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ORIGINAL ARTICLE

Seabuckthorn (*Hippophae rhamnoides* and *Hippophae salicifolia*) Seed Oil in Combating Inflammation: A Mechanistic Approach

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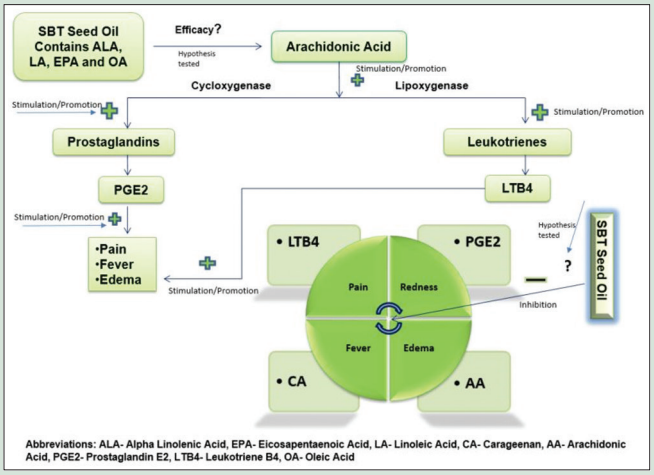
ABSTRACT

Objective: This study assessed and compared *in vivo* anti-inflammatory activity of *Hippophae rhamnoides* (HR) and *Hippophae salicifolia* (HS) seed oil. **Materials and Methods:** HR and HS seed oil was extracted by Soxhlet apparatus and characterized using gas chromatography mass spectroscopy. Wistar rats were used for predicting anti-inflammatory activity. **Results:** HR and HS (2 and 4 ml/kg, respectively) exhibited dose-dependent inhibition of carrageenan-, histamine-, prostaglandin-, bradykinin-, and arachidonic acid (AA)-induced paw edema. Significant leukotriene-induced inhibition was observed in HR. Myeloperoxidase (MPO) and lipid-peroxidase (LPO) assays were performed and HR and HS seed oil significantly decreased the level of MPO and LPO at 4 ml/kg dose ($P < 0.001$). **Conclusion:** Dual inhibition of AA metabolism in HR and cyclooxygenase inhibition in HS was observed that might be attributed to the presence of polyunsaturated fatty acids (PUFAs), specifically, a correct balance of n-3 and n-6 PUFAs. However, the findings should be interpreted in the light of limitation of this study. Detailed experimentation at enzymatic levels would further help in substantiating the results inferred in this study.

Key words: Antibradykinin, antihistaminic, arachidonic acid, leukotriene, polyunsaturated fatty acid, prostaglandin

SUMMARY

- In summary, this study provides an important insight into the anti-inflammatory potential of *Hippophae rhamnoides* and *Hippophae salicifolia* seed oil. This study not only evaluates and compares the potential of these seed oil but also provides a plausible mechanistic and biochemical explanation behind the therapeutic potential.



Abbreviations Used: AA: Arachidonic acid, ALA: Alpha linolenic acid, COX: Cyclooxygenase, EPA: Eicosapentaenoic acid, GCMS: Gas chromatography-mass spectroscopy, HR: *Hippophae rhamnoides,* HS: *Hippophae salicifolia,* ip: Intraperitoneal, LOX: Lipoxygenase, LPO: Lipid peroxidase, LTB_4: Leukotriene B_4, LTs: Leukotrienes, MPO: Myeloperoxidase, PGE_2: Prostaglandin E_2, PGs: Prostaglandins, PUFA: Poly unsaturated fatty acid, SBT: Seabuckthorn

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INTRODUCTION

Arachidonic acid (AA) (20:4 n-6) is one of the most influential poly unsaturated fatty acids (PUFAs) in positively modulating the inflammatory process.[1] Dietary long-chain n-3 PUFAs can decrease tissue AA levels and eicosanoid production, both *in vitro* and *in vivo.* This decrease results in alteration of plasma phospholipid fatty acid composition. In addition, n-3 PUFAs increase competition for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby decreasing pro-inflammatory prostaglandins (PGs) and leukotrienes (LTs), which subsequently can result in anti-inflammatory activity.[2] Treatment of inflammation includes extensive use of nonsteroidal anti-inflammatory drugs (NSAIDs). However, their use makes the patients more vulnerable to gastrointestinal and liver toxicities.[3,4] Therefore, herbal anti-inflammatory treatments having alternative mechanisms are preferred as substitutes to NSAIDs.

