GC-MS Phytochemical Profiling, Amelioration Of Pain And Inflammation With Chloroform Extract Of Marchantia Polymorpha L. Via Modulation Of Inflammatory Biomarkers

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Introduction

- Inflammation is a physiological litigate, take place inside a living body which is often dedicated in the excusatory reactions of the tissue trauma, perchance condign to some of the physical or caloric impairment, mechanical stultification, antigen–antibody responses.

Commonly symptoms are swelling, extreme painful sensation, loss of function of effectuated region and heat.[1}

- Bryophytes are often a group of lower green land plants without well-developed vascular systems and have dominating leafy generation. People of some of the tribes inhabiting Himalayan regions employed a mixture of moss ashes, honey, and fat as conventional folk medicines for various ailments such as burns, wounds and other skin abrasions.
- Marchantia polymorpha L. (MP) a thallus liverwort of class Hepaticae with green to brown or purple coloured, hexangular marks on ramified branches of about 10 cm long and up to 2 cm in width. It has been observed that the people of Himalayan areas make use of a mixture of ashes made from Marchantia polymorpha and Marchantia palmata plants, blended with honey and small quantity of fat for healing cuts, burns and other skin injuries .[2]
- A restrain amount of scientific examining of various indigenous bryophytic species have been done so far, particularly the Marchantia polymorpha L. fail to perceive such studies in our country. The reports about the anti-inflammatory and analgesic potentials of this species and their mechanisms of action have also been lacking in the literature.
- The present study is innovated to vindicate the folk employment of bryophytic plant species such as MP, by executing its anti-inflammatory and analgesic activeness scientifically.

Material & Methods

- The plant material was collected from the Northern areas of Pakistan. It is then dried, garbled, pulverized and placed in a glass container. The ground plant material was extracted successively with three major solvents such as n-hexane, chloroform, and methanol. Chloroform extract was further utilized.
- Phyto chemical analysis was done by GC-MS spectrometer. The results were compared with the standard NIST databse.
- Biological Assay: Invitro Anti-infammatory activity was assessed using the inhibition of albumin denaturation technique at different concentrations.
- In vivo Anti-inflammatory assay was performed using Carrageenan-induced hind paw edema (acute model) and Formalin-induced edema in rat paw (chronic model) at different dose levels (250, 500 and 750 mg/kg). The serum was then tested for the presence of serum enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH). In addition, TNF-α, IL-6, IL-4, IL-10, and hematological assays were also performed.
- Analgesic activity was performed using Hot plate and Tail flick methods at different doses (250, 500, 750 mg/kg).

Results

Table 1: GC-Mass Spectra of the Phytochemicals

{ No.	RT (Min)	Compound Name	Compound Class	Molecular Formula	Molecular Mass	Area
1	1.20	1 Methane, oxybis[dichloro		/ $_2H_2Cl_4O$		
2	5.97	Longifolenaldehyde	Aldehyde	$C_{15}H_{24}O$	220.35	0.49
3	5.97	Thunbergol		$C_{20}H_{34}O$	290.48	0.49
5	6.11	Pentadecanoic acid, methyl ester	Fatty acid ester	$C_{16}H_{32}O_2$	256.42	0.32
6	6.19	Neophytadiene	Terpene	$C_{20}H_{38}$	278.51	0.94
7	6.51	7,10,13-Hexadecatrienoic acid	Fatty acid	$C_{17}H_{28}O_2$	264.4	0.79
8	6.96	cis-Vaccenic acid	Fatty acid	$C_{18}H_{34}O_2$	282.5	0.91
9	7.12	n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256.42	1.11
10	7.70	Phytol	Diterpene alcohol	$C_{20}H_{40}O$	296.93	1.37
11	8.35	Arachidonic acid	Fatty Acid	$C_{20}H_{32}O_2$	304.47	0.85
12	8.96	Hexadecanoic acid, 2-hydroxy-1	Fatty acid	$C_{19}H_{38}O_4$	330.5	2.04
13	9.97	Fumaric acid, 3,5-difluorophenyl hexyl ester	Ester	$C_{16}H_{18}F_2O_4$	312.31	0.75
14	10.68	Squalene	Triterpene	C? ? H? ?	410.73	0.79
15	11.58	24-Norursa-3,9(11),12-triene	Hydrocarbon	$C_{29}H_{44}$	392.65	0.69
16	12.17	Vitamin e tocopherol	Vitamin	$C_{29}H_{50}O_2$	430.7	1.34
17	12.98	Campesterol	Plant sterol	$C_{28}H_{48}O$	400.68	2.87
18	13.17	Stigmasterol	Sterol	$C_{29}H_{48}O$	412.69	1.93
19	13.69	Gamma-Sitosterol	Sterol	$C_{29}H_{50}O$	414.70	2.09
20	16.11	Lupeol	Triterpenoid	$C_{30}H_{50}O$	426.71	2.02

