

In-vitro antioxidant, Xanthine oxidase-inhibitory and *in-vivo* Anti-inflammatory, analgesic, antipyretic activity of *Onopordum acanthium*

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Abstract

Onopordum acanthium (Scotch thistle) belong to Asteraceae (Compositae). *O. acanthium* is a flowering biennial plant native to Europe and Western Asia with coarse spiny leaves 20-50 cm in width with conspicuous and spiny-winged stems. We have previously reported pro-apoptotic and cytotoxic effect of *Onopordum acanthium* crude extract against glioblastoma U-373 cells. The present study was designed to evaluate the cytotoxicity, antioxidant, xanthine oxidase inhibition, anti-inflammatory, analgesic, antipyretic activity of butanolic extract of *Onopordum acanthium*. Cytotoxicity of different solvent (methanolic, butanol, chloroform and petroleum ether) extract studied by brine shrimp lethality bioassay, total flavonoid and phenolic, antioxidant, xanthine oxidase inhibition activity was studied by *in-vitro* whereas anti-inflammation studied by carrageenan-induced paw edema model, antipyretic with 20 % brew yeast injection induced pyretic model, analgesic with 1 % acetic acid induced analgesic model investigated in *in-vivo* in wistar rats. Good antioxidant activity was found with IC₅₀ = 134.4 µg/ml with considerable amount of total phenolic and flavonoid content. Xanthine oxidase inhibition effect was weak with IC₅₀ = 572.9 µg/ml. Oral administration of *O. Acanthium* butanolic extract (OA) showed minimum lethality of brine shrimp nauplii henceforth OA butanolic phases was selected for further *in-vivo* studies. OA 200 and 400 mg/kg body weight decreased the oedema by 37.78 % and 40.52 %, respectively; standard aspirin 100 mg/kg decreased 42.62 % at 5th hour of Carrageenan injection. OA 200 and 400 mg/kg significantly decreased acetic acid-induced abdominal writhes when compared to standard aspirin. OA have shown dose and time dependent decrease in body temperature in yeast induced pyrexia, comparable to standard, aspirin. The present results demonstrate that OA has notable anti-inflammatory, antipyretic, analgesic activity related to presence of phenolic compounds as from literature it has been demonstrated that isolated compounds from aerial parts of *Onopordum acanthium* had strong activity in *in-vitro* assay.

Keywords: *Onopordum acanthium*, Flavonoids, Xanthine oxidase inhibition, antiinflammation, analgesic and antipyretic.

Introduction

First anti-inflammatory drug was isolated from extract of willow bark *Salix alba* was salicylates and the acetyl salicylates was synthesized from it, likewise other synthetic anti-inflammatory agents such as propionic acid derivatives like ibuprofen, flurbiprofen, naproxen; anthranolic acid derivatives like mephenamic acid, oxycam derivative piroxicam, pyrrole, indole; and pyrazolone derivatives like ketorolac, indomethacin, and phenylbutazone was discovered [1]. These synthetic agents have side-effects like gastric irritation, ulceration, bleeding, renal failure,

interstitial nephritis, hepatic failure, headache, thrombocytopenia, hemolytic anaemia, asthma exacerbation, skin rashes, angioedema, and pruritus [2]. Investigating *in-vivo* pre-clinical anti-inflammatory and antipain activity from plant source can be a lead for new generation of anti-inflammatory agents devoid of adverse effects.

Onopordum acanthium L. commonly named Scotch thistle, is a medicinal plant belonging to the genus *Onopordum* that is naturalized in various parts of Europe and Asia. The plant has been used traditionally for its antibacterial, cardiotoxic, hemostatic, hypertensive, anticancer properties, treatment of inflammation of the bladder and the respiratory and urinary systems [3,4].

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Sesquiterpenes, flavonoids, triterpenes, sterols, lipids, nitrogen-containing compounds, phenolic acids, and coumarins has been reported in this species [5]. Pharmacological investigations revealed its antioxidant, angiotensin-converting enzyme (ACE) inhibitory activities and the ability to activate natural killer cells *in vitro* against tumor cells [6,7]. Formerly, the ant proliferative activities of extracts prepared from roots and aerial parts with solvents of different polarity were evaluated on four human tumor cell lines (HeLa, MCF7, A431 and U-373), and the chemical investigation of the roots resulted in the isolation of sesquiterpene lactones, a neolignane, steroids, and fatty acids. Ant proliferative activity of *O. acanthium* was due to some of these isolated compounds. [8,9,10]. Lajjter et al reported *in-vitro* COX-2 and NF- κ B1 gene expression, iNOS, 5-LOX, and COX-1 and COX-2 enzymes inhibitory effects of *O. acanthium* [11]. Previously we reported *in-vitro* pro-apoptotic and cytotoxic effect of *Onopordum acanthium* crude extract against glioblastoma U-373 cells (10). As continuation for authentication of traditional use of phytomedicine [12-14] the present study was designed to evaluate the antioxidant, xanthine oxidase inhibition activity, anti-inflammatory, antipyretic, analgesic and of *Onopordum acanthium* extract. Furthermore, in our findings we report *in-vitro* and *in-vivo* studies for the above activities.

