Anti-inflammatory Effects of Hydroalcoholic Extract of *Berberis chitria* Roots in Experimental Animals

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Abstract :

The aim of the study is to evaluate the anti-inflammatory effect of root extract of *Berberis chitria* Linn. by carrying out the pharmacological studies with the hydroalcoholic extract of root of *Berberis chitria* Linn. While over-the-counter painkillers can be really helpful, and in some cases may be the best option, they can also cause gastrointestinal upset and definitely aren't the best long-term strategy for coping with chronic pain. The goal is, of course, to try and help relieve pain by developing a new herbal remedy for the inflammatory action. Among the various Complementary and alternative medicine, "herbal medicine" is the most popular and fastest growing approach used to treat various ailments worldwide. The key to reduce chronic inflammation in our body starts with our diet and being liberal in the use of high-quality herbs and spices is one simple way to boost the quality of our food.

KEYWORD : chronic inflammation , anti-inflammatory effect, hydroalcoholic extract.

Introduction :

• **Inflammation:** Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues.

Thus, inflammation is distinct from infection—while inflammation is a protective response by the body to variety of etiologic agents (infectious or non-infectious), while infection is invasion into the body by harmful microbes and their resultant ill effects by toxins. Inflammation involves the two basic processes with some overlapping, viz. early inflammatory response and later followed by healing..

• **Types of inflammation**

Depending upon the defense capacity of the host and duration of response inflammation can beclassified as acute and chronic.

• **Acute inflammation**

Its denotes, short duration (lasting less than 2 weeks) and represents the early body reaction, resolves quickly and is usually followed by healing. The main features of acute inflammation are:

- Accumulation of fluid and plasma at the affected site;
- Intravascular activation of platelets; and
- Polymorphonuclear neutrophils as inflammatory cells.

Sometimes, the acute inflammatory response may be quite severe and is termed as fulminant acuteinflammation.

Table 01-Mediators of acute inflammation

Mediators	**Vasodilation**	**Vascular permeability**	**Chemotaxis**	**Pain**
Histamine	++	+++	-	-
Serotonin	+	+	-	-
Bradykinin	+++	+	-	+++
Prostaglandin	+++	+	+++	+
Leukotrienes	-	+++	+++	-

(+++ severe, ++ moderate, + mild, - absent)

- **Chronic inflammation**

Its have longer duration and occurs either after the causative agent of acute inflammation persists for a long time, or the stimulus is such that it induces chronic inflammation from the beginning. Avariant, chronic active inflammation is the type of chronic inflammation in which during the course of disease there are acute exacerbations of activity. The characteristic feature of chronic inflammation is presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages, granulation tissue formation, and in specific situations as granulomatous inflammation. In some instances, the term subacute inflammation is used for the state of inflammation between acute and chronic. [12]

