China Petroleum Processing and Petrochemical Technology

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2508-2518

PHYTOCHEMICALS AND EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIALS OF *SWERTIA CHIRATA* LEAVES EXTRACT IN ANIMAL MODEL

Minakshi Pandey

*[1]Assistant Professor, Sharda School of Pharmacy, Sharda University, Greater Noida- 201306 India

Vijay Kumar

[2]Research Scholar, Ch. Devi Lal College of Pharmacy, Bhagwangarh, Yamunanagar, Haryana-135003, India

Dharamveer Singh

[3]Assistant Professor, BIU College of Pharmacy, Bareilly International University, Bareilly-243006 UP, India

Ravinder Kaur

[4]Research Scholar, Ch. Devi Lal College of Pharmacy, Bhagwangarh, Yamunanagar, Haryana-135003, India

Pankaj Kumar

[5]Associate Professor, Bhagwati College of Pharmacy, Baraut, Bagpat, Uttar Pradesh- 250611 India

Yasmin Khatoon

[6]Professor, Institute of Pharmacy, Shri Ramswaroop Memorial University, Barabanki Uttar Pradesh- 225003, India

Vrinda Goel

[7]Research Scholar, Ch. Devi Lal College of Pharmacy, Bhagwangarh, Yamunanagar, Haryana-135003, India

Ritu

[8]Assistant Professor, Ch. Devi Lal College of Pharmacy, Bhagwangarh, Yamunanagar, Haryana-135003, India

***Corresponding author:** Dr Minakshi Pandey

[1]*Assistant Professor, Sharda School of Pharmacy, Sharda University, Greater Noida- 201306 India

Email id: minakshi.pandey@sharda.ac.in

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2508-2518

Abstract

Since ancient times, herbal medications are being used across the globe. There is a lot of potential for using plants to treat and control diseases. The present study was emphasized on the screening of phytochemicals and evaluation of anti-inflammatory potential of *Swertia chirata* leaves extract in animal model. The indomethacin, carrageenan and ethanol were obtained from the local chemical store. Evaluation kit for antioxidant activity was purchased from Thermofisher Scientific. The fresh leaves were harvested from the Noida region in UP. The plant was authenticated by a Botanist at Botanical Survey of India, in Allahabad UP with Ref. no. of 2022-23/01794. The leaves were shade-dried and then pulverised in coarse powder and soaked into 500ml ethanol and water (1:1) solvent for 15 days. Thus, it was extracted through maceration process with gradual stirrings. Wistar rats of either sex weighing 130-160g were obtained from the Animal House, Sharda School of Pharmacy, Sharda University, Greater Noida, UP, India. The animals were maintained in proper conditions, at room temperatures of 25±1°C with 12-hour light/dark cycle. Animals were divided in 5 groups; group 1 given only normal saline, group 2 given carrageenan (2%) intradermally, group 3 given carrageenan (2%) + indomethacin (10mg/kg, p. o.), group 4 given carrageenan (2%) + ethanolic leaves extract of *Swertia chirata* (ELSC) (200mg/kg, p. o.) and group 5 given carrageenan (2%) + ethanolic leaves extract of *Swertia chirata* (ELSC) (400mg/kg, p. o.) for 21 days. As the results indicate that ELSC exhibited significant antioxidant and anti-inflammatory activity and the response was noted as dose-dependent. When calculated with theoretic yield the % yield of the herbal extract was found to be 64.67%. Carrageenan (2%) + ELSC (200mg/kg, p. o.) and Carrageenan (2%) + ELSC (400mg/kg, p. o.) treated rats exhibited 58.20±0.16**µg/ml and 73.49±0.46***µg/ml, respectively. Percentage inhibition was estimated as 68.292±0.11 and 71.64±0.37 in the Carrageenan (2%) + ELSC (200mg/kg, p. o.) and Carrageenan (2%) + ELSC (400mg/kg, p. o.), respectively. In conclusion, *Swertia chirata* extract might be much significant in reducing the inflammation and counter it. Additional research was required to confirm its mechanism of action for reducing inflammation. Owing to the readily available *S. chirata*, its manufacture would be affordable and accessible to all patients, even those from lower socioeconomic classes.