Material and methods

Chemicals

LC-MS grade water (H₂O), acetonitrile (ACN), cinnamic acid and naringin were obtained from Sigma-Aldrich/Supelco (Milan, Italy). LC-MS grade acetic acid was attained from Riedel-de Haën (Seelze, Germany). Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck chemicals; methanol from Baker Analyzed Reagent; FeCl₂ was obtained from Carlo Erba (Milan, Italy). Acetic acid (glacial, 100% extrapure) was used for induction of pain in writhing test on mice and diluted with distilled water before administration. The chemicals ethanol, chloroform, methanol, and acetic acid were procured from Sigma-Aldrich (Milan, Italy). Aspirin was purchased from local pharmacy was used as reference standard in analgesic test. Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Animals

72 male Westar rats (200 gm each) were used for the present study obtained from Pasteur Institute of Algiers, Algeria. The rats were fed with standard pellet diet and water *ad libitum*. All animals were acclimatized for at least one week before the experimental session. All the experimental procedures were done as per following the guidelines of the Institutional Animals Ethics Committee (IEAC).

Plant material

The plant material was collected in June 2014 from Khenchelaregion (north east of Algeria). The plant was taxonomically identified by Professor Benayache Samir, voucher specimen (OA-1) of the plant material is deposited at Laboratory of

Development of Natural Resources and Synthesis of Bioactive Substances, University Mentouri, Constantine, Algeria.

Preparation of extracts

400 g air-dried powdered plant material were extracted with 80% aqueous methanol (4x3 L) under shaking at room temperature. The obtained extracts were filtered, and evaporated to dryness in Vacuo at 40°C; the yield of the extract was calculated in percentage (3.5%). The resulting dried substance was suspended in distilled water and then partitioned between n-hexane, chloroform, ethyl acetate and n-butanol. The fractions each were collected, and evaporated to dryness in Vacuo at 40°C; yields were calculated. All fractions were stored at 4°C till testing.

Artemiasalina Leach lethality bioassay

The Artemiasalina Leach (brine shrimp) lethality bioassay was employed to predict the toxic dose of *O. acanthium*. Medium lethal concentration (LC₅₀) determination was carried according to the method of Meyer et al. [15]. The sample, suitably dissolved and then diluted in artificial seawater, was tested at the final concentrations of 10, 100, 500 and 1000 µg/mL prepared in single vials. Ten brine shrimp larvae, taken 48 h after initiation of hatching in artificial seawater, were transferred to each sample vial, and artificial seawater was added to obtain a final volume of 5 mL. After 24 h of incubation at 25-28 °C, the vials were observed using a magnifying glass, and surviving larvae were counted. The assay was carried out in triplicate, and LC₅₀ was determined using the probit analysis method. LC₅₀ was calculated and organic phase with least LC₅₀ is selected for further experiments.

Identification of phenolic compounds by HPLC-PDA analysis

HPLC-PDA analyses were performed described by method by Habibatni et al on a LC system (Shimadzu, Milan, Italy) equipped with 2 LC-10ADVP pumps, a CTO-20AC column oven, a SCL-10AVP system controller, and SPD-M10AVP photo diode array (PDA) detection [16]. Data acquisition was performed by Shimadzu LabSolution Software ver. 1.12. For chromatographic separations, an Ascentis Express C₁₈ column (15 cm x 4.6 mm I.D.) packed with 2.7 µm partially porous particles, was employed (Supelco, Bellefonte, PA, USA). The injection volume was 2 µL, and the mobile phase consisted of water/acetic acid (0.075 %) at pH=3 (solvent A) and ACN/acetic acid (0.075 %) (solvent B), respectively in the following linear gradient mode: 0 min, 0% B; 60 min, 40% B; 80 min, 100% B; 81 min, 0% B. The mobile phase flow rate was 1.0 mL/min. PDA wavelength range was 190-400 nm and the chromatograms were extracted at 254 nm (sampling frequency: 6.25 Hz, time constant: 0.32 s). OA extract, n-But phases (10 mg) were dissolved in 1 mL of methanol (4:1 v/v) and filtered through a 0.45 µm membrane filters (Whatman, Clifton, USA).

