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# Anti-inflammatory and analgesic activities of methanol extract of *Helianthus annuus* Linn. (Asteraceae) leaf

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## Abstract

This study evaluate the anti-inflammatory and analgesic properties of *Helianthus annuus* Linn**.** in rats. Methanol extract of *Helianthus annuus* (HAE) leaf was used in this study. Formalin- and egg-albumin induced-paw edema were used to investigate the anti-inflammatory activities while acetic acid-induced writhing reflex and tail flick models were used to evaluate the analgesic properties. The doses of HAE used were 150, 300 and 600 mg/kg. Acetylsalicylic acid (ASA) was used as reference drug in the anti-inflammatory and writhing reflex models while pentazocine (reference drug) was used in tail flick model. The negative control was dosed 5% tween-20 (10 ml/kg). The HAE exhibited significant ($P < 0.05$) dose-dependent anti-inflammatory and analgesic activities. At 3 hour (h) post treatment, the HAE (300 mg/kg) produced 33.33% and 32.94% while ASA produced 36.36% and 35.29% reduction in paw volume in the formalin and egg-albumin induced paw edema models respectively when compared with negative control. In the acetic acid-induced writhing reflex, ASA and HAE (600 mg/kg) produced 67.89% and 35.78% reduction in the number of writhing, respectively when compared with the negative control. Pentazocine and HAE (300 mg/kg) caused 67.62% and 35.24% increase in pain reaction time when compared with the negative control. The study affirms the folkloric uses of *Helianthus annuus* in the management of pain and inflammation.

**Keywords:** Acetic acid, acetylsalicylic acid, analgesic, anti-inflammatory, *Helianthus annuus*

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## Introduction

Inflammation is an immunological reaction and implicated in the pathophysiology of many disease conditions (1, 2). Uncontrolled inflammatory reaction may aggravate pathological conditions and it is marked with pain, swelling, heat, redness and loss of function (3). Inflammatory processes are suppressed with anti-inflammatory drug in medical practice. Ethnomedical preparations improves clinical disease conditions by suppression of inflammatory processes (4-6). *Helianthus annuus* Linn**.** is one of the medicinal plants used in ethnomedical practice with suspected anti-inflammatory activity (7).

*Helianthus annuus* Linn. belong to the family Asteraceae. It has wide folkloric uses in the ethnomedical practice of South Eastern Nigeria, India and Pakistan (8). The plant is used in the treatment of pyrexia, inflammation, worm infestation, diabetes mellitus and other stomach problems (9). The phytochemical screening of the leaf have reported the presences of alkaloids, saponins, glycosides, terpenes, sterols, tannins and flavonoids (10, 11). Bioactive compounds such as Helivypolide G, annuionones a, b, c and helinorbisabone, 24α-Methyl-5α-cholest-7-en-3β-ol, Annuithrin, heliangolide, niveusin B and its ethoxy derivatives have been isolated from *H. annuus* (12, 13). There are literatures on the antidiabetic, antioxidant, antihepatotoxic, antimicrobial and antidiarrhoea activities of *H. annuus* (8, 14). The present study investigated the anti-inflammatory and analgesic properties of methanol extract of *Helianthus annuus* leaf in rat.

## Materials and Methods

### Plant collection and extract preparation

The leaf of *H. annuus* (voucher number; MOUAU/VPP/2015/16) was harvested from University of Nigeria Nsukka environment and identified by Mr. A.O. Ozioko. The leaves were air dried under shed and later ground into powder with manual grinder (Corona, China). Two hundred grams (200 g) of the powdered plant material were extracted using cold maceration method with 80% methanol, concentrated and the percentage yield was determined as described by Onoja *et al.* (15).

### Experimental animals

One hundred and twenty (120) Wistar albino rats of both sexes weighing 100-115 grams were used for this experiment. The environmental condition of the animal house and other management practices were as explained by Onoja *et al.* (15). The experimental protocol was approved by the University Animal Ethics Committee with reference MOUAU/CVM/EAEC/2015/010.

### Phytochemical analysis

The presence of tannins, saponins, alkaloids, flavonoids, glycosides and terpenes were determined using standard methods of Trease and Evans (16).

### Anti-inflammatory study

The *in vivo* anti-inflammatory activities of *Helianthus annuus* extract (HAE) was evaluated with formalin-and egg albumin-induced paw edema as described by Onoja *et al.* (15). Thirty rats were assigned to five groups (n = 6). Groups 1 and 2 received 5% Tween-20 (10 ml/kg) (negative control) and acetylsalicylic acid (ASA) (200 mg/kg; positive control), respectively. Groups 3, 4 and 5 received HAE at dose range of 150, 300 and 600 mg/kg, respectively. Volume displacement method was used in the determination of paw volumes.

