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Analgesic Activity and Anti-Inflammatory Activities of The Ethanolic Extract of Cassia *Auriculata* Leaves

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ABSTRACT
Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs present well known side and toxic effects. More over synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Interest in natural sources to provide treatments for pain, palliatives or curatives for a variety of maladies or recreational use reaches back to the earliest points of history. *Cassia auriculata* is used in *Ayurveda* for the treatment for antidiabetic**,** antiviral and anti spasmodic activities. The ethanolic extract of *Cassia auriculata* leaves has been reported for various biological activities like skin diseases, anticancer, laxative and purgative.In the present investigation focus to evaluate the analgesic and anti-inflammatory activities of ethanolic extact of *Cassia auriculata* leaves. Acute toxicity studies showed that the ethanolic extract is safe up to 3g/kg body weight. The phytochemical screening of ethanolic extract showed the presence of alkaloid, flavonoid, flavone glycosides, saponin and protein. The results of the analgesic activity carried out by Eddy's hot plate and tail flick methods showed that the ethanolic extract have significant analgesic activity ($p<0.001$) compared to control. The result of anti-inflammatory activity studies using carageenin induced hind paw edema method reveal that ethanolic extract showed to possess significant activity ($p<0.01$) in reducing rat paw edema volume at both the tested levels (300mg and 500mg/kg body weight). Flavonoids are known to target prostaglandins which are involved in the late phase of pain perception and acute inflammation and by its direct inhibition of inflammatory mediators and enzymes. Hence, the presence of flavonoid in *Cassia auriculata* leaves extract may be contributory to the analgesic and anti inflammatory activity.

Keywords: *Cassia auriculata; Flavonoid; analgesic; anti-inflammatory*

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INTRODUCTION
According to World Health Organization (WHO) more than 80 % of the world's population relies on traditional medicine for their primary health care needs[1].The medicinal value of plants is attributed to only few active constituents that produce a definite physiological effect. Alkaloids, flavonoids, tannins and phenolic compounds form a major group of such bioactive constituent [2]. Plants and herbs have attained a significant role not only as therapeutic agent but also as health maintaining agent. Any injury of infection is associated with inflammation which has a critical role in the defense and healing processes. Herbal medicines have long histories of use relative ease of administration, relatively low cost and excellent safety records and can be appealing alternatives to routinely used NSAID'S [3]. Available clinical evidence suggests that some herbs such as Aegle marmelos (Rutaceae), Ageratum conzoides (Asteraceae), Curcuma longa (Zingiberaceae), Butea monosperma (Leguminosae), Calligonum comosum (Poligonaceae), Caralluma tuberculata (Asclepidaceae), Cassia spp. (Caesalpinaceae), and Centaurea cyanus (Asteraceae) can significantly reduce the inflammation [4]. *Cassia auriculata* is generally found in warm and moist climate. In India it is found in the western region. As considered to the other parts of the globe is ground in southern parts of Pakistan and certain parts of Africa. Its habitat revolves around the tropical region *Cassia auriculata* is found throughout central and southern India, Also cultivated in Punjab, Haryana, Uttar Pradesh, Maharashtra, Gujarat, Rajasthan and Madhya Pradesh and west Bengal. The shrub usually occurs on roadsides, waste line, and railway Bankments. Leaves are anthelmintic and also used to treat ulcers, Skin diseases and leprosy. An aqueous extract of leaves possesses hypoglycemic activity[5].

Cassia auriculata profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and opthalmia[6]. It is one of the principle constituents of 'Avaarai panchaga chooranam'- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level[7]. The antidiabetic activity of aqueous extract of *C. auriculata* flowers has been documented previously[8]. Our *in vitro* studies revealed that the water-soluble fraction of ethanol extract has more antioxidant potential than aqueous extract of *C. auriculata* flowers i.e. the potential of scavenging the free radicals by water-soluble fraction of ethanol extract has more efficient than the aqueous extract. It is known that free radicals formation is elevated in diabetes and its complications[9].