Total flavonoid content

The total flavonoid content was determined according to the method described by Jothy et al, [17], with minor modification. OA

extracts (0.5 ml of 1 mg/ml) was added to 2 ml of dist. water. Then, 0.15 ml of 5% w/v NaNO₂ was added and allowed to stand for 6 min, 0.15 ml of 10% AlCl₃ was added and allowed to stand for 6 min. 2 ml of 4% w/v NaOH and 0.2 ml of distilled water were added and allowed to stand for 15 min at RT and the absorbance was measured at 510 nm. Distilled water was used as blank. Different concentrations of catechin were used as standard for plotting the calibration curve ($Y = 0.0041 + 0.0024X$, $R^2 = 0.9901$). The flavonoid content was estimated as mg of catechin/100 mg of dried extract.

Total polyphenolic content

The total phenolic content in the OA extract was measured based on procedure described by Singleton et al. [18], with some modifications. Where, 300 µL of 1 mg/ml of OA extract was mixed with 1500 µL of FolinCiocalteu reagent (1:10 dilution) and allowed to stand in darkness for 6 min. Then, 1200 µL of 7.5% sodium carbonate were added and the mixture was kept in dark at 40°C for 90 min. The absorbance of the blue color, that developed, was measured at 765 nm. Plants that produced absorbances higher than 1.5, a concentration of 100 µg/ml was repeated. The experiments were carried out in triplicates. Gallic acid was used for constructing the standard curve (10-100 µL) $Y = 0.0102X + 0.0215$; $R^2 = 0.9979$) and the total phenolic compounds concentration in each extract was expressed as mg of gallic acid equivalent per 100 mg of dried extract (mg GAE/100 mg OA extract).

DPPH-assay

Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrylhydrazyl): The radical scavenging assay was quantitatively carried out by the method described by Jothy et al [17], with minor modification. In a light-protected bottle, 500 µL of OA extract at concentrations ranging from 10 µg/ml to 3 mg/ml was added to 5 ml of 0.004% w/v solution of DDPH in 80% methanol. Ascorbic acid was used as standards and 80% methanol as blank whereas the DPPH solution in the absence of OA extract was used as negative control. The reaction mixture was incubated in dark at 37°C for 30 min and measured at 517 nm. All assays were measured in triplicates and DPPH scavenging effect was calculated according to the following equation:

$$\% \text{DPPH scavenging effect} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 is the absorbance of the negative control, A_1 is the absorbance of DPPH in presence of OA extract - the absorbance of OA extract in 80% methanol. IC₅₀ (concentration providing 50% scavenging effect) values, were calculated from the dose inhibition curve and results were recorded as average \pm SD.

Xanthine oxidase inhibition assay

Xanthine oxidase inhibition was assayed spectrophotometrically according to Samaha et al [19] using xanthine as substrate. A 4-ml mixture containing 1 ml of 100 µg/ml of OA extract or allopurinol, 1ml of xanthine substrate (0.6mM), and 1.9 ml of 150mM Potassium phosphate buffer pH 7.5, preincubated for 10 min at 25°C and the reaction was started by addition of 0.1 ml of XO

enzyme (0.1U/ml in phosphate buffer). The reaction was monitored spectrophotometrically at 25°C for 10 min every 30 seconds against phosphate buffer as blank. Allopurinol was used as standard enzyme inhibitors. The percent xanthine oxidase inhibition was calculated as per the following formula:

$$\% \text{inhibition} = 100 - \frac{(\Delta A_1 / \Delta t)}{(\Delta A_0 / \Delta t)} \times 100$$

Where $\Delta A_1 / \Delta t$ is the activity of the enzyme in presence of OA extract, $\Delta A_0 / \Delta t$ is the absorbance in absence of the OA extract. IC₅₀'s were determined for plants as concentration of OA extract at half maximal inhibitory activity.

Anti-inflammatory activity: edema carrageenan

Inflammation is caused by the injection of carrageenan 1% at the dose of 100 µL per rat paw [20]. The measures of the volumes of the right hind paw of each rat were taken before induction of edema and each 1 h, 2 h, 3 h, 4 h and 5 h after the injection of carrageenan. For anti-inflammatory activity, four groups of six Wistar rats were used. Negative control group received the physiological water (0.9%) orally, one hour before injection of carrageenan (0,1ml; 0.6%) in the plantar arch of the right paw of the rat. Positive control batch were treated orally with therapeutically used anti-inflammatory drug (Aspirin) at a dose of 100 mg / Kg, 1 hour before the injection of carrageenan. Test group butanolic OA extract was administered orally at a dose of 200 and 400 mg/kg respectively; 1h before the injection of carrageenan. Paw thickness were measured just before the carrageenan injection at zero hour and then at 1, 2, 3, 4, and 5 th hour after carrageenan injection, using a plethysmometer. Percentile edema inhibition was calculated according to the following formula:

$$\text{Percentile inhibition} = (1 - V_t / V_c) \times 100,$$

where V_t and V_c represent the mean difference in paw measurement between the treated and control groups.