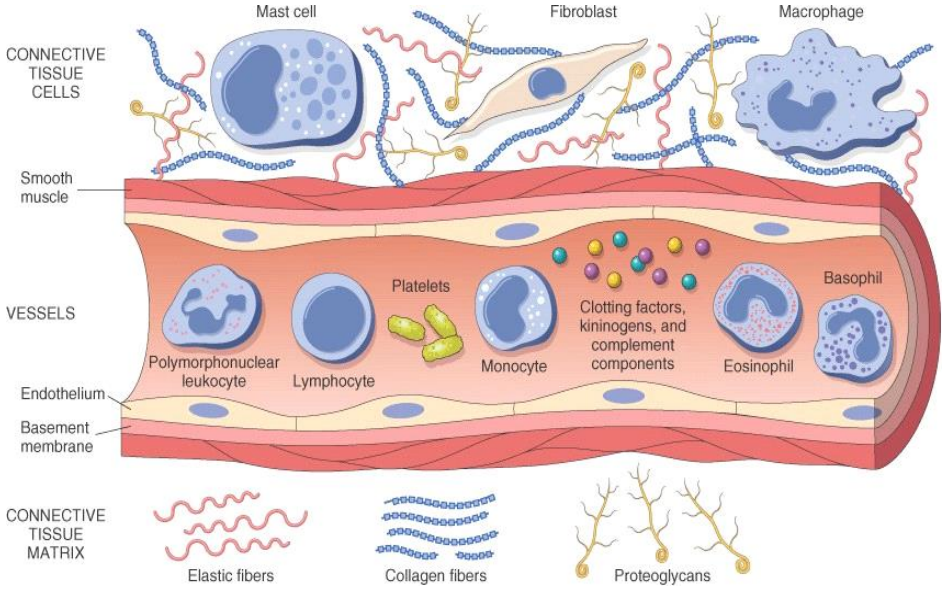


Figure 1: The components of acute and chronic inflammatory responses

Table 02: Mediators of chronic inflammation

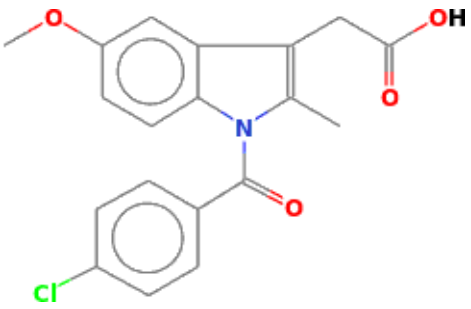
Mediators	**Sources**	**Primary effects**
IL-1, IL-2, IL-3	Macrophages, T-lymphocytes	Lymphocyte activation, prostaglandin production
GM-CSF	T-lymphocytes, endothelial cells, Fibroblast	Macrophages and granulocyte Activation
TNF-α	Macrophages	Prostaglandin
Interferons	Macrophages, endothelial cells, T- lymphocytes	Many
PDGF	Macrophages, endothelial cells, fibroblast, platelets	Fibroblast chemotaxis, Proliferation

- **DRUG PROFILE**

- **Indometacin**

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic properties. NSAIDs consist of agents that are structurally unrelated; the NSAID chemical classification of indometacin is an indole-acetic acid derivative with the chemical name 1- (p-chlorobenzoyl)25-methoxy-2-methylindole-3-acetic acid. [55]

Structure:



Molecular formula: $C_{19}H_{16}ClNO_4$

Molecular weight: 357.788

IUPAC Name: 1- (p-chlorobenzoyl) 25-methoxy-2-methylindole-3-acetic acid

- **Pharmacodynamics**

Indometacin is an NSAID with analgesic and antipyretic properties that exerts its pharmacological effects by inhibiting the synthesis of factors involved in pain, fever, and inflammation. Its therapeutic action does not involve pituitary-adrenal stimulation. Indometacin primarily works by suppressing inflammation in rheumatoid arthritis by providing relief of pain as well as reducing fever, swelling, and tenderness. This effectiveness has been demonstrated by a reduction in the extent of joint swelling, the average number of joints displaying symptoms of inflammation, and the severity of morning stiffness. Increased mobility was demonstrated by a decrease in total walking time and by improved functional capability seen as an increase in grip strength.

- **Mechanism of action**

Indometacin is a nonspecific and reversible inhibitor of the cyclo-oxygenase (COX) enzyme or prostaglandin G/H synthase. There are two identified isoforms of COX: COX-1 is universally present in most body tissues and is involved in the synthesis of the prostaglandins and thromboxane A2, while COX-2 is expressed in response to injury or inflammation. Constitutively expressed, theCOX-1 enzyme is involved in gastric mucosal

protection, platelet, and kidney function by catalyzing the conversion of arachidonic acid to prostaglandin (PG) G2 and PGG2 to PGH2. COX- 2 is constitutively expressed and highly inducible by inflammatory stimuli. It is found in the centralnervous system, kidneys, uterus, and other organs. COX-2 also catalyzes the conversion of arachidonic acid to PGG2 and PGG2 to PGH2. In the COX-2-mediated pathway, PGH2 is furtherconverted to PGE2 and PGI2 (also known as prostacyclin). PGE2 is involved in mediating inflammation, pain, and fever. [56]

- **Absorption**

Indometacin displays a linear pharmacokinetics profile where the plasma concentrations and area under the curve (AUC) are dose-proportional, whereas half-life (T1/2) and plasma and renal clearance are dose-dependent. Indometacin is readily and rapidly absorbed from the gastrointestinal tract. The bioavailability is virtually 100% following oral administration and about 90% of the dose is absorbed within 4 hours. The bioavailability is about 80-90% following rectal administration.

- **Volume of distribution**

The volume of distribution ranged from 0.34 to 1.57 L/kg following oral, intravenous, or rectal administration of single and multiple doses of indometacin in healthy individuals. Indometacin is distributed into the synovial fluid and is extensively bound to tissues. It has been detected in humanbreast milk and placenta. [57]

- **Protein binding**

Indometacin is a weak organic acid that is 90-99% bound to protein in plasma over the expected range of therapeutic plasma concentrations. Like other NSAIDs, indometacin is bound to plasma albumin but it does not bind to red blood cells.

