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In-vitro Anti-inflammatory Potential of *Cassia purpurea* (Roxb.) Leaves Extract on Human RBC and Analgesic Property on Swiss Albino Mice

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Phytochemical analysis of ethanolic extract of A. *Cassia purpurea* has indicated the presence of steroid, flavonoid and tri terpenoids, hydrolyable tannins types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check *Cassia purpurea* for possible anti-inflammatory activity by HRBC (Human Red Blood Cell) membrane stabilization method by the inhibition of protein denaturation method . The ethanolic extracts of the plant exhibited notable anti-inflammatory activity and remarkable analgesic activity, . The maximum membrane stabilization of *Cassia purpurea* was found to be 98.54%. Hence, the ethanolic extracts of *Cassia purpurea* demonstrated the anti- inflammatory, Analgesic activity. Therefore, our studies support the isolation and the use of active constituents from *Cassia purpurea* in treating inflammations.

Keyword: *Cassia purpurea*, Anti-inflammatory Activity, Membrane stabilization, Analgesic activity.

1. Introduction

Potential of medicinal plants are everybody becoming more and more obvious and new remedies to tackle dreadful disease are being discovered. In India a no. of governmental and nongovernmental organization and institution are at present engaged in the study of medicinal, in a scientific way. The traditional system of medicine is so in grained in our culture that, even now 75% of the Indian population depends on indigenous system for relief. With a huge section of an ever increasing population relaying on herbal remedies, it is impurities that the plant product which have been in huge for such a long time be scientifically supported for efficacy. The uses of traditional medicine wide spread medicine and plants still represent a large source of natural anti-oxidants that might serve as leads for the development of the novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant or radical scavenging mechanism as part of their activity[1]. The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species from activated neutrophil and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukine- I, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important

mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation[2-3].

Cassia purpurea (Family: Caesalpiniaceae) commonly called as Dev Tarota. It is one of the medicinal plants scientifically investigated by the medicinal plant researchers. It is a medicinally important plant used for the treatment of inflammation, Pain. The work on the chemical composition of the leaves revealed the presence of steroids and flavonoids. Many flavonoids have remarkable wound Healing activity. The present study was carried out to evaluating the anti-inflammatory and analgesic activity of the leaves of *Cassia purpurea*[4,5].

This document is set in 10-point Times New Roman. If absolutely necessary, we suggest the use of condensed line spacing rather than smaller point sizes. Some technical formatting software print mathematical formulas in italic type, with subscripts and superscripts in a slightly smaller font size. This is acceptable.

2. Material and Methods

2.1 Collection of Plant:

The leaves of *Cassia purpurea* were collected from the Gardenin the month of June 2010, from Sakegaon, jalgaon dist, Maharashratra, India . The plant material was identified and authenticated by Dr. Tanveer A. Khan, Botanist, M J College, Jalgaon. The Collected plant material was free from diseases and also free from contamination of other plants. A voucher specimen of the leaves itself is deposited in department for future reference. Collected fresh leaves was washed & use for study of organoleptic characteristics. The dried leaves where used for the determination of phytochemical investigation.

2.2 Chemicals:

All the chemicals used were of analytical grade like Ethanol. The standard drugs in injectable forrns were purchased from local market.

2.3 Experimental animals:

Healthy Eighter sex Swiss albino mice (150-250g) were used for investigation. An animal were issued from Departmental Animal House (Reg. No. -1310/ac/09/ CPCSEA). All animals were maintained under laboratory conditions of temperature (24°±2°C) and relative humidity (65±5%). Animals were fed with standard pellet food (Amrit animal feed, Pune, Maharashtra, India) and tap water ad libitum.

The animal experimentation was carried out according to the Committee for the Purpose of Control and Supervision of experimental animals (CPCSEA) guideline and Institutional Animal Ethical committee approved all the procedure for investing experimental pain and inflammatory conditions in conscious animals

2.4 Preparation of Ethanolic Extract:

For the extraction purpose the drug whose extraction want to be done are first it cut in to small peaces then it shade dried for 4 to 5 days as it required for drying or also it can be dried in hot air oven at a constant temperature (30-30oc) for 2 to 3 days. After the drying the well dried drug are grind in to coarse drug power with the help of grinder mixer. The coarsely dry powder was passed through sieve. The coarsely dry powder was taken & macerates it with Ethanol for 24 hour. The assembly for extraction was set & thimble made. 120 gram of powder was taken packed in to thimble & extraction was proceed. The extraction was carried out for 2 to 4 days, in the period of 2 to 3 days 20 cycle of extraction was carried out lastly the extract was concentrated by evaporating the solvent.