Analgesic activity

The abdominal cramp in mice in response to intraperitoneal injection of acetic acid is one of the well-established basic tests for analgesic activity [21]. Analgesic effect is assessed by counting these cramps for 30 min after the injection of the algogenic agent. For each test of the analgesic activity, four groups of six rats were used. These rats are of the same sex were fasted 16 hours before the test. Negative control batch rats received the vehicle solution (physiological saline) 30 minutes before the injection of acetic acid (1%) intraperitoneally. Positive batch were treated orally with an analgesic (the Aspirin 500 mg / Kg) 30 minutes before the i.p. injection of acetic acid. Test batch animals receive orally the butanolic extract of OA at different doses 200, 400 mg / kg, respectively 30 minutes before the injection of acetic acid. The mice with decreased number of writhes were considered protected. The percentage cramps inhibition was calculated using the following formula:

$$\text{Cramping \% inhibition} = \frac{[\text{witness Average} - \text{Average test}]}{\text{witness Avg.}}$$

Percent inhibition = $(1 - Wt/Wc) \times 100$,

where *Wt* and *Wc* represent the number of writhing movements, measured between the treated and control groups.

Antipyretic activity

Antipyretic activity was assessed by method described by Afsar et al [22]. Rats were divided into four groups of six rats, and they were trained to remain quiet in a restraint cage. The rectal temperature (RT) was recorded with 0.1 C precision by inserting a lubricated digital thermometer (external diameter 3 mm) into the rectum of each animal. After measuring basal RT, subcutaneous injection of a pyrogenic dose of Brewer's yeast 20% (1 ml / 100 g body weight) induced fever, and subsequently RT was recorded every hour. Two hours after injection of yeast, either water (as the negative control), or 500mg/kg aspirin (as the positive control) or butanolic OA (200, 400 mg/kg as the test group) was administered orally to each rat, and the RT was recorded every hour for another 5 hours. Results are expressed as the changes of the RT from basal RT.

Statistics

For analgesic and anti-inflammatory activity was analyzed by statistical comparisons among the groups were assessed by one-way analysis of variance (ANOVA) followed by One-way ANOVA followed by Dunnett's Multiple Comparison Test whereas antipyretic activity was by Two-way ANOVA followed by Bonferroni

multiple comparison test) using Grappad prism software. $P < 0.05$ was considered statistically significant.

Results and Discussion

Cytotoxicity

For the preliminary assessment of the toxicity of OA extract towards *Artemiasalina* (brine shrimps) larvae, LC50 value was determined. Methanolic, chloroform, petroleum ether extract had higher LC50 value more than 500 µg/mL whereas butanolic extract had less than 500 µg/mL. Henceforth butanolic extract was selected for further pharmacological testings.

Phenolic compounds identification by HPLC-PDA analysis

The HPLC-PDA analysis of OA extract, n-But phases revealed the presence of various polyphenolic compounds, belonging to different chemical classes viz. gallic acid, flavone, apigenin derivatives (Figure 1). respectively, thanks to the match with elution order and reference material data. Lajter et al identified three lignans: pinoresinol, medioresinol and (±)-syringaresinol and four flavonoids: hispidulin, nepetin, apigenin and luteolin from the aerial parts of *O. acanthium* [11]. These findings are similar to our identified phenolic contents, nepetin, apigenin and luteolin belongs flavone group of flavonoids, additionally gallic acid: a tannin was identified in our sample.