Seabuckthorn (SBT) is a parasol term used for most of the plant species of genus *Hippophae*, family Elaeagnaceae. Two of the most prominent Indian species of this plant are *Hippophae rhamnoides* (HR) and *Hippophae salicifolia* (HS), henceforth collectively referred to as SBT.[5] Previous studies have revealed the presence of considerable amount of n-3 and n-6 fatty acids, namely, alpha linolenic acid (ALA), linoleic acid (LA), and oleic acid (OA) in HR and HS seed oil which are precursors of other PUFAs such as AA and eicosapentaenoic acid (EPA) in SBT seed oil.[6,7] In view of the above, this study aimed to evaluate and

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compare the anti-inflammatory potential of HR and HS seed oil with standard of care. In addition, this study aimed to assess the probable role of HR and HS in AA inflammatory cascade.

MATERIALS AND METHODS

The details about the procurement of plant material, SBT seed oil extraction, characterization of SBT seed oil, physicochemical characteristics, and preparation of the sample have been reported in our previous study.[8] National Botanical Research Institute, Lucknow, via letter number NBRI/CIF/494/2015 and NBRI/CIF/528/2016, authenticated the HR and HS seeds, respectively, and seed oil was extracted using Soxhlet apparatus by slightly modifying the method of Cenkowski *et al.*, 2006.[9] To characterize the HR and HS seed oil, physicochemical and fatty acid profiling was performed. Wistar strain albino rats (142–150 g) were procured from Central Animal House facility of SHIATS, Allahabad. Animal Ethical Committee (IAEC/SHIATS/212) endorsed the experimental protocol.

Anti-inflammatory activity was performed using slight modifications in methods described by Kaithwas *et al.*, 2011.[10] Various phlogistic agents were used to induce paw edema, against their specific standard of care and the test, for drawing an inference about the plausible mechanism of action of HR and HS seed oil. Myeloperoxidase (MPO) and lipid peroxidase (LPO) assays were performed using slight modifications in methods described by Morumpudi *et al.*, 2014[11] and Ohkawa *et al.*, 1979.[12]

Statistical analysis was carried out using Graph pad prism software (5.0) (San Diego, CA). Data were presented as mean ± standard error of mean and analyzed by one-way ANOVA followed by Bonferroni/ Student's Newman test. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant.

RESULTS

The density, specific gravity, color, iodine value, acid value, and saponification value of HR and HS seed oil were measured for characterization and qualitative assessment of the samples. The gas chromatography-mass spectroscopy (GCMS) analysis of HR seed oil confirmed the presence of palmitoleic acid (6.72%), eicosanoic acid (11.18%), ALA (24.2%), and LA (39.71%). Around 34.93% of 9-octadecenoic acid, methyl ester (E), OA, and 26.7% ALA were found in HS.

The results of carrageenan-induced paw edema evinced a statistical ($P < 0.05$) significance among all the study groups when compared using Bonferroni multiple comparison test [Table 1]. HR and HS seed oil (2 and 4 ml/kg, each) exhibited dose-dependent inhibition of carrageenan (HR [39.7%, 76.9%]; HS [42.6%, 80.8%] $P < 0.05$)-, histamine (HR [55.6%, 64.5%]; HS [46.1, 46.1%] $P < 0.05$)-, PG (HR [19.1%, 73.5%]; HS [42.6%, 80.8%] $P < 0.05$)-, bradykinin (HR [71.0%, 78.3%]; HS [67.4%, 74.4%] $P < 0.05$)-, and AA (HR [61.4%, 41.6%]; HS [44.9%, 40.1%] $P < 0.05$)-induced paw edema. Significant LT-induced inhibition (HR [63.0%, 75.3%]; HS [12.3%, 9.6%] $P < 0.05$) was observed in HR.

The LT-induced paw edema model had a contrasting result as compared to other models. HR exhibited a statistically significant percentage inhibition of paw edema (63.01%; 75.34%; at 2 and 4 ml/kg of the dose, respectively) at all the doses as compared to HS (12.32%; 09.58%; at 2 and 4 ml/kg of the dose, respectively) which did not exhibit any significant difference ($P > 0.05$). These results were compared to normal control and positive control (ketoconazole [LT antagonist]) and the difference among groups was found to be statistically significant ($P < 0.05$).

The cumulative table for anti-inflammatory models showed consistent paw edema inhibition at 4 ml/kg dose for HR and HS seed oil. Percentage inhibition in aspirin (against PG, LT, and bradykinin-induced paw edema), chlorpheniramine maleate (against AA, histamine, and bradykinin-induced paw edema), cyproheptadine chloride (against AA-induced paw edema), and ketoconazole (against LT- and AA-induced paw edema) was statistically different ($P < 0.05$). Comparative analyses of both the doses across all phlogistic agents are illustrated in Figure 1. The results of biochemical estimation revealed that HR and HS significantly decreased the level of MPO and LPO at 4 ml/kg dose ($P < 0.001$) [Figure 2].