- **Metabolism**

Indometacin undergoes hepatic metabolism involving glucuronidation, O-desmethylation, and N- deacylation. O-desmethyl-indomethacin, N-deschlorobenzoyl-indomethacin, and O-desmethyl-N- deschlorobenzoyl-indomethacin metabolites and their glucuronides are primarily inactive and have no pharmacological activity.

- **Route of elimination**

Indometacin is eliminated via renal excretion, metabolism, and biliary excretion. It is also subject to enter the enterohepatic circulation through excretion of its glucuronide metabolites into bile followed by resorption of indometacin after hydrolysis.

- **Half-life**

Indomethacin disposition from the plasma is reported to be biphasic, with a half-life of 1 hour during the initial phase and 2.6–11.2 hours during the second phase.

- **Toxicity**

Acute oral LD50 is 2.42 mg/kg in rats and 13 mg/kg in mice. The oral LD50 of indomethacin in mice and rats (based on 14-day mortality response) was 50 and 12 mg/kg, respectively. [58]

Plant Profile :

Fig No 2 *Berberis chitria*

Table no 3 Morphological characteristics of *Berberis chitria* roots

S. No.	Character	Observation
1	Color	Greenish
2	Odor	None
3	Taste	Characteristic
4	Size	4-7 cm. length

- **Collection and Authentication of plant**

Berberis chitria roots were collected from Sanjeevani Gardan, Bhopal the state of Madhya Pradesh during the month of March. The plant has been identified and authenticated by Dr. Saba Naaz Head of the Department Botany at the Safia college of science, Bhopal (M.P.)

- **Drying, size reduction and storage of plant material**

The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This materialwas used for the further study.

- **Preparation of *Berberis chitria* roots extract**

Extraction of *Berberis chitria* was done by Soxhlet extraction method. [49]

Soxhlet Extraction: The coarse powder was packed tightly in the soxhlet apparatus and extracted with 99% ethanol and distilled water for 72 hours with occasional shaking maintained at 78°C throughout the extraction process. The extract was concentrated to ¼ of its original volume by evaporation. The resulting extract of *Berberis chitria* was subjected to phytochemical study.

Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Figure 3- Soxhlet Extraction of plants parts

- **Consistency and color:**

Table 04- Consistency and color of roots part of *Berberis chitria*

Extract	Color	Consistency
Ethanolic	Blackish	Semi solid

- **Practical & Percentage Yield:** In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used.

Table 05- Percentage yield of roots part of *Berberis chitria*

S. No	Extracts	Yield (gm)	Percentage Yield
1	Hydroalcoholic	11.50	13.25 %

Quantitative Phytochemical Estimation

- **Phytochemical screening:**

There is a presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins and saponins inhydroalcoholic extract of *Berberis chitria roots* part.

Table 06- Phytochemical screening of ethanolic extract of roots part of *Berberis chitria*

S. No.	Identification Test	Test name	Results
1	Alkaloids	Mayer's test	-
		Dragendroff's test	+
		Wagner's test	+
2	Glycosides	Killer-killani test	+
3	Carbohydrates	Molisch's test	+
		Fehling test	+
4	Tannins & Phenols	Gelatin test	+
		Ferric chloride test	+
5	Flavonoids	Shinoda test	+
		Alkaline reagent test	+
6	Steroids	Libermamm-Burchard test	+
		Salkowski test	+
7	Saponins	Foam test	+
8	Protein	Xanthoprotic	+
9	Gums & Mucilage	With 95% alcohol	-

TPC

The total phenolic content of *Berberis chitria* extract was determined using the Folin-Ciocalteu Assay. The *Berberis chitria* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mLwith distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction.This blue colour was measured spectrophotometrically.