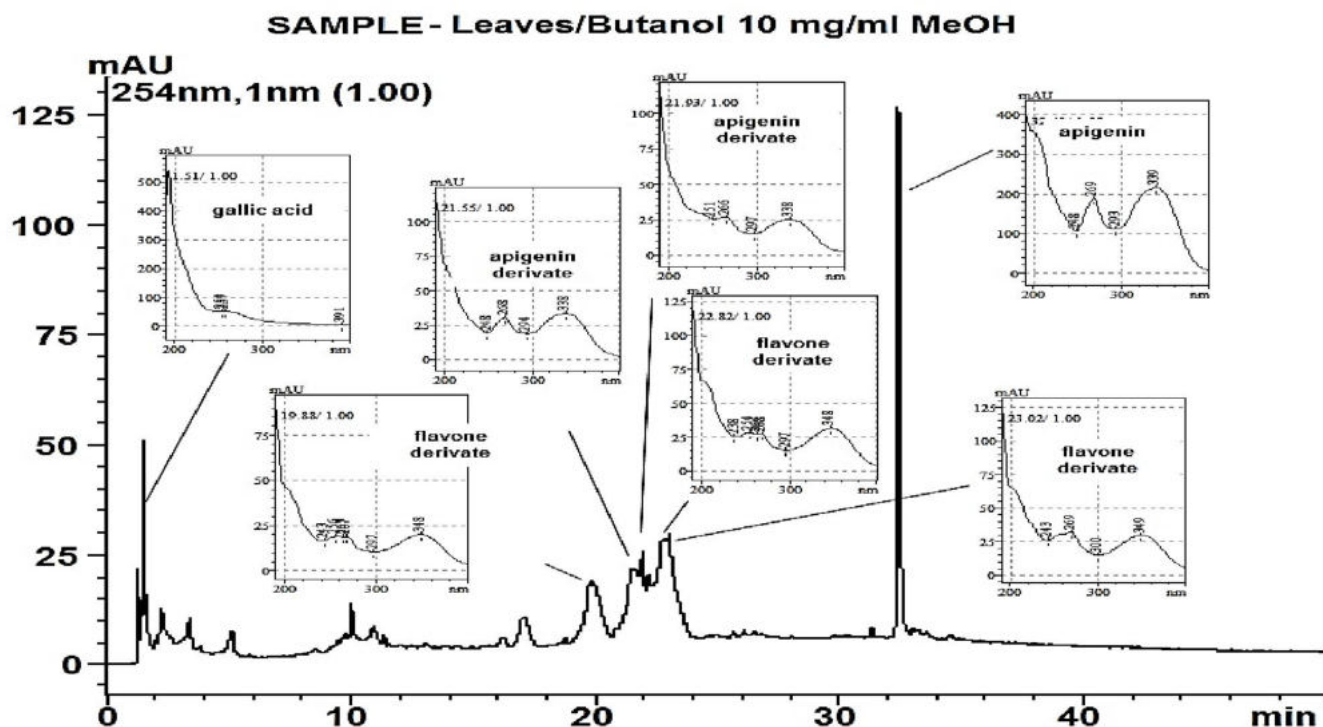


Figure 1: The HPLC-PDA analysis of *Onopordium acanthium* butanolic leaves extract revealed the presence of various polyphenolic compounds, belonging to different chemical classes viz. Gallic acid, flavones derivatives and apigenin derivatives (Figure 1).

Total flavonoid and phenolic contents

The total flavonoid content, calculated from the calibration curve ($R^2 = 0.9901$) was 3.93 ± 0.037 mg catechin equivalent/ 100 mg dried extract. The total phenolic content of the OA extract ($R^2 = 0.9979$) was 8.93 ± 1.33 gallic acid equivalents/ 100 mg dried extract (Table 1). Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites and these phenolic compounds have redox properties, which allow them to act as antioxidants [23]. Furthermore, flavonoids activate Antioxidant Responsive Element (ARE) through Nrf2 pathway to counteract proinflammatory mediator such as TNF and COX induced inflammation [24-26].

Antioxidant activity

Phenolics, flavonoids and carotenoids are plant secondary metabolites rich in antioxidant activity due to their redox properties and chemical structures. The DPPH radical is widely used in assessing free radical scavenging activity because of the ease of the reaction. DPPH scavenging activity IC₅₀ value of OA extract was $134.4 \mu\text{g/mL}$ while that of the control, ascorbic acid, was $21.4 \mu\text{g/mL}$ (Table 1). Flavonoids subdue reactive oxygen species, scavenge reactive species, chelate trace elements involved in free-radical production and up-regulate antioxidant protection [23]. Thanks to activation of a redox-sensitive gene regulatory network mediated by the NF-E2-related factor-2 (Nrf2) which is involved in

response to oxidative stress and xenobiotics, mediated through Antioxidant Responsive Element (ARE). Phenolic compounds such as flavonoids activate Antioxidant Responsive Element (ARE) through Nrf2 pathway to counteract proinflammatory induced inflammation [24-26]. Similarly, phenolics counteracting oxidative stress in various pathological conditions, few to be mentioned as in cancer cell lines and hypoxia for this reason increasingly being used as therapeutic agent. [27,28].

