Preliminary studies, anti-oxidants, acute oral toxicity and anti-arthritis activity of *Cassia Sophera*

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Abstract **- To determine the ameliorative potential of petroleum ether and ethanol extracts of *Cassia sophera* (*C.sophera*) against *in-vitro* antioxidant and *in-vivo* acute oral toxicity and arthritis using *albino wistar rats*, and its possible mechanism of action. Antioxidant potential was evaluated by DPPH and reducing power method and results was found to be significant. *Albino Wistar rats* (200±20g) under standard controlled conditions (22 ± 2°C, 30% humidity and 12:12 light/dark cycle). Acute toxicity studies were performed according to Organisation of Economic Co-operation and Development guidelines and no mortality was found. For anti-arthritis activity, the animal's groups were divided into 6 groups (n = 6/group) and assigned as control, reference standard (Indomethcine) and, group III and IV animal received crude extracts of *C.sophera*. Paw size was measured till 28th days and analysed for haematological and histopathological parameter. At 28th day, *C.sophera* showed paw edema 3.705±0.281 at the dose 400mg/kg wt similar to reference drug. Thus, the present study revealed that the extract of *C.sophera* offered significant protection against anti-oxidants, acute oral toxicity and arthritis.**

Index Terms - Cassia Sophera, **Arthritis, Anti-oxidant, Acute oral toxicity.**

INTRODUCTION

Cassia sophera Linn (Family Caesalpiniaceae), popularly known as kasundi, is a shrubby herb found throughout India and in most tropical countries. In the ethnobotanical claims, the leaves are considered to be used for their anti-inflammatory, antirheumatic, and purgative property, as an expectorant for cough, cold, bronchitis, and asthma, and in the treatment of liver disorders. Previous studies have investigated on its pharmacological activities of the seeds of *C. sophera* including analgesic and anticonvulsant(1), antidiabetic(2), inhibition of lipid peroxidation(3), herbicidal, and fungicidal(4) effects.

The chemical constituents of *C. sophera* include the flavonoids(5) and anthraquinone(6)(7). To the best of our knowledge, there is no scientific report of antiarthritis effect of *C. sophera*. Thus, the present study was to investigate the phyotochemical, antioxidants and antiarthritis activity of ethanol extract of leaves of *C. sophera* against Freund's adjuvant induced arthritis rat model.

MATERIALS&METHODS

Chemical and Reagents

Petroleum ether, ethyl acetate, methanol, sodium carbonate, potassium ferricyanide, DMSO, NaOH, were procured from SD fine chemicals Pvt. Ltd. Mumbai, India. Complete freund's adjuvant (CFA), gallic acid, rutin, folin-ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH),Nitro blue tetrazolium (NBT) and ascorbic acid was procured from Sigma Aldrich chemicals Pvt. Ltd, Hyderabad, India. Indomethacin was obtained from Akums Drugs and Pharmaceuticals, India. All other chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Lobo Chem, Ltd. (Mumbai, India), SRL Pvt. Ltd. (Mumbai, India) and Merck Life Sci. Private Ltd. (Mumbai, India). All other chemicals used in this study were obtained commercially and were of analytical grade.

Plant Material

Plant materials were collected from the local area of Bhopal (M.P.) India. Herbarium of plants species was prepared graciously and submitted to Department of Botany, Saifia College of Science, Bhopal India, for authentication. Plants were authenticated by Dr.Saba Khan, Department of Botany, Saifia College of Science, Bhopal, India. Plant authentication voucher number obtained for *Cassia sopher* respectively.

Preparation of extract

The plant material were washed with water to remove dust and sand. Then it was dried under shade at room temperature and was grinded into a fine powder in an electric blender and subsequently sieved for obtaining a fine powder. The plant powder was successively extracted with petroleum ether in a soxhlet extractor by continous hot percolation method. At the end of extraction, it was passed through Whatman filter paper. The petroleum ether extract was concentrated to dryness under vaccum on rotary evaporator at 40°C and weighed and percentage yield was determined.(8) Prepared extract were observed for organoleptic characters (percentage yield, colour and odour) and were packed in air tight container and labelled till further use

Qualitative Phytochemical Estimation

Preliminary screening tests for alkaloids, flavonoids, sterols, tannins, and other natural compounds were carried out on the basis of those reported method in order to determine the various classes of natural compounds in the ethyl acetate and methanol extract.(8)

Invitro Analysis

The in-vitro anti-oxidant capacity was evaluated by DPPH and Reducing Power Assay Method.

