GC-MS analysis, Anti-Inflammatory and Analgesic Evaluation of *Hackelia uncinata* (Royle Ex Benth) Ethanol Extract

Rifat Roshan[1], Muhammad Mohtasheemul Hasan[1], Erum Shah[2], Tehseen Quds[2], Norin Memon[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi-75270, Karachi, Pakistan.

[2]Dow College of Pharmacy, Dow University of Health Sciences-74200, Karachi, Pakistan.

Abstract- The current study aims to investigate GC-MS analysis and the anti-inflammatory and analgesic effects of the ethanol extract of *Hackelia uncinata* in mice using carrageenan-induction paw edema method, hot plate and acetic-acid induction methods. *Hackelia uncinata* is therapeutically used to treat inflammation, wounds, colds, blisters etc. The extract of plant was administered orally at 100, 200 and 400 mg/kg doses. A total of five groups with five animals each were allocated. In the carrageenan-induced inflammatory model, the ethanol extract exhibited significant anti-inflammatory effects ($p<0.05$) at all doses and was highly significant ($p<0.001$) after 3 hours at 400 mg/kg dose compared to control. During the investigation of analgesic effect by hot plate method, the dose of 400 mg/kg highly significantly ($p<0.001$) reduced pain at 60, 90 and 120 min, and lower doses of 100 and 200 mg/kg significantly ($p<0.05$) reduced pain while in an intraperitoneal injection of acetic acid-induced analgesia, the extract significantly ($p<0.001$) reduced pain. In conclusion, all these findings suggest that ethanol extract of *H. uncinata* possesses anti-inflammatory and analgesic effects. Gas chromatography-mass spectrometry analysis was done to study phytochemical compounds in the extract, a total of 30 different bioactive compounds were detected in ethanol extract.

Index Terms-*Hackelia uncinata*, ethanol, anti-inflammatory, analgesic, GC-MS analysis

I. INTRODUCTION

Inflammation is a protective mechanism of the body produced in response to pathogens and tissue injury resulting in the activation of immune cells and releasing various inflammatory mediators such as cytokines, nitric oxide, histamine, leukotrienes and prostaglandins which in turn rise the temperature and causes redness, edema and pain [1, 2]. On the other hand, pain is a response produced by the body upon the abolition of pain-producing factors. Pain could be acute, chronic, visceral and may be inflammatory, or neuropathic type depending upon the organ of origin [3].

Conventionally, inflammation and pain are treated by non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs inhibit the activation of cyclooxygenase (COX-I) and Cyclooxygenase (COX-II) enzymes, preventing the production and release of prostaglandins and thromboxanes by displaying the anti-inflammatory and analgesic effects [4]. However, these drugs are accompanied by severe adverse effects and low potency and efficacy, therefore there is an urge to develop new anti-inflammatory agents [5].

Plants are excellent sources of a wide range of medicines for humans as they possess remarkable analgesic, antipyretic anti-inflammatory, antimicrobial, anti-arthritic, anticancer, neuroprotective and antidiabetic properties [6]. Plants are recorded as a valuable source for the development of innovative pharmaceutical molecules used in the treatment of many life-threatening ailments. That is why herbal products are considered as an alternative pain management therapy [3]. More than 85-90 % of the population depends on plants for combating various diseases due to the presence of bioactive compounds [7].

Hackelia uncinata Royle ex Benth belongs to the *Boraginaceae* family and it is a perennial branched herb up to 90cm in height with pale blue to sky blue flowers. It is found in 2400-3500 m of forest zones [8]. The secondary metabolites of *Boraginaceae* are alkaloids, flavonoids, polyphenols, terpenoids and phytosterols with reported biological activities of anti-inflammatory, antimutagenic, antipyretic and diuretic potential [9]. *Hackelia uncinata* is used to treat cough and cold [10]. Its roots are used for piles [11], root paste is applied on boils, blisters, cuts and wounds and the whole plant is used for fractured bones and ligaments [12,13]. The present study aimed to evaluate the anti-inflammatory and analgesic properties in mice model along with the GC-MS analysis of ethanol extract of whole plant of *Hackelia uncinata*.