Keywords: *Swertia chirata*, antioxidant, anti-inflammatory, indomethacin, carrageenan-induced paw edema

INTRODUCTION

Inflammation is a reaction to tissue damage that causes venule dilatation, elevated permeability of the vascular wall, and the influx of cytokines, histamine, and other inflammatory agents [1][2]. Stress reactions lead to inflammation, which is an essential component of those reactions [3]. Inflammation and pain are brought on by a variety of mediators, including cytokines, IL, PG, PAF, and LT [4]. The body does not always benefit from inflammations. Dangerous inflammation arises when the immune system unintentionally targets the body's own cells in certain illnesses [5]. Inflammation also produces diseases as mentioned below-

- Ulcerative colitis

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2508-2518

- Inflammatory bowel illness
- Crohn's disease
- RA
- Psoriasis

The immune system's response to harmful stimuli like infections, injured cells, toxic substances, or radiation is inflammation, which starts the healing process and gets rid of the harmful stimuli. Thus, inflammation is an essential defence mechanism for overall health. During acute inflammatory responses, cellular and molecular activity and interactions typically effectively prevent impending injury or infection. This mitigating process helps both the resolution of acute inflammation and the restoration of tissue homeostasis. On the other hand, unchecked acute inflammation can become chronic and result in a number of chronic inflammatory illnesses [6].
The coordinated activation of signalling pathways that control the amounts of inflammatory mediators in both blood-borne inflammatory cells and local tissue cells is known as the inflammatory response. Inflammation is the root cause of many chronic illnesses, such as cancer, diabetes, gastrointestinal and cardiovascular disorders, and arthritis [7].

Plant profile

Swertia chirata is one of many medicinal herbs that have been utilised historically as hepatoprotective agents. Although the herb has been utilised for generations, it wasn't until 1839 that it was brought to Europe. When Roxburgh first described the genus Swertia in 1814, it was known as Gentiana chirata. There are about 135 represented annual and perennial plant species, both introduced and endemic. Many herbal treatments contain components of the common swertia species [8].

Morphology

The entire plant known as chirata is used for medical purposes. Fresh herb samples are completely covered in a beautiful yellowish tint. The stem has a maximum length of 1 m, a diameter of 6 mm, and a coloration ranging from yellowish-brown to purplish. Its surface is hair-free and smooth, devoid of protrusion. The lower part of the stem is cylindrical, with a huge, continuous, readily separated yellow pith; the top half of the stem is somewhat quadrilateral. The plant has opposite, acuminate, cauline, flattened, narrow, oval-shaped leaves that taper to a point at each end, and usually have five to seven plainly visible lateral veins [9].

Taxonomy

Kingdom : Plantae
Class : Magnoliopsida
Order : Gentianales
Family : Gentianaceae
Genus : *Swertia*

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2508-2518

Species : *chirata*

Chemical constituents

Chirata is a plant that is utilised medicinally throughout. Samples of fresh herbs are entirely covered in a stunning yellowish hue. The stem's diameter is 6 mm, its greatest length is 1 m, and its colour varies from yellowish-brown to purplish. Its surface is smooth, hairless, and devoid of protrusions. The top half of the stem is roughly quadrilateral, while the lower part is cylindrical and has a large, continuous, easily separable yellow pith. The leaves of the plant are opposite, acuminate, cauline, flattened, narrow, oval-shaped, and tapering to a point at each end. Five to seven clearly visible lateral veins are typically present in the leaves [9].