DISCUSSION

This study delineated the effect of HR and HS seed oil on overall inflammatory cascade with the objective of providing an insight into the plausible mechanism of action. The results of density, specific

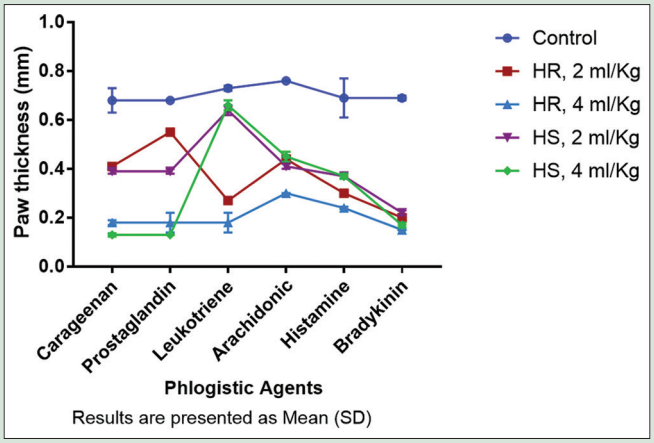

Figure 1: Effect of study groups on mean paw thickness against various phlogistic agents; results are presented as mean (standard deviation)

Table 1: Percentage inhibition of paw edema amongst study groups

	Aspirin	CPR	CHD	KTZ	HR (2 ml/kg)	HR (4 ml/kg)	HS (2 ml/kg)	HS (4 ml/kg)
Carrageenan	-	-	-	-	39.7*	76.9*	42.6*	80.9*a
Prostaglandin	79.2*	-	-	-	19.1	73.5*	42.6*[a]	80.9*[a]
Leukotriene	32.3*	-	-	82.2*	63.0*	75.3*	12.3[a]	09.6[a]
Arachidonic Acid	13.2	63.7*	71.6*	61.4*	41.6*	59.5*	44.9*[a]	40.1*[a]
Histamine	-	72.7*	-	-	55.5*	64.5*	46.1*[a]	46.1*[a]
Bradykinin	52.7*	64.2*	-	-	71.0*	78.3*	67.4*[a]	74.4*[a]

CPR, chlorpheniramine; CHD, cyproheptadine; KTZ, Ketoconazole. *Statistical significance for all experimental groups vs. control ($P<0.05$) by Bonferroni multiple comparison test. [a]Statistical significance for all dosage of HR vs. HS ($P<0.05$) by Bonferroni multiple comparison test where each dose of HR was compared to its equivalent dose in HS. The dosage of the study groups are as follows: control (1.5 ml/kg tween 80 + 1.5 ml/kg normal saline (ip); Aspirin, 100 mg/kg; Chlorpheniramine, 25 mg/kg; Cyproheptadine, 25 mg/kg; Ketoconazole, 14 mg/kg)

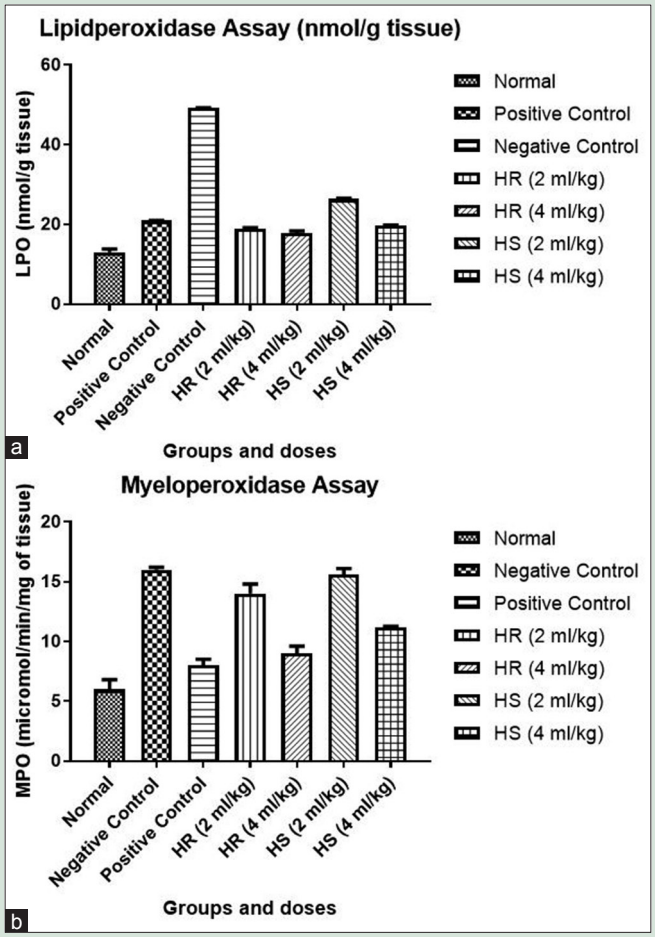

Figure 2: (a) Effect of *Hippophae rhamnoides* and *Hippophae salicifolia* seed oil on myeloperoxidase levels. (b) Effect of *Hippophae rhamnoides* and *Hippophae salicifolia* seed oil on lipid peroxidase levels. Results are expressed as mean ± standard deviation unless otherwise specified. *Post hoc* analysis was carried out using Bonferroni multiple comparison test