2.5 Phytochemical analysis

The phytochemical screening of extracts of *Cassia purpurea* showed the presence tannin, flavonoids, terpenoids, alkaloids, and Steroids[6,7,8].

2.6 *In vitro* Anti-Inflammatory Activity by HRBC Membrane Stabilization Method

Principle behind the membrane stabilization is when hypotonic solution was added to HRBC suspension the lysis of RBCs occurs. After

treatment of extract of *Cassia purpurea* it recovered the lysis of cells.

Table 1: Phytochemical analysis of ethanolic extract of *Cassia purpurea*

Sr No	Chemical constituent	Test	Result
1	Flavonoids	Shinoda test	+ve
2	Steroids	Salkowski test	+ve
3	Alkaloids	Dragondorff's test	+ve
4	Tannins	Iodine solution	+ve
5	Terpenoids	Libermann-Berchard test	+ve

2.6.1 Preparation of Red Blood Cells (RBCs) Suspension (HRBC)

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline. It is mixed with equal volume of sterilized Alsever solution[9,10].

Table 2: Composition of Alsever Solution

Ingredients	Percentage
Dextrose	2 %
Sodium Citrate	0.8 %
Citric Acid	0.5 %
NaCl	0.42 %
Distilled water	Qs

2.6.2 Reaction mixture (4.5 ml mixture) for human red blood corpuscles membrane stabilization method[11]

1. Test solution (4.5ml): 2ml of hypotonic saline (0.25%w/v) + 1ml of phosphate buffer (pH7.4) + 1ml of test extract (250mcg/ml) in saline + 0.5ml of 10% w/v HRBC in isotonic saline.
2. Product control (4.5ml): 2ml of hypotonic saline (0.25% w/v) +1ml of phosphate buffer (pH7.4) + 1ml of test extract (250mcg/ml) in saline + 0.5ml of isotonic saline.
3. Test control (4.5ml): 2ml of hypotonic saline (0.25%w/v) + 1ml of phosphate buffer (7.4pH) + 1ml of isotonic saline + 0.5ml of 10%w/v HRBC in isotonic saline.
4. Standard solution (4.5ml): 2ml of hypotonic saline (0.25%w/v) + 1ml of phosphate buffer (7.4pH) + 1ml of Diclofenac sodium (250mcg/ml) in saline + 0.5ml 10%w/v HRBC in isotonic saline.

The above solutions were incubated at 56 ^{0}C for 30 minutes. They were cooled running tap water for 30 minutes. After centrifuge the supernatant liquid was separated and the absorbance of supernatant solution was measured at 560nm by UV-spectrophotometer[12].

Percentage Stabilization = 100- [(Optical Density of Drug) ÷ (Optical Density of Control) × 100]

2.7 Analgesic Activity

2.7.1 Experimental Animals

Three months old Swiss albino mice of either sex weighing 150- 250g were used for the study. The animals were procured from Animal House Faculty, department Of Pharmacology, SSJIPER. Jamner. All the experimental procedures and protocols used in the study were reviewed and approved by the IAEC SSJIPER, Jamner and were in accordance with the guidelines of the CPCSEA. Registration No.1130/ac/09/CPCSEA

2.7.2 Eddy's Hot Plate Test

Experimental animals were selected and divided into five groups designated as group-I, group-II, group-III, group-control, group- standard and test sample groups were divided into 250mg/kg 500mg/kg , 1000mg/kg respectively. Each group received a particular treatment i.e. control (1% Distilled water), standard (Aspirin 100mg/kg, i.p.) and the test sample (ethanolic extract of 250 mg/kg, p.o. & 500 mg/kg, 1000 mg/kg respectively). The animals were positioned on Eddy's hot plate kept at a temperature of 55 ± 0.5 0C. A cut off period of 15 sec[8] was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples. The initial reaction times of all the animals of control

and test groups were recorded by putting them on the Hot plate. Thereafter, the control group was administered with distilled water while the test group was treated with test drug of various doses. Post treatment reaction time of each animal was recorded at 20 minutes interval for 120 minutes.

3. Results and Discussion

3.1 Anti-inflammatory activity

The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. The percentage protection of Ethanolic extracts was 85.12% at 1000µg/ml. It possesses significant activity comparable with that of the standard Diclofenac sodium *Cassia purpurea* has significant anti-inflammatory activity which may be due to presence of chemical profile such as Flavones, Tri-Terpenoids, Flavonones and tannins.