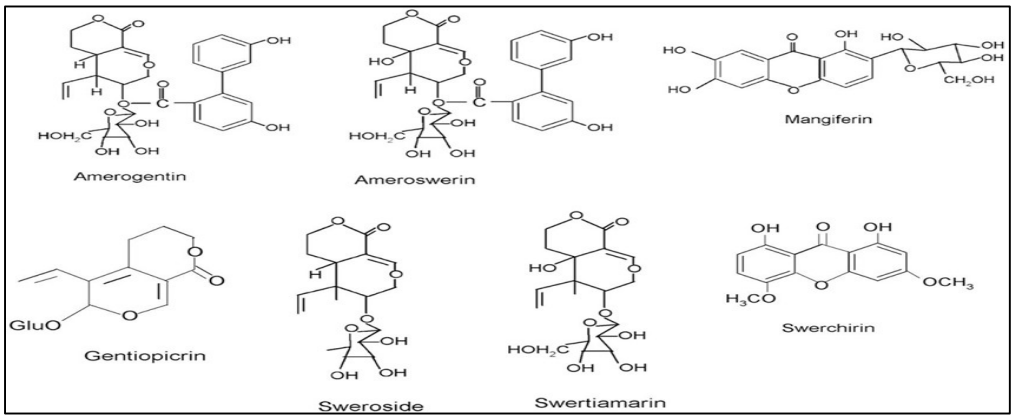

Fig 1. Structures of main chemical compounds of *S. chirata* extract

The most important medicinal properties of C. Swertia are anti-inflammatory, hypoglycemic, hepatoprotective, antibacterial, wound healing, antispasmodic, antioxidant, anti-diabetic, antipyretic, and antitussive activity. Additionally, many pharmaceutical compounds that have anticancer, antitumor, and anti-AIDS properties were identified from natural plants.

The present research was based on the evaluation of phytochemicals and antioxidant and anti-inflammatory potentials of S*wertia chirata* leaves extract in animal model.

MATERIALS AND METHODS

Chemicals and instruments

Carrageenan, Indomethacin, sodium phosphate, sulfuric acid, ammonium molybdate, distilled water, ethanol, UV-spectrophotometer, Water-bath, rotatory evaporator, weighing machine and ethanol.

Collection, Authentication and Extraction of plant

The fresh leaves were harvested from the Noida region in UP. The plant was authenticated by a Botanist at Botanical Survey of India, in Allahabad UP with Ref. no. of 2022-23/01794. The leaves were shade-dried and then pulverised in coarse powder and soaked into 500ml ethanol and water

(1:1) solvent for 15 days. Thus, it was extracted through maceration process with gradual stirrings. The produced brownish semisolid extract was dried using a rotary evaporator. The yield of the extract was calculated in percentage [11].

Screening of phytochemicals

To get better knowledge about the phytochemicals in the obtained extracts qualitative test alkaloids, terpenoids, proteins etc. were performed following standard chemical tests [12].

Saponins test

A modest amount of extract and water should be combined in a test tube for the foam test. When saponin is present, vigorous shaking produces foam that lasts for around 10 minutes.

Alkaloids

Chloroform is used to dissolve plant extract in the Dragendroff test. After the chloroform has been acidified and evaporated, add a few drops of Dragendroff's agent.

Mayer's test

Adding Mayer's reagent to 2–3 ml of filtrate yields ppt.

Wagner's test

When Wagner's reagent is added to 2–3 ml of filtrate, a reddish-brown colour develops. The presence of alkaloids in plant extracts was determined by adding a few drops of Hager's reagent and observing the resulting yellow precipitate.

Carbohydrate analysis

The Fehling's test

Boiling for one minute a millilitre of each of Fehling's A and B solution mixtures. A similar quantity should be added to the solution of test extract. Immerse for about 5-10 minutes maximum. Carbohydrates are present as indicated by the appearance of a precipitate of orange red colour.

Benedict's test

Combine test extract with Benedict's reagent in a test tube at a volume ratio of 1:1. Bring to a boil and let sit for five minutes. The consensus appears to be positive. The test solution's hue will be either yellow or red, depending on the concentration of disaccharide present.

Flavonoid Analysis

Ferric chloride test

Mix a few drops of diluted ferric chloride with the extract's ethanol solution. The colour green represents the presence of flavonoids. Add 5ml (95% ethanol), a few drops of hydrochloric acid, and 0.5g (magnesium powder) to a dry extract to perform the Shinoda test. There's a hint of pink

there. The yellow hue of test solutions is heightened after being treated with sodium hydroxide solution, but it disappears when a weak acid is added. Adding a few drops of lead acetate to the test solution causes a greenish-lemon precipitate to form.