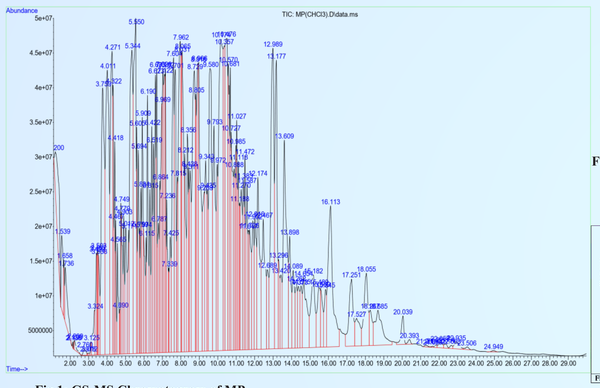

Fig 1: GS-MS Chromatogram of MP

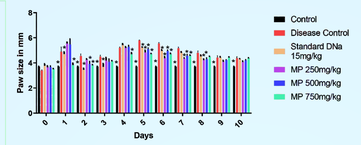

Fig 3: Paw thickness in formation-induced edema

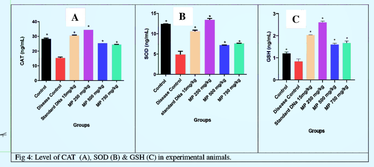

Fig 4: Level of CAT (A), SOD (B) & GSH (C) in experimental animals.

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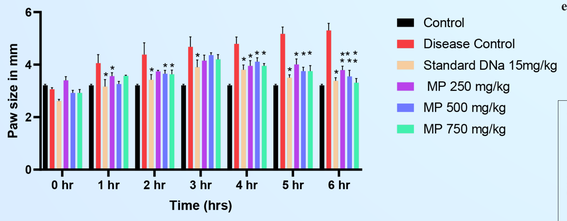

Fig 2: Paw diameter in carrageenan-induced edema

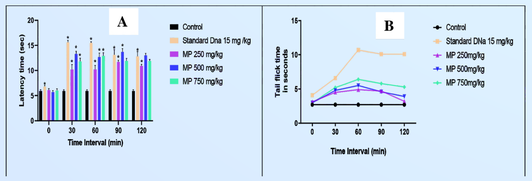

Fig 6: Latency time in different treatment groups, tail-immersion method (A), tail-fick method (B) at various time intervals

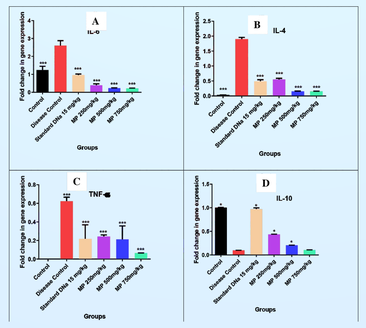

Fig 5: Genetic expression changes in IL-4 (A), IL-6 (B), TNF-a © IL-10 (D) in various treated experimental groups.

Discussion

- The phytochemical profiling by GC–MS identified presence of established anti-inflammatory and analgesic compounds like Hexadecanoic acid, Arachidonic acid, Fumaric acid Phytol, Stigmasterol, Campesterol etc Fig 1 & Table 1.
- The chloroform extract of MP inhibited protein denaturation and the results were comparable with standard diclofenac sodium. From the results of a linear response, the MP demonstrated significant in vitro anti-inflammatory activity.
- Results showed that MP reduced the inflammation caused by carrageenan which may have blocked the production of prostaglandins in the second phase of acute inflammation (Fig. 2).
- Chronic inflammation was induced with formalin. Antioxidant enzymes i.e. CAT, SOD and GPx are the most significant enzymes that protect cells from oxidative damage. Results showed that MP treatment significantly upregulated antioxidant enzymes and protects against oxidative damage in chronic inflammation (Fig. 3 & 4).
- Inflammatory mediators were also downregulated with prior MP treatment as compared to the diseased group. The levels of inflammatory mediators including IL-6, IL-4 and TNF-α were declined and IL-10 was increased (Fig. 5).
- Hematological findings supported the protective effect of MP on different parameters like RBC, hemoglobin, WBC and C-reactive protein.
- Hot-plate and tail-flick assays were employed to evaluate the central analgesic effectiveness of MP. These are acute models of pain and both types of stimuli induce pain by heat mediated damage of tissues and are selective for chemicals like opioids. Results revealed that mice treated with doses of 250, 500, and 750 mg/kg MP experienced a significant analgesic effect (Fig. 6).

Conclusion

The current phytochemical and biochemical investigations point to the potential analgesic and anti-inflammatory action of MP. Therefore, it can be concluded that this plant will make a viable choice for the treatment of illnesses linked to inflammation and pain. However, further investigation of molecular targets reducing inflammation may be elucidated in future.

Refrences

[1] Bouyahya A, Guaouguaou FE, El Omari N, El Menyiy N, Balahbib A, El-Shazly M, Bakri Y (2022) Anti-infammatory and analgesic properties of Moroccan medicinal plants: phytochemistry, in vitro and in vivo investigations, mechanism insights, clinical evidences and perspectives. J Pharm Anal 12(1):35–57

[2] Sabovljevic MS, Vuji i M, Wang X, Garraffo HM, Bewley CA, Sabovljevi A (2017) Production of the macrocyclic bis-bibenzyls in axenically farmed and wild liverwort Marchantia polymorpha L. subsp. ruderalis Bischl. et Boisselier. Plant Biosyst 151(3):414–418