II. MATERIAL AND METHODS

Collection of Plants- The whole plants of *Hackelia uncinata* (Fig. 1.) were collected from Muzaffarabad, Azad Jammu and Kashmir. The plant was authenticated at the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad and the voucher specimen (RR-3182) was deposited in the herbarium.

Fig.1. *Hackelia uncinata* Royle ex Benth

After washing with clean water, the plants were dried in shade and cut into pieces. The chopped pieces were soaked in ethanol for 1 week and filtered. The filtered solvent was evaporated on a rotary evaporator at 40 ºC to get a semi-solid mass which was stored in bottles for pharmacologic activities and future use.

Selection of Animals- Male Swiss Albino mice (10 weeks old, 24-35 g) were housed in polypropylene cages under suitable environmental conditions of 25 ± 2 ºC, 30-70% humidity with 12 hr light and 12 hr dark cycle. All the animals had free access to laboratory diet *ad libitum* and were allowed free access to drinking water during the acclimatization period. The experiments were conducted after overnight fasting but there was free access to water.

Grouping of Animals- To carry out anti-inflammatory and analgesic activities animals were grouped into five with five animals each. Group I was treated with normal saline (10 ml/kg), group II was treated with a standard drug (diclofenac sodium 10 mg/kg), and groups III, IV and V received extract at the dose of 100 mg/kg, 200 mg/kg and 400 mg/kg.

Anti-inflammatory effects

Anti-inflammatory effects were determined by carrageenan-induced paw edema with slight changes. After the thirty minutes of oral administration of treatments to respective groups, freshly prepared 0.05 ml carrageenan solution was injected subcutaneously in the right hind paw of each animal of each group. The paw volume of mice was measured by a digital plethysmometer at 0, 1, 2, 3, and 4 hours of carrageenan injection and percent inhibition was calculated [14].

Analgesic effects

Hot plate method-In the hot plate method, after the thirty minutes of oral treatments to mice of each group, animals were placed individually in a preheated hot plate set at 55 ± 0.1 ºC by taking the cut-off time of 15 seconds. The latency time was noted at 0, 30, 60, 90 and 120 minutes and percent analgesia was determined [15].

Acetic-induced method- In acetic-induced method, animals were given oral treatments and after one-hour writhes were produced by injecting 1% of 10 ml/kg acetic acid into the peritoneum. After five minutes of acetic acid injection, abdominal elongation and contraction known as writhes were counted for 10 minutes and percent inhibition was determined [15].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis- The ethanol extract of *Hackelia uncinata* was subjected to GcC-MS and components of the extract were identified by comparing the retention times with the known components from NIST and Wiley libraries.

Statistical Analysis

The results were described as mean ± SEM, statistical analysis was performed by Microsoft Excel and One-way ANOVA followed by Tukey test. The level of significance was calculated by taking the *p<0.05 as significant, **p<0.01 as more significant, *** p<0.001 as highly significant.

III. RESULTS AND DISCUSSION

Anti-inflammatory effects- The ethanol extract of whole plants of *Hackelia uncinata* produced significant effects at all doses in carrageenan-induced paw edema of mice. Extract at the doses of 100, 200 and 400 mg/kg significantly reduced the edema at 1, 2, 3 and 4 hours compared to vehicle control as shown in Fig 2. This method is mostly used for the screening of anti-inflammatory compounds due to a greater degree of reproducibility. In animals, the inflammatory response is characterized by a biphasic response due to the formation of several inflammatory mediators in the first phase such as serotonin, histamine and bradykinin whereas prostaglandins and nitric oxide are released in the second phase [16].

Furthermore, intraplantar injection of carrageenan stimulates the release of inflammatory indicators such as histamine, bradykinin, prostaglandin, thrombin and reactive oxygen species [4]. All these mediators cause swelling, pain, and redness at the site of production. NSAIDs are commonly prescribed drugs worldwide for suppressing pain and inflammation. However, the excessive use of NSAIDS is associated with gastrointestinal tract, liver, kidney, cardiovascular and respiratory problems [17].

