. The scientific definition of pain by the ISAP also related pain to an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage[10]. A drug's ability to either inhibit or attenuate the degree of evolution of any of the cardinal signs of inflammation would strongly suggest the existence of anti inflammatory potentials in such an agent. For some time it has been known that during inflammatory pain, prostaglandins are generated at the peripheral terminals of sensory neurons and cause hyperalgesia[50,51]. This is accompanied by the production of pro inflammatory cytokines (IL-1,IL-8 and TNF-a) and most probably by induction of Cox 2 in inflammatory cells if not in the nerve terminal[52,53]. Considering the folkloric use of the leaves of *H. annus* following tissue injuries it is possible that its analgesic potential may be related to an anti-inflammatory action[10]. Its acclaimed effectiveness in treating fever also points to a possible anti-inflammatory action.

This study confirmed the existence of anti-inflammatory and analgesic potentials of the ethanol extract of the leaves of *H. annus* in Wistar rats and therefore validated its folkloric use and acclaimed effectiveness in treating several painful and inflammatory conditions especially following tissue injuries.

Further *in vivo* and *in vitro* studies should be carried out to elucidate the exact mechanisms underlying the analgesic and anti-inflammatory actions of the leaves of *H. annus*. Investigation of these effects using other pain and inflammation models should be done to corroborate the findings in our study.

Limited clinical trials could also be carried out in human subjects patronizing the use of the plant in potentially painful and inflammatory situations in different traditional medical systems.

Conflict of interest statement

We declare that we have no conflict of interest.

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