Protein analysis

Biuret test

Add 2 ml of the biuret solution, mix it well, and heat it in a water bath. Red or violet hues indicate the presence of proteins. Mix 3 ml of extract with a few drops of a 15 CuSO4 solution and 4 percent NaOH. It gives off a pink/violated vibe.

Million's test

A reddish-brown precipitate is formed when a standard solution is added to Million's Reagent and then heated. The xanthoprotein test is boiling the test solution in concentrated nitric acid to create a yellow precipitate.

Ninhydrin test

It involves a reagent called ninhydrin, which gives the test solution a bluish tint.

Glycosides identification

Keller-Kelliani test

The test solution was made by mixing two millilitres of ferric chloride solution with a few drops of glacial acetic acid. Sulfuric acid dripping down the sides of the test tube causes the top layer to turn blue-green, and then the two layers to separate and show a reddish-brown colour.

Amino acids test

Bring 3 ml of extract and 3 drops of a 5% Ninhydrin solution to a boil in a water bath, then let it sit for 10 minutes. The sky turns violet or blue. Warm 3 ml of extract and add 3 drops of Million's reagent to conduct the tyrosine test. The answer turns a deep crimson hue when mixed.

Steroid analysis

Add 3 milliliters of extract to 3 milliliters of acetic anhydride to perform the Liebermann reaction. Calm and comfortable Sulfuric acid in the form of a few drops Seems to be a blue colour.

Preparation of animals

Wistar rats of either sex weighing 130-160g were obtained from the Animal House, Sharda School of Pharmacy, Sharda University, Greater Noida, UP, India. The animals were maintained in proper conditions, at room temperatures of 25±1°C with 12-hour light/dark cycle.

Group design

Animals were divided in 5 groups (n=6) and treated for 21 days, as follows [13]:

- Group 1 given only normal saline.

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2508-2518

- Group 2 given carrageenan (2%) intradermally.
- Group 3 given carrageenan (2%) + indomethacin (10mg/kg, p. o.).
- Group 4 given carrageenan (2%) + ethanolic leaves extract of *Swertia chirata* (ELSC) (200mg/kg, p. o.).
- Group 5 given carrageenan (2%) + ethanolic leaves extract of *Swertia chirata* (ELSC) (400mg/kg, p. o.).

METHODS

1. Determination of total antioxidant activity

The total antioxidant activity of fractions was calculated using Prieto et al's method [14[0.3 mL sulfuric acid combined with 28 mm sodium phosphate, 4 mm ammonium molybdate, and a little quantity of extract. The reaction mixture was incubated at 95°F for 90 minutes under a water bath. The absorbance of each sample combination was measured at 695 nm. The total antioxidant activity was determined by measuring the milligrammes of ascorbic acid equivalents per gramme of extract.

2. Carrageenan- induced paw edema

The rats were divided into 4 groups, each weighing 180–220 g. The studied chemicals were given intra-peritoneally in the dose of 0.01 mmol/kg (body weight), suspended in the water added few drops of Tween 80 and pulverized in a mortar before use. The right foot pad received 0.1 ml of carrageenan (2%) intradermally, with the left paw served as a control. Simultaneously with the phlogistic agent, indomethacin (the reference medication) was given intraperitoneally. Both hind paws are diagnosed, just above the ankle joint and recorded for the volume of inflammation. The medication treatments are repeated at 5, 10, 15, and 21 days [15].

RESULTS AND DISCUSSION

Percentage yield

When calculated with theoretic yield the % yield of the herbal extract was found to be 64.67%.