C*assia* species are rich sources of polyphenols, anthraquinone derivatives, flavanoids and polysaccharides [**10,** 11, 12], saponins, tannins and steroids**[13].** Some of the *Cassia* species are rich in glycerides with linoleic, oleic, stearic and palmitic acids[**14,** 15]. *Cassia* species are well known for their laxative and purgative constituents, and are also used for the treatment of skin diseases[**16].** Their medicinal properties are due mainly to the content of hydroxyanthraquinone derivatives[**17].** Although *Cassia* species have been used widely to treat diseases, they have shown marked toxicity to man and livestock resulting in fatalities following overdoses of remedies involving the plants[**18].**

MATERIALS AND METHODS

Collection of Plant Material

Cassia auriculata leaves were collected freshly from Gulbarga District, Karnataka, India in the month of Janaury. The plant was identified and authenticated at the Herbarium of Botany Department in Gulbarga University Gulbarga and voucher specimen (HGUG 222) was deposited. The collected leaves were shade dried and them powered to the 22mesh size and stored in an airtight container.

Method of Extraction

The powdered leaves of *Cassia auriculata* were subjected to sequential Soxhlet extraction with Petroleum ether (40-60^{0}C), chloroform, ethanol and distilled water. The extracts thus obtained were concentrated and evaporated under reduced pressure and controlled temperature. The presence of phytoconstituents inCassia auriculata leaves exract are summarised in table 1.

Animals

All the experiments were carried out with male albino rats aged seven to eight weeks (180-250 g), obtained from the Central Animal House, Luqman College of Pharmacy, Gulbarga, Karnataka, India. The animals were housed in polypropylene cages and provided with water and standard pellet diet (Karnataka Agro Food Corporation Limited, Bangalore, India). The animals used in the present study were approved by the institutional animal ethical committee (IAEC).

Acute Toxicity Study

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD_{50} of the ethanolic extract. The acute toxicity study was carried out in adult female albino mice by "up and down" method (OECD-425 guidelines). Swiss albino mice of either sex weighing 18-25 g were used for the study. The alcohol extracts were administered orally to different groups of overnight fasted mice at the doses of 300,500, 1000 and 3000 mg/kg body weight. And isolated compound administered orally to different groups of over night fasted mice at the doses of 10, 50, 200, and 500mg/kg body weight. After administration of the extracts and isolated compound, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs. Further the animals were under investigation up to a period of one week[19]. The toxicity study showed that the ethanolic extract at a minimum dose of 300mg/kg onwards shows the reaction in experimental animals and isolated compound at a minimum dose of 10 mg/kg onwards shows the reaction in experimental animals. However, no mortality was reported even after 72hours. This indicates that the ethanolic extract is safe up to a single dose of 3g/kg and isolated compound is safe up to 100mg/kg body weight.

Selection of doses

For the assessment of analgesic activity, two dose levels were chosen in such a way that, the first dose is approximately one ten of the maximum dose during acute toxicity study and second dose may be approximately double of the minimum dose.

Analgesic activity

Analgesic activity of ethanolic extract and isolated compound of *Cassia auriculata* leaves were studied by Eddy's hot plate and Tail flick method.

Eddy's hot plate method

Principle

In this method heat is used as a source of pain. Animals were individually placed on a hot plate maintained at constant temperature (55 ^{0}C) and reaction of animals, such as paw licking or jump response was taken as the end point. The test compound if analgesic increases the reaction-time. The method was first described by Eddy and Leimbach (1953).

a) Analgesic activity of ethanolic extract

The animals were divided into four groups of 6 animals each.
Group I served as control.
Group II served as standard and were injected with ketoprofen (3 mg/kg) intraperitonially.
Group III and IV were treated orally with ethanolic extract of 300 and 500 mg/kg body weight respectively.
The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds to avoid tissue damage. The results of the studies are compiled in Table and graphically depicted in figure

Tail flick method [20]