Xanthine oxidase inhibition

Inhibition of xanthine oxidase resulted in a decreased production of uric acid, which was measured spectrophotometrically. OA extract had lower inhibition of xanthine oxidase enzyme activity with IC₅₀ value of $572.9 \mu\text{g/mL}$ when compared with standard drug allopurinol (IC₅₀= $3.9 \mu\text{g/mL}$). Hypouricemic agents are commonly employed for the treatment of chronic gouty arthritis, which includes xanthine oxidase inhibitors. The crystallization of uric acid, often related to relatively high levels in the blood, is the underlying cause of gout leading to pain in joints and pain. An ideal agent to cure gout would be a drug possessing anti-inflammatory, analgesic, antipyretic and xanthine oxidase inhibition properties [29]. In our findings, OA extract shown lower xanthine oxidase inhibition, indeed OA extract can act as synergism in combination with other bioactive compounds.

Table 1: Total flavonoid content, total phenolic content, antioxidant and xanthine oxidase inhibition activity

Plant	Total Flavonoid Content mg catechin/100 mg dried extract	Total phenolic content mg Gallic acid/100mg dry extract	DPPH scavenging activity IC ₅₀ (μg/ml)	%Xanthine oxidase inhibition (100 μg/ml)	Xanthine oxidase IC ₅₀ (μg/ml)
OA extract	3.93 ± 0.037	8.93 ± 0.133	134.4	7.0%	572.9
Ascorbic acid	-	-	21.4	-	-
Allopurinol	-	-	-	-	3.9

Anti-inflammatory activity

This is the first research on *in-vivo* xanthine oxidase inhibition antipyretic, analgesic and anti-inflammatory activities of OA leaves extract, to the best of our knowledge. Present findings revealed that the OA extract had noteworthy antipyretic, analgesic and anti-inflammatory properties with a rational preventive profile. The hind paw carrageenan injection induced a gradual edema attaining its maximum at 4 hours. In case of negative control group animals had shown significant increase of paw thickness at consecutive hour ($P < 0.05$). At initial zero hours, the thickness was 1.414 ± 0.053 cm, then increased to 2.314 ± 0.0178 cm at $t = 3$ hours. The thickness at 5 hours was 2.65 ± 0.146 cm. The paw thickness of positive control animals was 1.302 ± 0.217 cm which manifested a mild increase at the end of 0.5 hour, that is, 1.704 ± 0.078 cm. After the 0.5 hour, it decreased to 1.694 ± 0.448 cm, 1.528 ± 0.293 cm, 1.526 ± 0.255 cm,

1.526 ± 0.243 cm and 1.47 ± 0.207 cm at the end of 1, 2, 3, 4 and 5 hours, respectively ($P < 0.05$). OA 200 mg/kg treated animals had shown an increase up to the 2nd hour, at the end of third hour thickness was found to be 2.01 ± 0.332 cm, then decreased to 1.788 ± 0.265 cm and 1.594 ± 0.152 cm at 4 & 5 hours respectively ($P < 0.05$). In OA 400 mg/kg treated animals, paw thickness was increased to 1.346 ± 0.043 cm ($t = 0$ hours), 1.55 ± 0.154 cm ($t = 0.5$ hours), 1.74 ± 0.116 cm ($t = 1$ hours), and 1.928 ± 0.225 cm ($t = 2$ hours) and it decreased after the second hour to 1.702 ± 0.338 cm ($t = 3$ hours), 1.544 ± 0.344 cm ($t = 4$ hours), 1.524 ± 0.342 cm ($t = 5$ hours). These values were found to be statistically significant at $P < 0.05$. OA 400 mg/kg shown dose dependent effect than OA 200 mg/kg. (Figure 2). The inflammation inhibition by OA extracts could be contributed by the presence of active constituents. Lajter et al identified and isolated, three lignans, pinosresinol, medioresinol and (±)-syringaresinol, four flavonoids, hispidulin, nepetin, apigenin and

luteolin from the aerial parts of *O. acanthium*. The isolated compounds from aerial parts were tested for inhibition of COX-2 and NF-κB1 gene expression, iNOS, 5-LOX, COX-1 and COX-2 enzymes in *in-vitro* assays, among the flavonoids, notable inhibitory activities (> 50% inhibition) were recorded for luteolin, nepetin, and hispidulin. Luteolin shown most potent 5-LOX inhibition. Moreover, luteolin was moderately inhibited COX-2 and NF-κB1 gene expression and iNOS assays, only moderate activities were observed for the lignans [11,30]. In our studies, in the leaves extract, gallic acid and flavone derivatives was identified

that includes nepetin, apigenin and luteolin. This explains that *in-vivo* anti-inflammatory studies was comparable with standard drug aspirin at both doses OA 200 mg/kg and 400 mg/kg. Furthermore, Gallic acid shown anti-inflammatory activity by interfering with the polymorphonuclear leukocytes (PMNs) functioning and active NADPH oxidase assembly. Presence of Gallic acid in OA extract might be play a part in strong anti-inflammatory activity like , O-dihydroxy group of gallic acid is significant for the inhibitory activity *in-vitro* [22].