Table 07- Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.155
2.	40	0.180
3.	60	0.198
4.	80	0.226
5.	100	0.275

Absorbance :

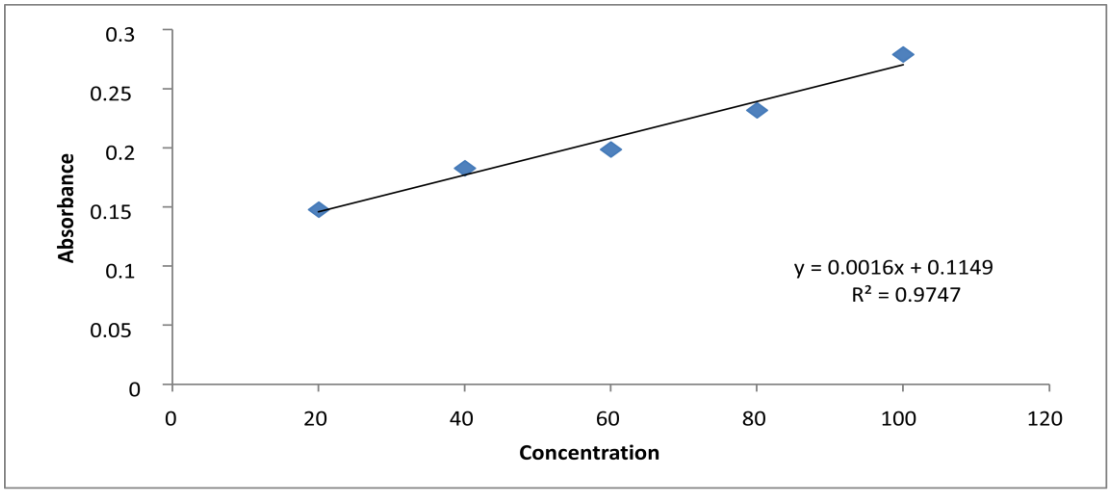


Table 08: Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.135	49.33 mg/gm
2	0.182	
3	0.194	

Table 09 -Total Phenolic Content of extract *Berberis chitria*

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Hydroalcoholic	49.33

TFC

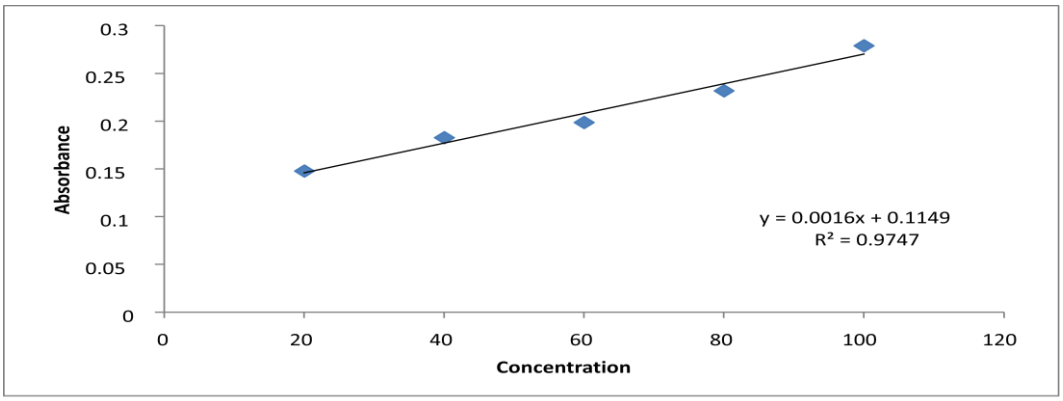
The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Berberis chitria* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%)was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared

- **Total Flavonoids content (TFC) estimation**

Table 10- Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.182
2.	40	0.205
3.	60	0.283
4.	80	0.325
5.	100	0.337

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.483	51.457
40	0.435	56.281
60	0.339	65.929
80	0.287	71.155
100	0.144	85.527
Control	0.995	
IC50		21.30



Total Flavonoid Content in extract

Table 11: **Total Flavonoid Content**

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.155	18.16 mg/gm
2	0.168	
3	0.197	

Table 12 : **Total Flavonoid Content of extract *Berberis chitria***

Extracts	Total Flavonoid content (mg/gm equivalentof rutin)
Hydroalcoholic	18.16

DPPH

The antioxidant activity of *Berberis chitria* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared.Different concentration of *Berberis chitria* extracts /standard (20 – 100µg/ml) were prepared from 1mg/mL stock solution and 2mL of 0.1mM solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using UV spectrophotometer (Shimadzu 1700) at 517 nm. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm**.**

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]