gravity, and other parameters such as color, iodine value, acid value, and saponification value of both the seed oil samples were in corroboration with the previous studies.[13,14] The result of GCMS profiling is discussed in detail in our previously published study.[8] The corroboration of GCMS findings with previous reports substantiates the characterization and authentication of obtained oil samples.

There has been a lot of focus on fixed oils in the recent past in terms of analyzing their therapeutic potential since these are rich in PUFAs. Our study makes a holistic effort to analyze the mechanism behind the anti-inflammatory potential of HR and HS seed oil. SBT seed oil contains the 18-carbon n-3 fatty acid, ALA, which could be converted after ingestion into the 20-carbon n-3 fatty acid, EPA. EPA may act as a competitive inhibitor of AA conversion to PGE_2 and LTB_4, thereby decreasing the synthesis of one or both of these eicosanoids. Similar to the effect of n-3 fatty acids, inclusion of the 20-carbon n-9 fatty acid, eicosatrienoic acid, and OA in the diet also results in decreased synthesis of LTB_4.[15,16]

Carrageenan is a nonspecific phlogistic agent and does not help in comprehending any specific phase inhibition. In addition, it does not provide any insight as to how the anti-inflammatory action is mechanized. In the biphasic response of carrageenan, the first phase is associated with the release of histamine, serotonin, and bradykinin. The second phase is attributed to the overproduction of PG and release of bradykinin, protease, and lysosomal enzymes in tissues.[17] Hence, we focused on subsequently released mediators that induced inflammation to understand the overall pattern of response.

The oil significantly inhibited the LT-, histamine-, and bradykinin-induced inflammation, whereas, aspirin, a COX inhibitor, failed to inhibit LT and bradykinin-induced inflammation. Tissue inflammation originates from AA, which is metabolized by COX and LOX pathways. Activation of COX pathway produces PGs, while LOX pathway yields LTs, and both PGs and LTs are pro-inflammatory. Both HR and HS oil inhibited inflammation caused by exogenous PGs. Only HR inhibited LOX pathway.

Since AA is a substrate for production of PGs and LTs, the effect of oil was evaluated against inflammation induced by AA. In AA-induced edema, significant edema inhibition was observed with the oil, chlorpheniramine, cyproheptadine, and ketoconazole, while aspirin did not inhibit the edema formation, and the inhibitory effect of oil was much greater than the rest. The edema inhibition by chlorpheniramine (antihistaminic) and cyproheptadine (antihistaminic/antiserotonin agent) appears to be due to inhibition of mast cell mediator release, indicating that mast cell mediator release may partly contribute toward AA-induced paw edema. The results suggest that HR is a dual inhibitor of AA metabolism. HS specifically gives motivating results as a specific cox inhibitor. Further, antihistaminic and antibradykinin effects of the oil, also, could contribute toward anti-inflammatory effect.

Evidently, MPO, a hemoprotein abundantly expressed by polymorphonuclear neutrophils and secreted during activation, possesses potent pro-inflammatory properties and may contribute directly to tissue injury.[18] Zhang *et al*., 2002, demonstrated a principal role for MPO in the promotion of oxidant stress at sites of inflammation.[19] The significant decrease by HR and HS seed oil in MPO levels suggests that the fixed oil may have additional anti-inflammatory action as well. Generation of LPO is also known to cause inflammation.[20] LPO levels were decreased by HR and HS seed oil. A majority of studies do not indicate that n-3 PUFAs increased LPO.[21] A diet rich in n-3 PUFAs could balance reactive substances under low oxidative conditions.[22] HR and HS seed oil, being rich in n-3 PUFAs, decreases the LPO levels across all doses (2 and 4 ml/kg, respectively).

CONCLUSION

Our study results show that HR and HS seed oil, at 4 ml/kg intraperitoneal dose, responds positively against all the inflammatory mediators except HS in LT-induced inflammatory response. This may be due to lack of positional specificity of the constituents in HS. This study also substantiates the role of PUFAs in HR and HS seed oil in mediating inflammation. However, further investigation is required to comment on this finding more precisely.

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Conflicts of interest

There are no conflicts of interest.

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