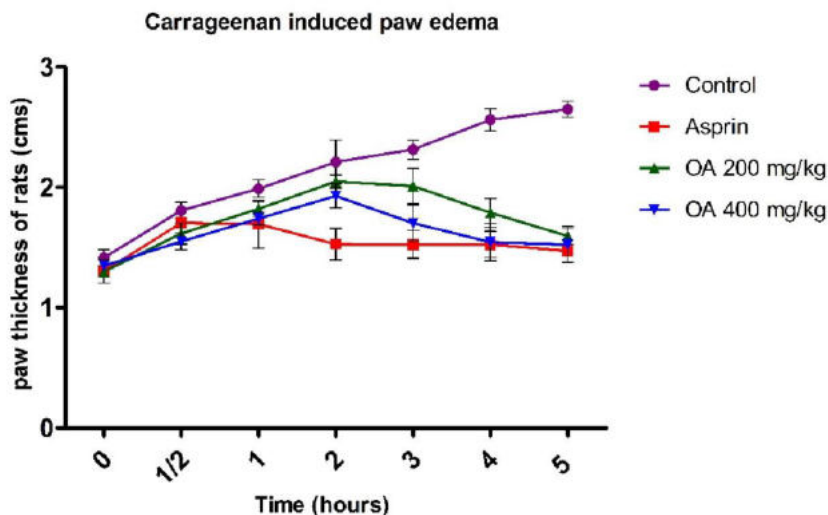


Figure 2: Anti-inflammatory activity of *Onopordum acanthium*

Data are expressed as mean ± standard error of five rats per group. Aspirin treated group is at statistically **p < 0.01, OC 100 mg/kg treated group is at *p < 0.05, OC 300 mg/kg is at **p < 0.01 versus control group (One-way ANOVA followed by Dunnett's Multiple Comparison Test).

Analgesic activity

Prominent writhing movements was caused by intraperitoneal acetic acid injection in the rats. In OA treated test groups, the extract significantly (P < 0.5, P < 0.001) inhibited acetic acid induced writhing response in a dose dependent manner. OA extract produced 72.0% and 76.57% writhing protection at the dose of 200 and 400 mg/kg body weight, respectively. Where as the standard drug aspirin produced 86.29 % protection in positive control animals at the dose of 500 mg/kg body weight. From the results, it was observed that the percent protection of writhing was increased with the doses of the extracts. Evaluating analgesic activity potential for any medicinal agents, acetic acid induced writhing testis a well proposed method. Pain sensation in acetic acid

induced writhing model is evoked by producing a localized inflammatory response by releasing of free arachidonic acid from tissue phospholipids catalyzed by COX and increasing prostaglandins synthesis particularly PGE2 and PGE2 [31-33]. These prostaglandins and lipoxygenase cause swelling and pain by the increasing capillary permeability and releasing endogenous substances that prompt pain nerve endings. NSAIDs inhibits COX enzyme in the peripheral tissues and affect the transduction mechanism of main afferent nociceptors [34]. In our findings, acetic acid-induced abdominal constriction assay exhibited an eminent reduction in writhing reflex. The analgesic effect observed at 400 mg/kg dose was comparable with the NSAID standard drug Aspirin (Figure 3).