The dose of 400 mg/kg produced highly significant effects (p<0.001) at 3 hours of administration and significant effects (p<0.05) were observed at the dose of 100 mg/kg and 200 mg/kg after 2 hours of administration. While the standard drug diclofenac sodium produced more significant (p<0.01) effects at 1 hour and highly significant (p<0.001) at 3 and 4 hours, respectively. In our experiment, the extract produced more significant effects in the second phase indicating the inhibition of mediators and we postulated that edema may be associated with the release of prostaglandins.

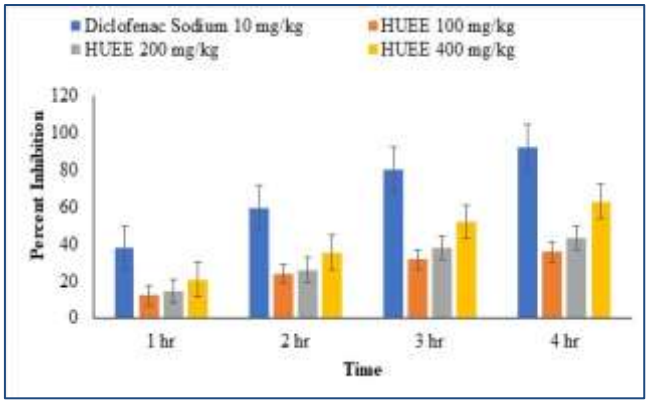

Fig. 2. Carrageenan-induced anti-inflammatory effects of *Hackelia uncinata* ethanol extract in mice at different time intervals

Analgesic effects-Although there are various methods to determine the analgesic effects, we performed hot plate and acetic acid-induced writhing methods to determine the central and peripheral effects in animal models [18].

In the hot plate method, the extract of *H. uncinata* produced analgesic effects after 30 minutes of administration, and the dose levels of 100, 200 and 400 mg/kg reduced pain at 30, 60, 90 and 120 minutes as shown in Fig 3. The high doses of *H. uncinata* produced highly significant effects while the lowest dose produced minimal effects in experimental animals. The *H. uncinata* extract at the doses of 200 and 400 mg/kg produced highly significant ($p<0.001$) effects after 60 minutes of treatment, the extract at 100 mg/kg dose produced a significant effect ($p<0.05$) throughout the experiment. The standard drug exhibited significant ($p<0.05$) analgesia after 30 minutes and highly significant ($p<0.001$) after 60 minutes to 120 minutes.

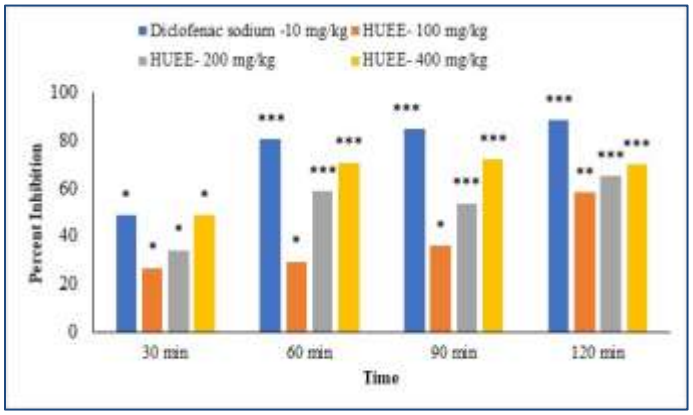

Fig. 3. Hot plate-induced analgesic effect of *Hackelia uncinata* ethanol extract in mice at different time intervals

The writhing test is a chemical-induced method by the injection of acetic acid in mice to determine peripheral analgesic effects. The ethanol extract of *Hackelia uncinata* significantly inhibited the abdominal contractions and elongations and the percent inhibition by three doses is shown in Fig 4. In this test, contraction of abdomen and extension of hind limbs and arching of the back are the key signs of reflexes. Acetic acid produces pain by releasing endogenous substances such as serotonin, prostaglandins, histamine and bradykinins [19]. The *H. uncinata* whole plant produced a significant decrease in writhing indicating the ability to inhibit the endogenous substances in mice. Intraperitoneal injection leads to an increased level of prostaglandins in the abdominal cavity and our extract possibly inhibited the production of prostaglandins due to bioactive compounds in extract as *H. uncinata* crude extract is a mixture of several therapeutic useful compounds. The extract at 400 mg/kg dose highly significantly ($p<0.001$) inhibited the writhes in mice and the doses of 100 and 200 mg/kg significantly ($p<0.05$) inhibited acetic acid-induced writhes. While the standard drug exhibited highly significant ($p<0.001$) effects. These findings suggest that ethanol extract of *H. uncinata* has peripheral analgesic activity and mechanism of action may be mediated through the inhibition of cyclooxygenase enzymes in the peritoneal region.