Phytoconstituents

It has demonstrated for diverse phytoconstituents as follows-

Table 2. Phytochemicals of *H. vittatum* extract

Tests	Observation
Alkaloids	++
Saponins	+
Tannins	+
Proteins	+
Glycosides	++
Amino acids	++
Steroids	+
Flavonoids	++

++= High +=Moderate

Evaluation of pharmacological activities

1. Determination of total antioxidant activity

In determination of total antioxidant activity, carrageenan (2%) + indomethacin (10mg/kg, p. o.) treated animals showed 79.18±0.52***µg/ml. Whereas, Carrageenan (2%) + ELSC (200mg/kg, p. o.) and Carrageenan (2%) + ELSC (400mg/kg, p. o.) treated rats exhibited 58.20±0.16**µg/ml and 73.49±0.46***µg/ml, respectively. However, Carrageenan (2%. intradermally) administered rats showed antioxidant level as 26.30± 0.33**µg/ml. Therefore, at both the doses herbal extract significantly demonstrated anti-oxidant potential that indicates for its anti-inflammatory effect when compared with disease control.

The following table depicts the total anti-oxidant activity of *S. chirata*-

Table 1. Determination of total antioxidant activity of ELSC

Treatment	Total antioxidant activity (µg/ml)
Normal saline	37.29± 0.42*8
Carrageenan (2%, intradermally)	26.30± 0.33**
Carrageenan (2%) + indomethacin (10mg/kg, p. o.)	79.18± 0.52***
Carrageenan (2%) + ELSC (200mg/kg, p. o.)	58.20± 0.16***
Carrageenan (2%) + ELSC (400mg/kg, p. o.)	73.49± 0.46***

Significance level was represented by *; P<0.05

n=4; readings were given in Mean± SEM

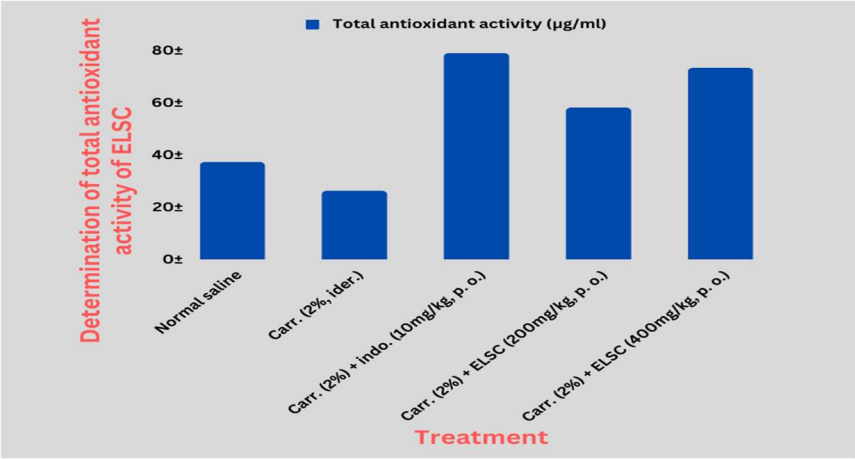

Fig 2. Graphical data of total antioxidant activity of ELSC

2. Carrageenan-induced paw oedema

All the treated groups were estimated for left hind paw inflammation at 30, 60, 120 and 180 mins. The negligible reduction in paw oedema was noted in the control group and maximum in carrageenan induced-oedema among rats. At 180 min, the volume of left hind paw was estimated as 4.09±0.27** and 4.26±0.03** in Carrageenan (2%) + ELSC (200mg/kg, p. o.) and Carrageenan (2%) + ELSC (400mg/kg, p. o.) treated rats, respectively. While, highest inflammation was

observed in Carrageenan (2%, intradermally) administered rats in contrast to control (normal saline) fed animals. The anti-inflammatory effect was observed in dose-dependent manner.