Principle

In the labortory commonly used procedures are tail-flick (tail withdrawal from the radiant heat) method using analgesiometer. The prescreened animals (reaction time: 3-4 second) were divided into groups as shown in Table. Ketoprofen 3 mg/kg acted as the standard drug. The drugs were administered orally. The tail flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 second to avoid tissue damage.

a. Analgesic activity of ethanolic extract:

The animals were divided into four groups of 6 animals each.
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Group III and IV were treated orally with ethanolic extract of 300 and 500 mg/kg body weight respectively.

b. Anti-inflammatory activity by Carrageenan – Induced oedema method:

Principle

The anti-inflammatory activity was evaluvated by carrageenan induced rat paw edema method. Thereby measuring the carrageenan induced inflammation by plethysmometer. Inflammation is response of the tissue to an infection, irritation or due to any foreign substance e.g. carrageenan. The term inflammatory reaction refers to the events that occur in the tissue in response to an invading pathogen (disease causing organism) or the presence of noxious stimulant. The carrageenan induced rat paw edema is standard method for testing anti-inflammatory drugs. One of the cardinal signs of inflammation is the presence of edema.

Method

Paw oedema was induced by injecting 0.1ml of 1% Carrageenan in physiological saline into the subplantar tissues of the left hind paw of each rat. The extracts of cassia auriculata leaves (300 & 500 mg/kg) were administered orally 30 min prior to Carrageenan administration. The paw volume was measured at 60, 120, 180, 240 minutes by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the control group. Ketoprofen (3 mg / kg orally) was used as standard.

RESULT AND DISCUSSION

Phytochemical investigation

Results of qualitative chemical investigation of leaves of Cassia auriculata indicates the presents of following active principles in various extracts.

Table 1: Phytoconstituents present in the leave of *Cassia auriculata.*

		Tests	Inference		
			Ethanolic	Pet.ether	Chloroform
1.	Alkaloids	(a) Dragendroff' test	+ve	-ve	+ve
		(b) Hager's test	+ve	-ve	+ve

2.		(c) Wagner's test	+ve	-ve	+ve
		(d) Mayer's test	+ve	-ve	+ve
3.	Carbohydrates	(a) Anthrone test	+ve	+ve	+ve
		(b) Benedict's test	+ve	+ve	-ve
		(c) Fehling's test	+ve	+ve	-ve
		d) Molisch's test	+ve	+ve	-ve
4.	Starch	Iodine test			-ve
5.	GLYCOSIDE	Keddes test	+ve	-ve	-ve
		Killer killani test	+ve	-ve	-ve
6.	Flavanoids	(a) Shinoda's test	+ve	-ve	-ve
		(b) lead acetate test	+ve	-ve	-ve
		(c) Ferric chloride test	+ve	-ve	-ve
7.	Triterpenoids (a) Libermann-Burchard's test		-ve	-ve	-ve
8.	Resins			-ve	-ve
9.	Saponins		+ve	+ve	-ve
10.	Steroid	(a) Libermann Burchard's test	-ve	-ve	-ve
		(b) Salkowaski reaction	-ve	-ve	-ve
11.	Protein	Millon test	+ve	-ve	-ve
		Biuret test	+ve	-ve	-ve
12.	Tannins	Ferric chloride test	+ve	-ve	-ve

Eddy hot plate method

The result obtained from Eddy hot plate method showed that ethanolic extract at both the tested dose level posses analgesic activity that beginning from 30 minutes reading itself. The ethanolic extract tested at 300mg/kg body weight showed equipotent analgesic activity compare to ketoprofen (3mg/kg body weight) where are the ethanolic extract tested at 500mg/kg body weight showed to posses analgesic activity throughout that was the study superior in both test to ketoprofen (3mg/kg body weight).