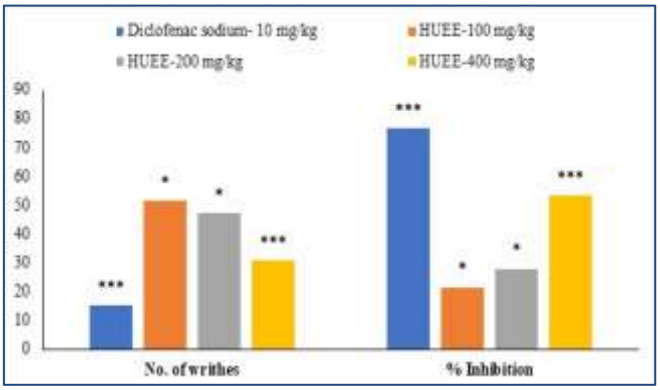

Fig. 4**.** Acetic acid-induced writhing effects of *Hackelia uncinata* ethanol extract in mice

GC-MS Analysis- GC-MS analysis was performed to identify the components present in the ethanol extract of the plant. The total number of identified components was thirty, among the identified bioactive compounds tetradecyl ester of isovaleric acid, methyl mannose, myoinositol, phthalic acid and 1,2-cyclo-hexanedicarboxylic acid ester were present in the highest concentration of 4.13%, 4.08%, 5.95%, 5.01% and 35.99% respectively (Fig. 5, Table 1.). The ethanol extract of *Hackelia uncinata* showed many compounds with biological effects such as indole derivatives are which have anti-diabetic properties [20]. The presence of fatty acids such as hexadecanoic acid and its esters help in regulating the anti-inflammatory pathways and act as inhibitors towards enzymes [21], similarly octadecanoic acid and its esters have antioxidant, antimicrobial, anti-cancerous and anti-inflammatory effects [22]. Benzamide derivatives are reported to have anti-inflammatory and analgesic properties, imidazoles have anti-cancer and anti-microbial activities. Thiazoles are one of the important classes of heterocyclic compounds from which many potent drugs have been introduced in the market. Thiazoles are effectively used to treat inflammation, allergies and various infections [23].

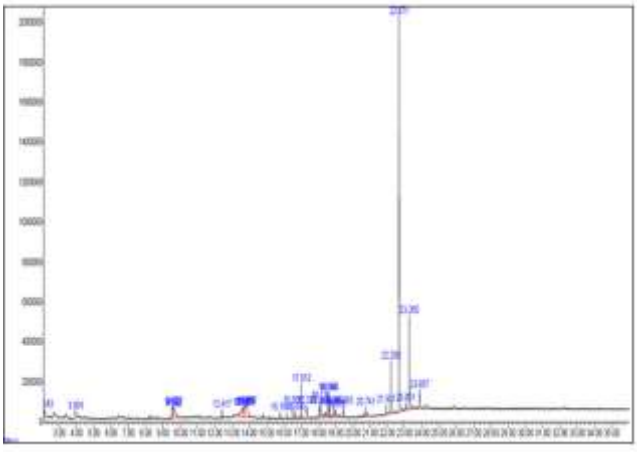

Fig 5. GC-MS Chromatogram of the whole plant of *Hackelia uncinata*

Table 1. Phytochemical analysis of ethanol extract of *Hackelia uncinata* by GC-MC analysis