Graph 03: DPPH radical scavenging activity of Std. Ascorbic acid

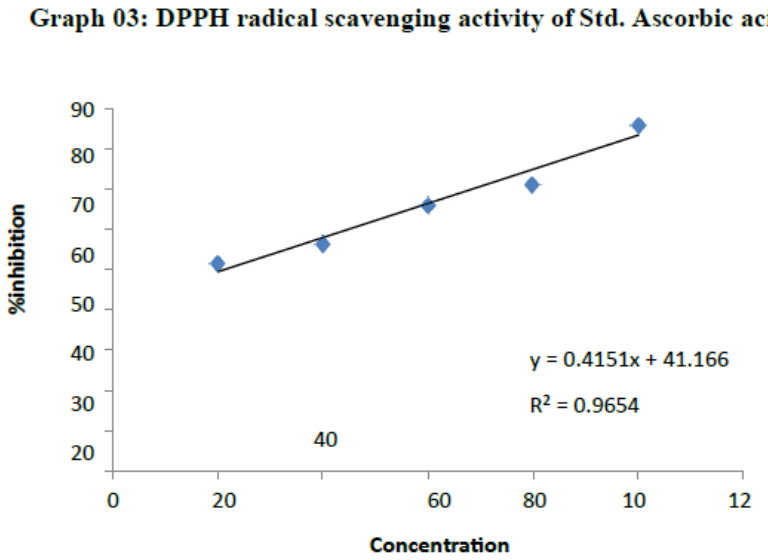
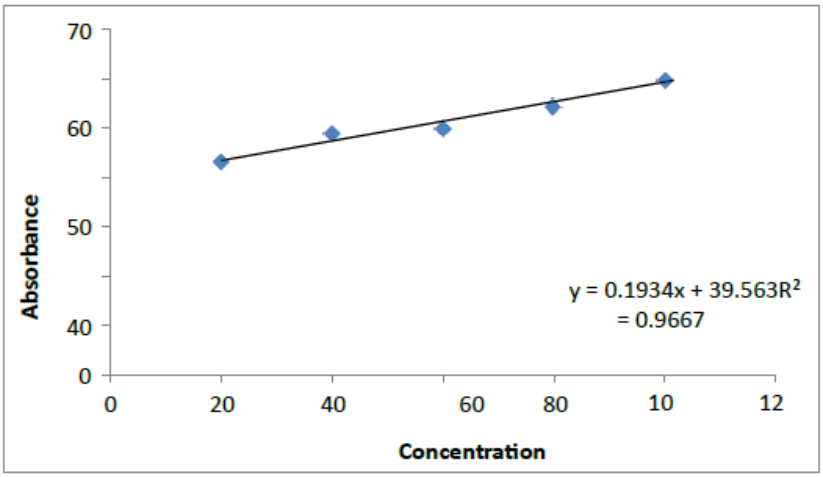


Table 13: DPPH radical scavenging activity of methanol extract of *Berberis chitria*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.521	43.060
40	0.468	48.852
60	0.458	49.945
80	0.418	54.316
100	0.369	59.672
Control	0.915	
IC50		54.09

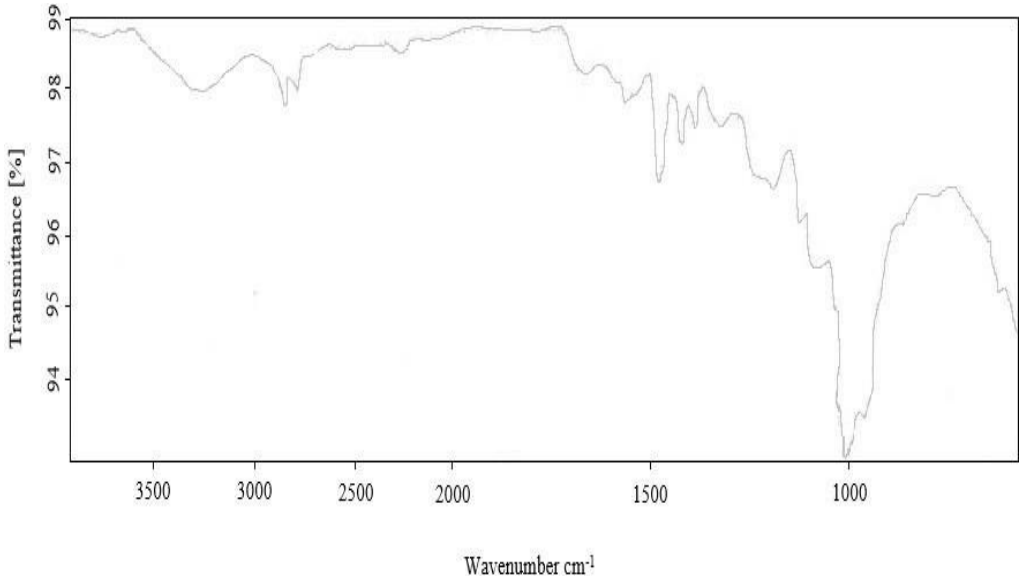
Graph 04: Represents the Percentage Inhibition *Vs* Concentration of extract of *Berberis chitria*