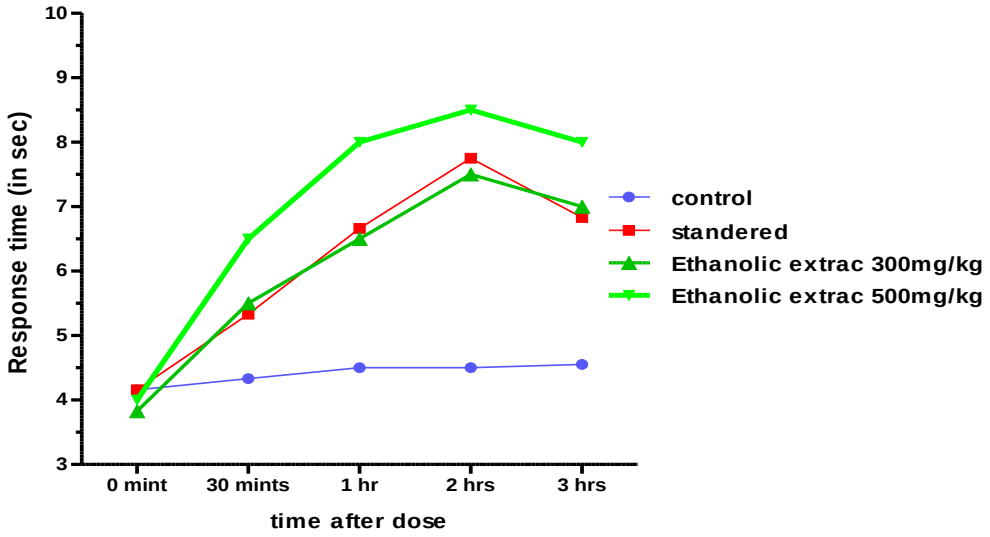
Table2: Analgesic activity of the ethanolic extract of Cassia auriculatsa leaves in rats by Eddy's hot plate method

N==6 in each group *P<0.0001 compared to control

Drug	**DOSE**	**Response Time (In Sec)**				
		0 mints	**30 mint**	**60mint**	**2hrs**	**3hrs**
Control	1ml/kg	4.16±0.30	4.33±0.24	4.50±0.25	4.50±0.18	4.55±0.19
Ketoprofen	3mg/kg	4.16±0.30	5.33±0.42	6.66±0.42	7.75±0.25	6.83±0.40
Ethanolic Extract	300mg/kg	3.83±0.21	5.50±0.42*	6.50±0.50**	7.50±0.50***	7.00±0.44***
Ethanolic Extract	500mg/kg	4.00±0.28	6.50±0.42**	8.00±0.36***	8.50±0.42***	8.00±0.63***

Group	Drug dose mg/kg, orally	Predrug (mean ± SEM reaction time (in sec)	Response time(in second)			
			30 min	1hr	2hrs	4hr
Control	1ml/kg	3.43±0.18	3.33±0.16	3.90±O.85	4.46±0.14	4.20±0.12
Ketoprofen	3mg/kg	3.68±0.25	4.41±0.14	7.01±0.11***	7.75±0.08***	6.50±0.14***
Cassia ariculata leaves extract	300mg/kg	3.66±0.17	3.91±0.14***	6.50±0.33***	6.33±0.29***	5.60±0.27***
Cassia ariculata leaves extract	500mg/kg	3.43±0.29	4.55±0.24*	7.55±0.46**	8..50±0.29***	7.93±0.20***

Figer 13: Analgesic activity of the ethanolic extract of Cassia auriculata leaves by Eddy's hot plate method in rats



2 Tail flick method

The analgesic activity of the ethanolic extract of *Cassia auriculata* leaves using Tail flick method at both the tested doses showed that these were less potent when compared to standard ketoprofen; even though the activity shown was significant when compared to control. The result of tail flick method for the ethanolic extract revealed that maximum activity has been shown at 2nd hrs of study at all the tested dose level that may be pertaining to the half life of the active principal present in the ethanolic extracted .

Table no10 Analgesic activity of the ethanolic extract of *Cassia auriculata* leaves on tail flick response in rats
N=6 in each group; *P<0.0001 compared to control

Anti inflammatory activity

The inhibitory activity of ethanolic extract of cassia auriculata leaves (300mg/kg and 500mg/kg body weight) and its isolated compound (10mg/kg and 20mg/kg body weight) were study on Carrageenan – Induced oedema method using Diclofenac sodium as standard (3mg/kg body weight). The result of the study revealed that even though the ethanolic extractt all the tested dose level posses significant anti-inflammatory activity were found to be potent than the standard Diclofenac sodium.