1,1- diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH (1,1- diphenyl-2-picrylhydrazyl) assay method is based on the reduction of DPPH, a stable free radical. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of extracts. The change in color was measured at 517 nm wavelength using spectrophotometer (UV-Systronics) with methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. All the tests were carried out in triplicates. The percentage inhibition of free radical DPPH was calculated.

Reducing Power Assay

1 ml of various concentrations of extract was mixed with 2.5 ml phosphate buffer solution (0.2 M,pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The solution was properly mixed and placed in incubator for 20 min at 50ºC. After incubation, the resulting solution was cooled and 2.5 ml of 10% trichloro acetic acid was added to reaction mixture, followed by centrifugation at 3000 rpm for 10 min. After centrifugation 2.5 ml of supernatant was mixed with equal volume of distilled water and finally 0.5 ml of 0.1% ferric chloride was added. The reaction mixture was shaken and kept at room temperature for 10 min. The absorbance was measured at 700 nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample(9)

In-vivo Analysis

Experimental animals

*Albino Wistar*rats at 5-6 weeks of age (weighing approximately 200±20 g) were obtained and selected randomly from the Animal house, Pinnacle Biomedical Research Institute (PBRI), Bhopal, India. The rats were acclimatized to the animal holding facility at PBRI for 7 days before the start of the experiments. The rats were housed in individual propylene cages with sterile husk as bedding. Relative humidity of 30.7 % at 22±2°C and 12:12 light and dark cycle was maintained in the animal house and were given food standard pellets (Golden Feeds, New Delhi, India) *ad libitum*and had free access to distilled water. All of the animal procedures were subjected to review and approval by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal. Separate group (n=6) of rats was used for each set of experiments.

Toxicity evaluation

Acute toxicity study

The acute toxicity study was conducted to determine the adverse effects of *C.sophera* feeding on the vital organs (liver, kidney, heart and pancreas) and the biochemical parameters (alanine aminotransferase, alkaline phosphate, creatinine, urea and total protein). This was carried out according to the Organisation of Economic Co-operation and Development (OECD)

guideline 423. Prior to dosing with the extract, the rats were fasted overnight from food, but were allowed free access to water. A single dose of 5, 50, 300 and 2000 mg/kg of *C.sophera* was orally gaved in healthy Wistar rats.. All of the experimental animals were maintained under close observation for any signs of toxicity and mortality immediately after dosing, at 4 h and at 24 h and intervals, and twice daily for 14 days. After overnight fasting, on day 15th, the rats were anaesthetized using ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) and blood samples were collected *via* cardiac puncture. All the animals were sacrificed by cervical dislocation. The vital organs mainly liver, kidney, heart and pancreas was removed, cleaned with saline, weighed and preserved in 10% formalin for further histopathology observation. Relative organ weight was calculated as (weight of organ/body weight of rat on day of sacrifice) × 100%

Anti-Arthritis Study

Freund's adjuvant induced arthritis model(10) was used to assess the anti-arthritic activity of the ethanolic extract of *Cassia sophera*in Wister rats. Animals were randomly divided into six groups of six animals each (n=6). Group I animals received 0.2 ml CFA (containing 10 mg/ml of heat-killed *M. tuberculosis*) served as an arthritic control injected into left hind paw of all the animals, Group II animals received Indomethacine (10 mg/kg p.o.) served as reference standard, Group III and IV animals received the crude ethanolic extract of *Cassia sophera* (200 and 400 mg.kg-1). The paw size is an indicator of arthritic condition. Paw size was measured on 0th, 7th, 14th, 21st and 28th day by using electronic digital calipers. After 28th days blood samples were collected by puncturing the retro-orbital plexus into heparanized vials and analysed for hematological parameters.