Table 2. Volume of left hind paw in ELSC treated rats

Treatment	Volume of left hind paw (Mean± SEM)			
	30 min	60 min	120 min	180 min
Normal saline	2.81±0.12**	3.57±0.18***	4.21±0.31**	4.77±0.12**
Carrageenan (2%, intradermally)	4.39±0.17	4.68±0.29	5.11±0.41	5.69±0.32
Carrageenan (2%) + indomethacin (10mg/kg, p. o.)	2.13±0.18***	2.43±0.19***	2.74±0.26**	3.40±0.21**
Carrageenan (2%) + ELSC (200mg/kg, p. o.)	2.29±0.27**	2.79±0.21**	3.84±0.11**	4.09±0.27**
Carrageenan (2%) + ELSC (400mg/kg, p. o.)	2.42±0.23**	2.72±0.64***	4.05±0.30**	4.26±0.03**

Significance level was represented by *; P<0.05
n=4; readings were given in Mean± SEM

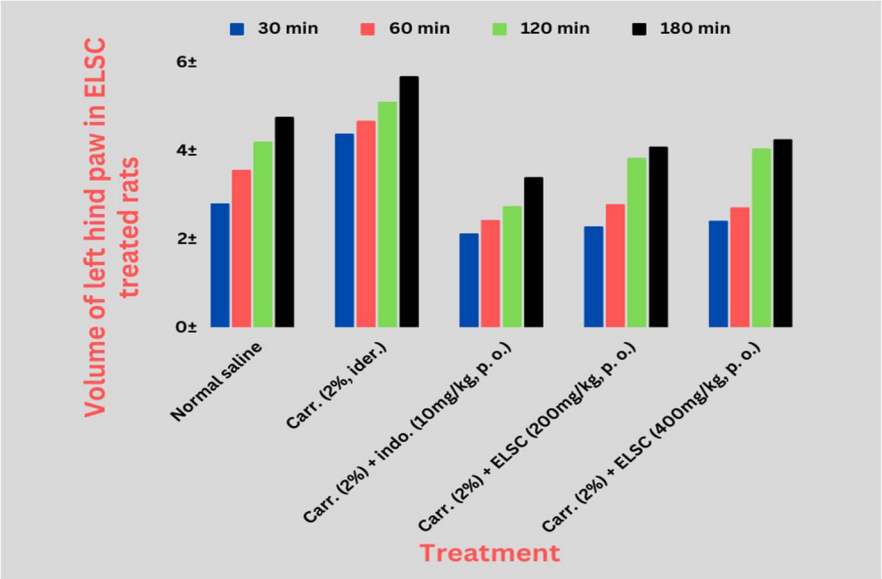

Fig 3. Graphical data of volume of left hind paw in ELSC treated rats

% Inhibition of Inflammation

The anti-inflammatory response is always observed based on percentage inhibition. It was found maximum (79.64±0.20) in the Carrageenan (2%) + indomethacin (10mg/kg, p. o.) treated group as a sign of potent COX (cyclooxygenase) inhibitor. Percentage inhibition was estimated as 68.292±0.11 and 71.64±0.37 in the Carrageenan (2%) + ELSC (200mg/kg, p. o.) and Carrageenan (2%) + ELSC (400mg/kg, p. o.), respectively.

However, % inhibition was observed Nil in control and disease control group.

Table 3. % Inhibition in ELSC treated rats

Treatment	% Inhibition (Mean ± SEM)
Normal saline	Nil.
Carrageenan (2%, intradermally)	Nil.

Carrageenan (2%) + indomethacin (10mg/kg, p. o.)	79.64±0.20
Carrageenan (2%) + ELSC (200mg/kg, p. o.)	68.292±0.11
Carrageenan (2%) + ELSC (400mg/kg, p. o.)	71.64±0.37

Mangiferin has also been found to have significant anti-inflammatory action. Herpes virus infections can be treated with mangiferin, both orally and topically. Mangiferin has been shown to prevent liver damage in rats exposed to hypoxia at high altitudes. However, it was found that xanthone-O-glycosides have a CNS depressive effect in mice and rats. Most Swertia species had mutagenic properties in their extract. The antimalarial medication AYUSH-64 includes the bacterium *S. chirayita*. According to research, *S. chirayita* xanthones can cause central nervous system depression. It has been reported that the O-glycoside norswertianolin possesses antitubercular action. Albino rats and mice have been shown to have CNS depression after being exposed to S. purpurescens O-glycosides. Isolated from the hexane fraction of Swertia chirayita, 1,8-dihydroxy-3,5-dimethoxyxanthone (swerchirin) significantly reduces blood sugar levels in fasting, fed, glucose-loaded, and tolbutamide-pretreated albino rats [16].