- **FT-IR**

The IR spectra of *Berberis chitria* powder confirmed the presence of berberine (Figure). The IR spectrum gave absorption at 1032 cm^{-1} and small peak is observed at 1103 cm^{-1} indicating the presence of (O-CH_2-O) group. A peak at 1442.75 cm^{-1} in *Berberis aristata*, showed CH bending. A major peak was seen at 1597.6 cm^{-1} in *Berberis aristata* powder indicates aromaticC-C stretching. A small peak was observed at 2826.8 cm^{-1} in powder indicates C-H stretching in aromatic functional group. A standard berberine

hydrochloride sample was also analyzed and it showed a similar spectrum indicating the presence of berberine in the sample



Graph 05: FTIR of *Berberis chitria*

Acute Toxicity Study

The acute oral toxicity study was performed according to OECD 423 guidelines. A single oral administration of a starting dose of 2000 mg/kg body weight, of hydroalcoholic extract of roots of *Berberis chitria* Linn. (HEBC) was administered to 3 male rats and observed. There was no lethality, mortality or any toxic reactions found at any selected dose level until the end of the study period. The results of acute oral toxicity studies are shown in Table.

Table 14- Results of Acute oral toxicity study of HEBC

Group name	**Animal mark**	**Dose mg/kg**	**y weight(gm)**			**Observa -tion**	**Mortality (If any)**
			1 Day	**7 Days**	**14 days**		
Control	H	Normal saline (0.91%)	153	148	146	No sign of toxicity & al. l animals Survive	No mortality occurs
	B		155	150	152		
	T		138	135	132		
Test	HT	2000 mg/kg of extract	205	208	202		
	BT		190	185	180		

	NM	(Once dosing at start of acute oral toxicity study)	175	165	168	d.	

- **Experimental work**

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC).

Animal used

Weight 200±250 gm

Strain Rat

Sex Male

Housing condition

Male rat aged 2-3 months and weighing 200-250 were taken for the study. The animals were maintained in standard animal house conditions with ad libitum access to food and water under a 12 hour light dark cycle prior to treatment.

- **Experimental**

Male Wistar rats weighing 200-250 g are used for animal studies, divided into group of 6 animals per cage. The animals were grouped in polyacrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2°C) and relative humidity (50 ±5 %) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Bangalore, India) and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC). The rats were acclimatized to laboratory condition for 14 days before commencement of experiment. The animals were fed with Gold Mohar commercial feed manufactured by Hindustan Lever Limited, Bangalore.

Chemicals

Test drug Carrageenan, Standard drug Indomethacin

- **Methodology**

Group I: Served as control (received normal saline). Group II: Rats were received 0.1 ml of 1 % carrageenan. Group III: Rats were received indomethacin (10 mg/kg/p.o) Group IV: Rats were received 1.0ml of hydroalcoholic extract of roots of *Berberis chitria* Linn. (200 mg/kg/p.o) and 0.1 ml of carrageenan. Group V: Rats were received 1.0ml hydroalcoholic extract of roots of *Berberis chitria* Linn. (400 mg/kg/p.o) and 0.1 ml of carrageenan.

Carrageenan Induced Inflammation

Acute inflammation or edema was induced by injection of 0.1 ml of carrageenan (1 % in 0.9 % sterile saline solution) into the rat's sub plantar surface of right hind paw region. The vehicle was administered 30 min. prior to injection of carrageenan and indomethacin was orally administered 1h prior to the injection of carrageenan. The pedal volume up to the ankle joint was measured using a digital plethysmometer at 1st, 2nd, 3rd, 4th, 5th and 6th h.

The percentage inhibition of edema volume between drug treated and carrageenan alone treated groups were calculated as follows:

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc-Vt and Vc represented the mean increase in paw edema volume in control and drug treated groups.