Measurement of arthritic score

The severity of arthritis in paw of animals was evaluated and then it was graded from 0 to 4. Grade 0 indicates absence of swelling; grade 1 denotes mild swelling or erythema in one of the fingers in paw; grade 2 shows swelling in one or more fingers of paw; grade 3 displays swelling of wrist or ankle; grade 4 specifies severe arthritic swelling in fingers and wrist. Score 8 is the highest arthritic score fixed for rats which are induced with CFA.

Histopathological analysis

The paw was immersed in 10% formalin solution for histopathological examination. These tissues were processed, dehydration in different grades of alcohol, cleared in toluene, and impregnated in molten paraffin wax for specified periods, processed tissues were embedded in fresh molten paraffin wax and allowed to set. Sections were eosin to demonstrate general tissue structure. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam. Stained sections were examined under microscope for histopathological change.

RESULTS&DISCUSSION

Percentage Yield

Table 1 shows the percentage yield of crude successive extracts (petroleum ether, and ethanol) of *C. sophera*. Ethanolic extract exhibited higher yield (4.80%) followed by ethanol (0.424%).

Table 1: Percentage yield of Cassia sopheraextract

S. No.	Solvent	Colour of extract	Weight of Plant material (gms)	Weight of extract (gms)	% yield
1.	Petroleum ether	Dark green	100.20	0.425	0.424
2.	Ethanol	Dark green	97	4.66	4.80

Qualitative analysis of phytochemicals

The present study revealed that the extracts of *C. sophera* contained carbohydrates, alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and proteins and amino acid (Table 2). Compared to both solvent extracts, ethanolic extract had higher number of secondary metabolites while the pet. Ether extract contained only saponins and triterpenoids and steroids.

Table 2 Results of qualitative phytochemical analysis of various extracts acquired from *C. sophera*.

Chemical Constituent	Pet. Ether Extract	Methanol Extract
Carbohydrates	-	+
Alkaloids	-	+
Terpenoids and steroids	+	+
Flavonoids	-	+
Tannins and Phenolic Compounds	-	+
Saponins	+	-
Protein and Amino acids	-	-
Glycosides	-	+

Soxhlet extraction method, + and – indicates the presence and absence of corresponding SMs in the test extract

In vitro antioxidant activity

DPPH radical scavenging assay

Quantitative antioxidant activity was performed by DPPH-free radical scavenging assay where the IC50 values of pet. ether and ethanol crude extract of *C. sophera* were found to be 93.54 µg/ml and 47.37 µg/ml, whereas the standard(ascorbic acid) showed the value as 10 µg/ml [Figure1]

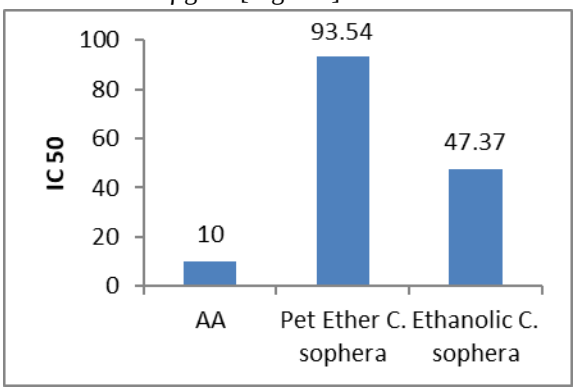


Figure 1: Percentage inhibition of extract and AA in DPPH study

Reducing Power Assay

The reducing power of extracts is shown graphically by depicting absorbance as a function of concentration. The reducing power of all the extracts increased with increase in concentration. Reducing power of ethanol extract is highest which is comparable to standard compound ascorbic acid.