Table 3: Percentage transmittance values for the ethanolic extract of *Cassia purpurea*

Conct. (mcg/ml	0 min	1 min	2 min	3 min	4 min	*5 min*
50	27.65	28.43	31.86	30.29	27.59	26.23
100	47.11	48.36	49.48	50.67	49.16	47.06
250	60.86	61.25	61.96	62.19	60.38	59.25
500	71.92	72.06	72.88	73.17	71.35	71.26
1000	93.79	93.65	93.25	92.97	92.54	92.03
Diclofenac (250 mcg/ml)	94.86	94.27	93.32	93.01	92.88	92.59

Table 4: Effect of *Cassia purpurea* on HRBC membrane stabilization

Concentration(mcg/ml)	Percentage Stabilization
50	96.48
100	98.03
250	98.44
500	98.71
1000	99.00
Diclofenac(250mcg/ml)	99.99

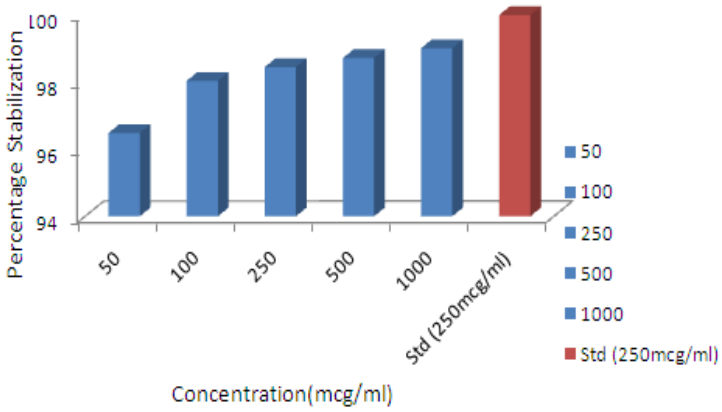

Fig 1: Effect of *Cassia purpurea* on HRBC membrane stabilization

3.2 Analgesic Activity

3.2.1 Eddy's Hot Plate Test

Aspirin produced a significant ($p<0.01$) increase in the reaction time at 30, 90, 120 and 150 min following administration as compared with control. The Ethanolic extract shows significant analgesic activity.

Table 5: Analgesic effects of Leaves of *Cassia purpurea* on albino mice by Hot plate Method

Gr.	Treatment	Avg. Reaction				
		0 min	30 min	90 min	120 min	150 min
I	Ethanolic extract (250 mg/kg)	5.85	7.24	8.08	10.23	13.56
II	Ethanolic extract (500 mg/kg)	6.29	8.08	13.65	14.29	17.87
II	Ethanolic extract (1000 mh/kg)	6.45	13.8	16.58	18.45	20.23
Control	Saline solution	6.2	6.43	5.19	5.01	5.16
Standard	Aspirin(100mg/kg)	6.47	12.81	18.23	19.35	21.79

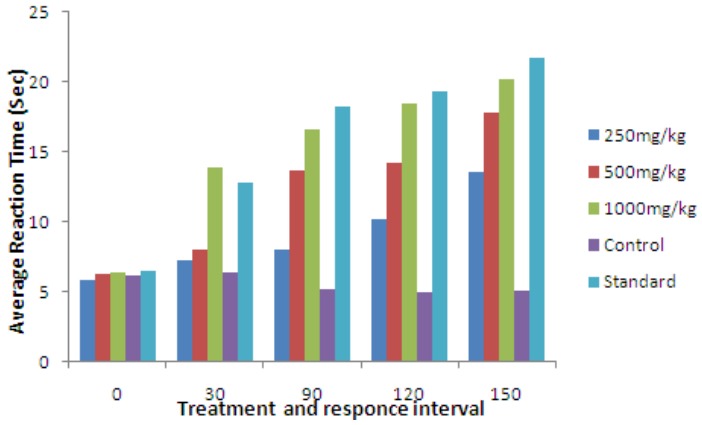

Fig 2: Effect of Ethanolic extract of *Cassia purpurea* by Eddy's Hot Plate

4. Conclusion

In vitro studies on *Cassia purpurea* demonstrate suppression of both inflammation and pain. The ethanolic extracts of the leaves of *Cassia purpurea* must contain some principles, which possess anti-inflammatory, analgesic activities. From the preliminary screening study, it showed the presence of steroids, Flavonones, and, Tri-Terpenoids,. Hence proper isolation of the active principles might help in the findings of new lead compounds in the fields of and anti-inflammatory drug research. Studies related to active constituents on lipid derived eicosanoids, enzyme expression (COX-2, lipoxygenase) and cytokines are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity. Hence it can be used as a potent agent against it.

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