No.	Compounds	RT (min)	Area	Area (%)	Mol. weight	Mol. formula
1	1-Deoxy-d-arabitol	2.143	860980	0.83	136.15	$C_5H_{12}O_4$
2	Glycerin	3.901	1839662	1.77	92.09	$C_3H_8O_3$
3	Isobutyl isovalerate	9.525	1964258	1.89	158.24	$C_9H_{18}O_2$
4	2-Oxazolidinethione	9.582	1097889	1.05	103.143	C_3H_5NOS
5	Isovaleric acid, tetradecyl ester	9.622	4300630	4.13	298.5	$C_{19}H_{38}O_2$
6	2,4-Di-tert-butylphenol	12.417	679011	0.65	206.32	$C_{14}H_{22}O$
7	4-O-Methylmannose	13.605	4251197	4.08	194.18	$C_7H_{14}O_6$
8	Neodecanoic acid	13.683	1674919	1.61	172.26	$C_{10}H_{20}O_2$
9	Thiophene, tetrahydro-2-methyl-	13.773	2753717	2.64	102.19	$C_5H_{10}S$
10	Myo-Inositol, 4-C-methyl-	13.858	6193920	5.95	194.18	$C_7H_{14}O_6$
11	4-Isopropylphenol, TMS derivative	15.788	720200	0.69	208.37	$C_{12}H_{20}OSi$
12	Cyclononasiloxane, octadecamethyl	16.506	1003377	0.96	667.38	$C_{18}H_{54}O_9Si_9$
13	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	16.695	575745	0.55	276.4	$C_{17}H_{24}O_3$
14	Hexadecanoic acid, methyl ester	17.012	2872154	2.76	270.45	$C_{17}H_{34}O_2$
15	n-Hexadecanoic acid	17.328	1756684	1.69	256.42	$C_{16}H_{32}O_2$
16	N-Benzyl-N-ethyl-p-isopropylbenzamide	18.043	968125	0.93	281.4	$C_{19}H_{23}NO$
17	Cyclohexanecarboxylic acid, 4-butyl-, 4-methoxyphenyl ester	18.131	3203339	3.08	290.4	$C_{18}H_{26}O_3$
18	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.56	1956151	1.88	294.47	$C_{19}H_{34}O_2$
19	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.594	2319779	2.23	292.45	$C_{19}H_{32}O_2$
20	9-Octadecenoic acid (Z)-, methyl ester	18.641	1021699	0.98	296.48	$C_{19}H_{36}O_2$
21	Heptadecanoic acid, 16-methyl-, methyl ester	18.902	2028789	1.95	298.5	$C_{19}H_{38}O_2$
22	N-[[2-p-Tolylsulfonyl] ethyl]phthalimide	19.016	852020	0.82	329.4	$C_{17}H_{15}NO_4S$
23	Cyclodecasiloxane, eicosamethyl-	19.455	842386	0.81	741.5	$C_{20}H_{60}O_{10}Si$
24	Cyclononasiloxane, octadecamethyl-	20.741	900207	0.86	667.38	$C_{18}H_{54}O_9Si_9$
25	1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino-	21.931	1205605	1.16	207.12	$C_8H_{13}N_7$
26	Phthalic acid, di(2-propylpentyl) ester	22.2	5216048	5.01	390.55	$C_{24}H_{38}O_4$
27	1,2-Cyclohexanedicarboxylic acid, bis(2-ethylhexyl) ester	22.671	37478747	35.99	396.6	$C_{24}H_{44}O_4$
28	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	23.051	979549	0.94	355.4	$C_{21}H_{25}NO_4$
29	4[1H]-Pyridone 2,6-dimethyl-1-[2-[4-methyl-1-piperazinyl]ethyl]-	23.265	10258557	9.85	249.35	$C_{14}H_{23}N_3O$
30	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	23.857	2367387	2.27	390.62	$C_{24}H_{38}O_4$

IV. CONCLUSION

In conclusion, the ethanol extract of the whole plants of *Hackelia uncinata* displayed anti-inflammatory and analgesic properties by inhibiting prostaglandins and cyclooxygenase. Furthermore, the results of this study also supported the traditional use of plants in pain and inflammation management. In GC-MS analysis, several compounds were identified which can be responsible for anti-inflammatory and analgesic effects. Further studies are needed to isolate and identify active compounds responsible for the suppression of inflammatory and pain mediators. Once the bioactive compounds have been isolated the mechanism of activity can be examined.

Ethical approval

Study was approved by the Animal Ethical Committee of the University of Karachi (IBC KU-212/2021).

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Conflict of interest

Authors declare no conflict of interest

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Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

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Corresponding Author – Rifat Roshan