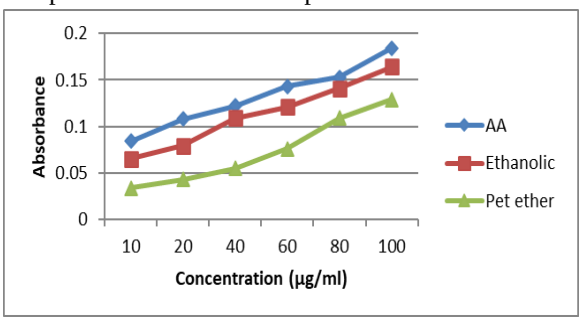


Figure 2: Reducing power assay of Cassia sophera and ascorbic acid

In vivo analysis

Acute oral toxicity (OECD)

In case of acute oral toxicity study when animals were treated with 5, 50, 300, and 2000 mg/kg b.w. of dose. There was no mortality, and any behavioral changes thus selected dose will be on the basis of LD50, 200 and 400 mg/kgb.w.

Induction of arthritis

The anti-arthritic action of *Cassia sophera* following the injection of CFA has been depicted in (Figure 3). Following CFA administration there was an increase in joint diameter which was maximum on day 21. After that, there was a progressive decrease in joint diameter in all the groups except the arthritic-treated where it was increased up to some extent after the 7th day. Treatment with indomethacin and *Cassia sopher* resulted in a remarkable decline in paw edema which was statistically significant in comparison to arthritic treated group.

The anti-arthritic activity of *Cassia sophera* against CFA induced paw edema has been shown in figure 3 and the results were comparable to that of standard drug indomethacine, a protype of non-steroidal anti-inflammatory agent. The *Cassia sophera* extract showed paw edema (3.705±0.281) at the dose 400 mg/kg body wt. after extract treatment in CFA induced paw edema and the reference drug (indomethacine) produced similar effects (3.561±0.066) paw size. Both standard and extract treatment groups were observed as effective when compared to CFA induced arthritic control group.

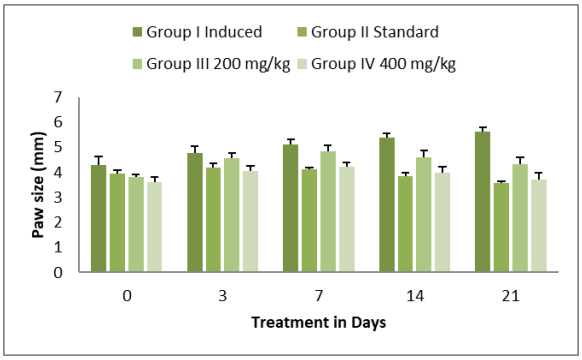


Figure 3:Effect of Cassia sopheraextract on Paw edema

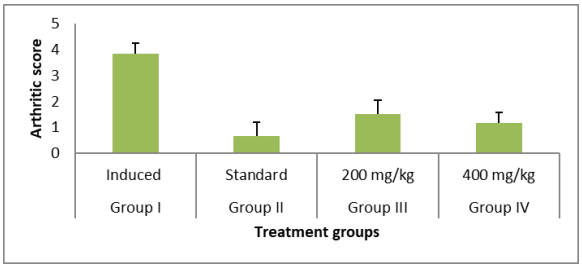


Figure 4: Arthritic score in treatment groups

Hematology parameters

Induction of arthritis resulted in significant decrease in RBC count (cells/mm3), Hb% and an increase in total WBC count and ESR. Treatment with *Cassia sophera* extract showed significant increase in Hb% and RBC count and decrease in total WBC count and ESR

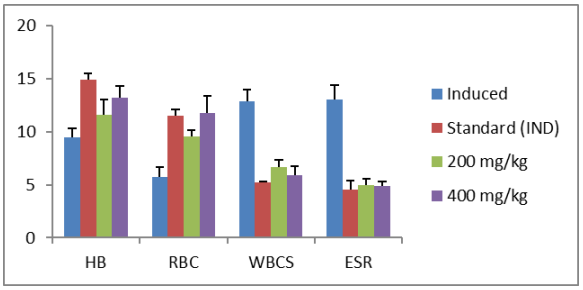
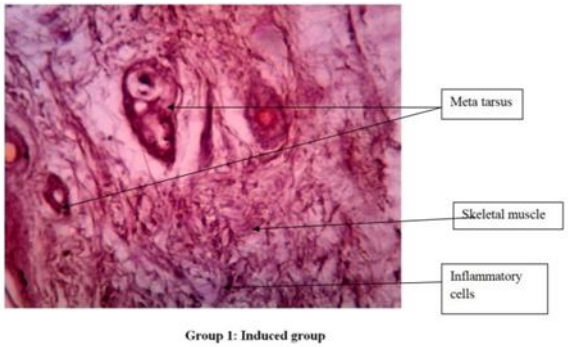