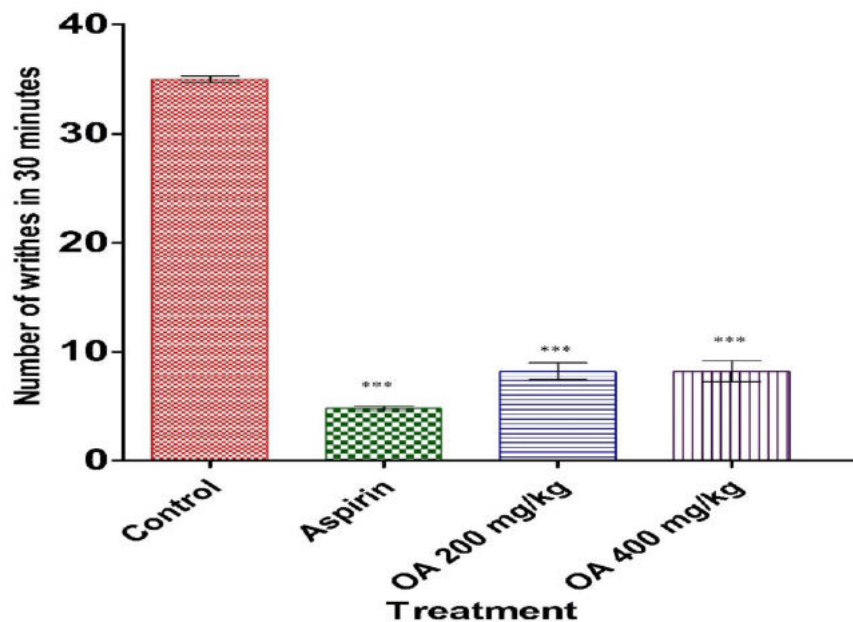


Figure 3 : Analgesic activity *Onopordum acanthium*

Data are expressed as mean \pm standard error of five rats per group statistically***p < 0.001 versus control group (One-way ANOVA followed by Dunnett's Multiple Comparison Test).

Antipyretic effect

The antipyretic activity results(Baker's yeast-induced fever) are depicted inTable 2. Animals of negative control group that received Baker's yeast (20 %)had a significant increase in the rectal temperature 3 hours after the yeast injection and the increase in TR was seen until the end of the experiment. The positive group animals treated with aspirin significantly decreased animal's TR during the tested period. Animals treated with either 200 or400 mg/kg OA showed a significant decrease in TR at 4 and 5 hours after the yeast injection when compared to the negative control at the same time point. This reduction was comparable to positive control. Increase in prostaglandins synthesis is induced by a

subcutaneous injection of Brewer's yeast causes pyrexia (called as pathogenic fever). Inhibition of prostaglandin biosynthesis is a particular characteristic of antipyretic drugs or compounds and it is considered as a useful test for the screening of plant materials as well as synthetic drugs for their antipyretic potential [35]. Inhibition of prostaglandin synthesis by blocking the cyclooxygenase enzyme activity could be the possible mechanism of antipyretic action [36]. The observed activity might be due to the presence of pharmacologically active metabolites that might interfere with the release of prostaglandins. Luteolin, a flavonoid isolated from OA aerial parts was the most potent in the inhibition of 5-LOX. Moreover, luteolin also moderately inhibited on the COX-2 and NF- κ B1 gene expression and COX and iNOS enzymes [11].

Table 2:Antipyretic activity of *Onopordum acanthium* Data values shown represent mean \pm SEM (n = 5)

Treatment	T0	T1/2	T1	T2	T3	T4	T5
Control	36.38 \pm 0.33	37.32 \pm 0.31	38.04 \pm 0.26	38.36 \pm 0.34	38.52 \pm 0.08	39.16 \pm 0.27	39.5 \pm 0.16
Aspirin	36.38 \pm 0.24	37.14 \pm 0.37	37.1 \pm 0.37 ***	38.35 \pm 0.32	37.92 \pm 0.31 **	37.42 \pm 0.31 ***	37.22 \pm 0.19 ***
OA 200 mg/kg	37.58 \pm 0.34 ***	37.8 \pm 0.34 *	38.26 \pm 0.35	38.46 \pm 0.21	37.92 \pm 0.37 **	37.54 \pm 0.32 ***	37.38 \pm 0.08 ***
OA 400 mg/kg	37.22 \pm 0.35 ***	37.38 \pm 0.33	37.86 \pm 0.11	38.36 \pm 0.25	37.92 \pm 0.08 **	37.44 \pm 0.11 ***	37.34 \pm 0.23 ***

statistically significant at *p < 0.05, **p < 0.01, ***p < 0.001 versus only control group (Two-way ANOVA followed by Bonferroni multiple comparison test).

Conclusion

Onopordum acanthium extract shown good antioxidant activity in DPPH test assay. The antioxidant properties of OA leaves extract are associated to the phenolic content. Xanthine oxidase inhibition effect was weak but with these data suggest that OA extract can be combined with other phytomedicine for considerable antigout activity. Indeed, needs to be tested with isolated bioactive compounds like flavonoids from this plant. Notable *in-vivo* anti-

inflammatory, analgesic and antipyretic properties was found, again these activities have been related to presence of phenolic compounds that has been reported in literature for isolated compounds tested in *in-vitro* assays. For authenticating OA extract as a potent anti-inflammatory, analgesic and antipyretic drug, active bioactive compounds should be isolated and studied *in-vivo*. In conclusion, from our results, the leaves of OA could be considered as encouraging candidate as safe antioxidant, anti-inflammatory, analgesic and antipyretic agent.

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