Table: 15 Animals are grouped into five categories which are as follows :

S.NO	GROUPS	TREATMENT	ROUTE
I	CONTROL	1.0 ml (Normal saline) –	p.o
II	CARRAGEENAN	0.1 ml (1% in 0.9% sterile saline solution)	i.p
III	INDOMETHACIN + CARRAGEENAN	10 mg/kg	p.o
IV	HEBC 200 mg/kg + CARRAGEENAN	1.0 ml	p.o
V	HEBC 400 mg/kg + CARRAGEENAN	1.0 ml	p.o

Measurement of Paw Edema

Table 19 indicates that the change which occurs due to the treatment of hydroalcoholic extract of roots of *Berberis chitria* Linn. in carrageenan induced paw edema by measuring the displacement value of mercury in plethysmometer.

Table 16: Effect of hydroalcoholic extract of roots of *Berberis chitria* Linn. on carrageenan induced paw edema

Group	Initial Paw Volume	Paw volume After Induction(ml) as measured by mercury displacement at					
		1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
I	1.180 ±	1.180 ±	1.180 ±	1.180 ±	1.180 ±	1.180 ±	1.180 ±
	0.005	0.005	0.005	0.005	0.005	0.005	0.005
II	1.193 ±	1.908 ±	2.253 ±	2.368 ±	2.437 ±	2.577 ±	2.692 ±
	0.007	0.036^{****}	0.025^{****}	0.035^{****}	0.008^{****}	0.020^{****}	0.122^{****}
III	0.990 ±	2.088 ±	1.535 ±	1.427 ±	1.292 ±	1.168 ±	1.182 ±
	0.008	0.033^{****}	0.023^{****}	0.022^{****}	0.061^{*}	0.006^{***}	0.006^{***}
IV	1.157 ±	1.372 ±	1.390 ±	1.280 ±	1.240 ±	1.190 ±	1.165 ±
	0.216	0.026^{ns}	0.157^{ns}	0.051^{ns}	0.040^{ns}	0.026^{ns}	0.007^{ns}
V	1.000 ±	1.597 ±	1.640 ±	1.492 ±	1.350 ±	1.230 ±	1.123 ±
	0.057	0.060^{**}	0.051^{***}	0.080^{**}	0.026^{***}	0.024^{*}	0.011^{ns}

Values (ng/ml) are expressed as mean ± SEM (n=6). Values comparison were made between Group I Vs Group II, III, IV, V ($^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.1$, ns- Non Significant).

Percentage inhibition

Table 20 indicates that the change which occurs due to the treatment of hydroalcoholic extract of roots of *Berberis chitria* Linn. in inhibition of carrageenan induced paw edema.

Table 17: Effect of hydroalcoholic extract of roots of Berberis chitria Linn. on percentage inhibition of carrageenan induced paw edema

Group	Initial Paw Volume	6 hr (mm)	Difference in Paw	Inhibition percentage
I	1.180 ± 0.005	1.180 ± 0.005	0.00	100
II	1.193 ± 0.007	2.692 ± 0.122****	1.499	43.12
III	0.990 ± 0.008	1.182 ± 0.006***	0.192	85.57
IV	1.157 ± 0.216	1.165 ± 0.007^{ns}	0.008	84.42
V	1.000 ± 0.057	1.123 ± 0.011^{ns}	0.123	87.24

Values (ng/ml) are expressed as mean ± SEM (n=6). Values comparison were made between Group I Vs Group II, III, IV, V (****p <0.0001, ***p <0.001, ns- Non-Significant).

Conclusion :

Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities. These also decrease cardiovascular complications. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. The hydroalcoholic roots extract of *Berberis chitria* Linn. showed significantly higher inhibition percentage (stronger hydrogen- donating ability) positively correlated with total phenolic content

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral, and anti-cancer activities. The present investigation suggests that the hydroalcoholic roots extract of *Berberis chitria* Linn. shows good antioxidant activity, reducing power, free radical scavenging activity and hepatic protection. Phytochemical screening of the hydroalcoholic roots extract of *Berberis chitria* Linn. reveals the presence of Alkaloids, Carbohydrate, Steroids, Phenols, Flavanoids, Terpenoids.

In the present study it was confirmed that, the hydroalcoholic roots extract of *Berberis chitria* Linn. (400 mg/kg) possess anti-inflammatory activity. The result also supports the plant in the treatment of inflammation related disease traditionally. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of roots extract of *Berberis chitria.*

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