Figure 5: Haematological analysis of *Cassia sophera*extract treatment in CFA induced arthritis model

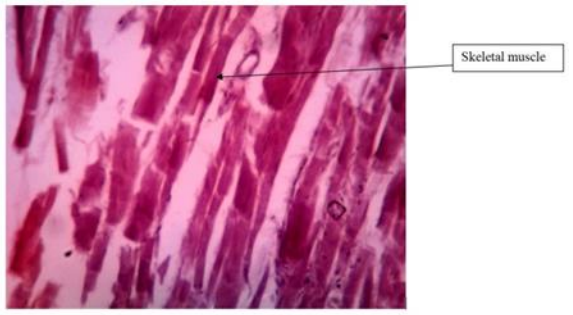
Histopathology of Paw

Slides represents the changes observed in the hind paw of the experimental groups. Group I showed abnormalities in the hind paw, edema, infiltrated inflammatory cells accumulation, degeneration of cartilage and bone marrow destruction. Cassia sophera 200 and 400 mg /kg treated rats (Group III, IV) showed less inflammatory signs such as improved bone marrow, absence of edema, and less cellular infiltration. Indomethacin (Group II) showed marked reduction of inflammation, no of destruction in cartilages and bone marrow found to be normal with minimal cellular infiltrates.

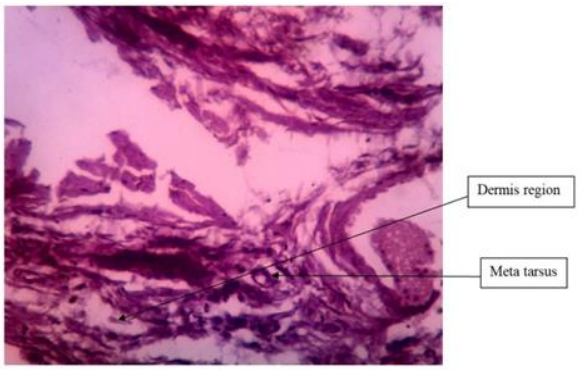
Haematoxylin and eosin staining of sections from the hind paws of rat variations groups. Group I:Induced ; Group II: Standard group; Group III: Arthritis induced + Cassia sophera 200 mg/kg group and Group IV: Arthritis induced + Cassia sophera 400 mg/kg group



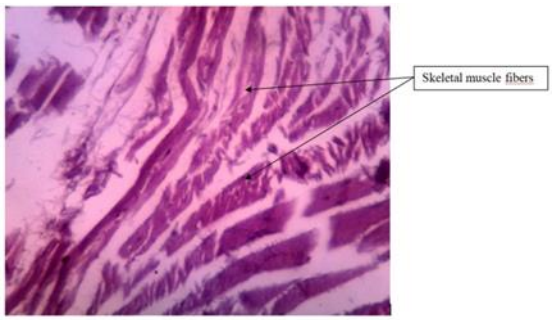
Group 1: Induced group



Group 2: Standard



Group 3: 200 mg/kg



Group 4: 400 mg/kg

Figure 6: Histopathology of Paw

CONCLUSION

In conclusion, this study provides the scientific evidence of the effectiveness of ethanol extract of Cassia sophera leaves as anti-inflammatory, and anti-arthritic medication supporting the common traditional beliefs and uses. The analysis of various arthritic assessment parameters used in this study revealed that Cassia sopheraextract has a considerable effect in preventing development or ameliorate arthritis disease severity. Moreover, the ethanol extract of Cassia sophera leaves revealed significant anti-oxidant activity.

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