S. chirata herbal extract exhibited significant anti-inflammatory activity. The response was noted as dose-dependent. Maximum percentage inhibition (antioxidant and anti-inflammatory properties) was recorded in the dose of 400mg/kg of ethanolic leaves extract of *S. chirata* (ELSC). This may be because prostaglandins, which are actually the cytokines that cause inflammation and edoema, are suppressed. Presumably, these compounds inhibited the LOX and COX enzymes.

CONCLUSION

In conclusion, *Swertia chirata* extract might be much significant in reducing the inflammation and counter it. Since they are primarily derived from plant sources, widespread availability will be facilitated.

To obtain the maximum potency and intrinsic activity, it recommends isolating and formulating that particular chemical moiety and developing it in the intended dosage form. Additional research was required to confirm its mechanism of action for reducing inflammation. Owing to the readily available *S. chirata*, its manufacture would be affordable and accessible to all patients, even those from lower socioeconomic classes.

FUNDING

Nil.

CONFLICT OF INTEREST

Authors have declared for none conflict of interest.

REFERENCES

Sahlmann C O, Ströbel P. Pathophysiology of inflammation. Nuklearmedizin, 2016; 55(1): 1-6.

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2508-2518

Ridker PM. C-reactive protein, inflammation, and cardiovascular disease: clinical update. Tex Heart Inst J. 2005;32(3):384-6.

Leonardi GC, Accardi G, Monastero R, Nicoletti F, Libra M. Ageing: from inflammation to cancer. Immun Ageing. 2018;15:1.

Mantovani A. The inflammation - cancer connection. FEBS J. 2018 Feb;285(4):638-640.

Singh Brij Mohan, Gaurav Negi , Praveen Bhole , Manju Jaiprakash. Pain And Inflammation: A Review. IJPSR, 2012; 3(12): 4697-09

Libby P. Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutr Rev.* 2007;65:S140–S146.

Zhou Y, Hong Y, Huang H. Triptolide Attenuates Inflammatory Response in Membranous Glomerulo-Nephritis Rat via Downregulation of NF-κB Signaling Pathway. *Kidney and Blood Pressure Res.* 2016;41:901–910.

Kumar V. and Van Staden J., A review of Swertia chirayita (Gentianaceae) as a traditional medicinal plant. Frontiers in pharmacology, 2016; 6:308.

Laxmi A., Siddhartha S. and Archana M., Antimicrobial screening of methanol and aqueous extracts of Swertia chirata. Int J Pharm Pharm Sci, 2011; 3(4):142-146.

Banerjee S. et. al. Assessment of the anti-inflammatory effect of *Swertia chirata* in acute and chronic experimental models in male albino rats. Indian Journal of Pharmacology. 32; 2000: 21-24.

Maji Soma. Role of Neem Leaves in Diabetes And Obesity. Role of phytochemicals in Human Physiology Disorders. Scholar's Press. 2020.

Patel R K, Vishal R P, Madhavi G Patel. Development and validation of a RP-HPLC method for the simultaneous determination of Embelin, Rottlerin and Ellagic acid in Vidangadi churna. Journal of Pharmaceutical Analysis, 2012; 2(5): 366-371.

Bhajoni et al. Evaluation of the Antiulcer Activity of the Leaves of Azadirachta indica: An Experimental Study. Integrative Medicine International, 2016; 3:10–16.

Misra H P, Fridovich I. The role of superoxide anion in the antioxidation of epinephrine and a simple assay for Superoxide dismutase. J Biol Chem, 247: 31-70, 1972.

Christos A. Kontogiorgis,KyriakosSavvoglou& Dimitra J. Hadjipavlou-Litina. Anti-inflammatory and antioxidant evaluation of novel coumarin derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry, 2005; 21(1): 21-29.

Jagmohan S. Negi, Pramod Singh and Bipin Rawat, 2011. Chemical Constituents and Biological Importance of Swertia: A Review. *Current Research in Chemistry, 3: 1-15.*