### Analgesic study

The antinociceptive properties of HAE was evaluated as described by Onoja *et al.* (15). Acetic acid-writhing reflex was employed in the evaluation of peripheral activity while tail flick test was adopted in the evaluation of central activity. The animal grouping and treatments were as stated in the anti-inflammatory study except that, ASA (200 mg/kg) was used as the positive control for the peripheral antinociceptive activity while pentazocine (3 mg/kg) was used as the positive control for the central antinociceptive activity.

### Data analysis

The results were presented as mean ± standard error of mean (SEM). Data obtained were analyzed using one way analysis of variance (ANOVAs) and the variant means were separated by Least Significant Difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$.

## Results

### Yield of the HAE

The percentage yield of the HAE was 10.31%.

### Phytochemical analysis

The test revealed the presence of tannins, saponins, alkaloids, flavonoids, glycosides and terpenes.

### Formalin-induced paw edema

The HAE elicited significant ($P < 0.05$) decrease in the paw volume in the treated groups when compared with the negative control group. At 3 h post treatment, the reduction in paw volume of ASA, HAE 150, 300 and 600 mg/kg treated groups were 36.36%, 24.24%, 33.33% and 33.33% reduction in paw volume, respectively when related to the negative control. The percentage inhibition in paw volume of HAE (300 and 600 mg/kg) treated groups were not significant ($p > 0.05$) compared to ASA treated group (**Table 1**).

### Egg albumin-induced paw edema

At 3 h post treatment the ASA, HAE 150, 300 and 600 mg/kg caused 35.29%, 29.41%, 32.94% and 31.76% reduction in paw volume in treated rats, respectively when compared with the negative control. The percentage inhibition in paw volume of HAE (300 and 600 mg/kg) treated groups were not significant ($p > 0.05$) compared to ASA treated group (**Table 2**).

**Table 1.** Formalin-induced paw edema

| Treatment (n = 6) | Mean increase in paw volume in ml ± SEM (% inhibition) | | |
|---|---|---|---|
| | 1 h | 2 h | 3 h |
| 5% Tween-20, 10 ml/kg | 0.39 ± 0.02 (-) | 0.44 ± 0.04 (-) | 0.33 ± 0.04 (-) |
| ASA 200 mg/kg | 0.27 ± 0.02*(30.77) | 0.21 ± 0.02*(52.27) | 0.21 ± 0.01*(36.36) |
| HAE 150 mg/kg | 0.36 ± 0.03 (7.69) | 0.29 ± 0.04*(34.09) | 0.25 ± 0.03*(24.24) |
| HAE 300 mg/kg | 0.28 ± 0.03*(28.21) | 0.24 ± 0.05*(45.45) | 0.22 ± 0.05*(33.33) |
| HAE 600 mg/kg | 0.31 ± 0.02*(20.51) | 0.24 ± 0.03*(45.45) | 0.22 ± 0.03*(33.33) |

*$p < 0.05$ compared with 5% Tween-20 treated group; HAE: *Helianthus annuus* extract, ASA: acetylsalicylic acid

**Table 2.** Egg albumin- induced paw edema

| Treatment (n = 6) | Mean increase in paw volume in ml ± SEM (% inhibition) | | |
|---|---|---|---|
| | 1 h | 2 h | 3 h |
| 5% Tween-20, 10 ml/kg | 0.85 ± 0.07 (-) | 0.83 ± 0.08 (-) | 0.85 ± 0.05 (-) |
| ASA 200 mg/kg | 0.83 ± 0.02 (2.35) | 0.78 ± 0.05 (6.02) | 0.55 ± 0.08*(35.29) |
| HAE 150 mg/kg | 0.93 ± 0.01 (-9.41) | 0.79 ± 0.02 (4.82) | 0.60 ± 0.04*(29.41) |
| HAE 300 mg/kg | 0.50 ± 0.03* (41.17) | 0.78 ± 0.06 (6.02) | 0.57 ± 0.09*(32.94) |
| HAE 600 mg/kg | 0.75 ± 0.08 (11.76) | 0.67 ± 0.09 (19.28) | 0.58 ± 0.07*(31.76) |

*p < 0.05 compared with 5% Tween-20 treated group; HAE: *Helianthus annuus* extract, ASA: acetylsalicylic acid

**Table 3.** Acetic acid induced writhing test

| Treatment (n = 6) | Mean writhing number | % inhibition |
|---|---|---|
| 5% Tween-20, 10 ml/kg | 21.80 ± 0.48 | - |
| ASA 200 mg/kg | 7.00 ± 0.58* | 67.89 |
| HAE 150 mg/kg | 19.80 ± 0.87* | 9.17 |
| HAE 300 mg/kg | 17.60 ± 0.66* | 19.27 |
| HAE 600 mg/kg | 14.00 ± 0.58* | 35.78 |

*p < 0.05 compared with 5% Tween-20 treated group; HAE: *Helianthus annuus* extract, ASA: acetylsalicylic acid

**Acetic acid induced writhing reflex**

The ASA and HAE 150, 300 and 600 mg/kg caused 67.89%, 9.17%, 19.27% and 35.78% reduction in writhing reflex in treated groups, respectively when compared with the negative control group (**Table 3**).

**Tail flick test**

Pentazocine and (HAE 150, 300 and 600 mg/kg) increased the PRT in treated rats by 67.62%, 19.05%, 35.24% and 22.86%, respectively when compared with the negative control. The PRT of HAE treated groups were low ($p < 0.05$) when compared to the pentazocine treated group (**Table 4**).

## Discussion

The HAE reduced ($p < 0.05$) paw edema induced with formalin and egg-albumin and reduced ($p < 0.05$) writhing reflex in treated rat. It also increased the pain reaction time in treated rats. It produced potent anti-inflammatory and analgesic activities which might be linked to the phytoconstituents. Onoja and Anaga (17) reported the presence of flavonoids, Tannins, alkaloids and saponins in the methanol extract of *H. annuus* leaf which is also in agreement with the results of the present study. The anti-inflammatory and analgesic properties of these phytochemicals have been documented (18, 19). The choice of the doses used in this study was based on previous reports and

**Table 4.** Tail flick test

| Treatment (n = 6) | PRT (sec) | % inhibition |
|---|---|---|
| 5% tween-20, 10 ml/kg | 2.10 ± 0.21 | - |
| Pentazocine 3 mg/kg | 3.52 ± 0.41* | 67.62 |
| HAE 150 mg/kg | 2.50 ± 0.08* | 19.05 |
| HAE 300 mg/kg | 2.84 ± 0.24* | 35.24 |
| HAE 600 mg/kg | 2.58 ± 0.15* | 22.86 |

*p < 0.05 compared with 5% Tween-20 treated group; HAE: *Helianthus annuus* extract, PRT: pain reaction time, SEC: second

the acute toxicity test was not conducted here because it has been documented (17). The plant material used in this study was collected from the same environment as the previous investigators; thus the repeat of the acute toxicity test was not necessary (18, 20).

The formalin- and egg-albumin induced paw edema were used as the inflammatory models while acetic acid-induced writhing and tail flick test were used as the analgesic models (21). The HAE exhibited its optimum pharmacological activities at 300 mg/kg (Tables 1, 2, 3 and 4) as an increase in dose beyond 300 mg/kg did not produce any appreciable increase in the magnitude of the activities. This might be due to receptor site saturation and inhibition (16). The HAE is a mixture of bioactive compounds that may have diverse pharmacological activities (12).

The mechanism of the analgesic and anti-inflammatory activities may be the inhibition of cyclooxygenase (COX) activity; same mechanism of ASA. ASA irreversibly inhibit the cyclooxygenase which catalyze prostaglandin production from arachidonic acid (22-24). Prostaglandins are pro-inflammatory mediators (25, 26). The production of prostaglandins are controlled by the level of COX(s) activity and also influenced by the relative cell-specific expression of the terminal synthases, some of which may be co-induced with COX-2 (27, 28).

The HAE demonstrated analgesic activity against both central and peripheral pain. The acetic acid-induced writhing is sensitive and useful in the screening of centrally and peripherally acting drugs while the tail flick test is used in the screening of centrally acting drugs (20). Another possible mechanism of the analgesic activity may be desensitization of the nociceptor and/or increase in the pain threshold in the hypothalamus (22).

In conclusion, the study affirms the folkloric uses of *Helianthus annuus* in the management of pain and inflammation. Further study is desired to isolate and characterize the active principle(s).

## Abbreviations

ASA: acetylsalicylic acid
LSD: Least Significant Difference
SEM: standard error of mean
ANOVA: one way analysis of variance
HAE: *Helianthus annuus* extract
COX: cyclooxygenase


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## Conflict of interest statement

The authors declare no conflict of interest

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