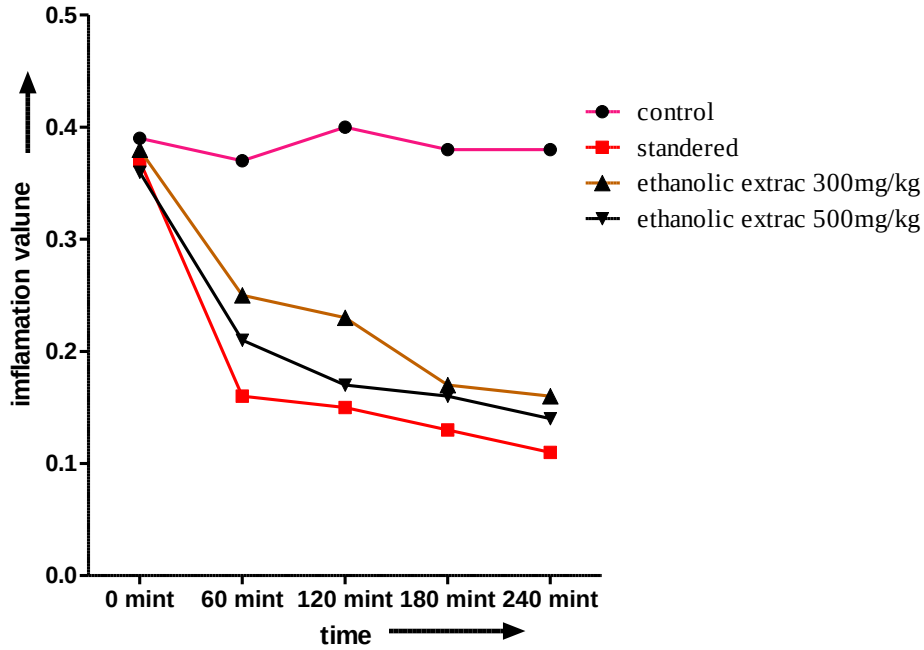
Table12: Anti-inflamatory activity of the ethanolic extract compound of *Cassia auriculata* leaves in rats

Drug	Dose (mg/kg)	Carageenan induced odema (Volume in ml)			
		60 min	120min	180min	240 min

Control	1ml/kg	0.37±0.20	0.40±0.01	0.38±0.01	0.38±0.01
Diclofenac	3mg/kg	0.16±0.01* (56.75)	0.15±0.01* (62.50)	0.13±0.01* (65.78)	0.11±0.01* (71.01)
Cassia ariculata leaves extract	300mg/kg	0.25±0.01* (32.43)	0.23±0.01* (42.50)	0.17±0.01* (55.26)	0.16±0.004* (57.89)
Cassia ariculata leaves extract	500mg/kg	0.21±0.01* (43.24)	0.17±0.01* (57.50)	0.16±0.01* (57.89)	0.14±0.01* (63.15)

N==6 in each group *P<0.0001 compared to control

Figer 18: Anti-inflamatory activity of the ethanolic extract of Cassia auriculata leaves in rats



CONCLUSION

The present study was undertaken to investigate extraction, phytochemical study and evaluation of analgesic and anti-inflammatory activity of *Cassia auriculata* leaves.

The dried leaves were subjected for soxhlet extraction using solvents sequentially according to increase in their polarity. The crude ethanolic extract was subjected to phytochemical screening to determine active constituent present in it.The phytochemical studies of *Cassia auriculata* leaves show the presence of alkaloid, flevonoid, flavon glycoside, saponin presence along with protein.The ethanolic extract at the tested dose level (300mg/kg and 500mg/kg body weight) was screened for analgesic and anti inflammatory activity. The analgesic activity with the higher dose of ethanolic extract was the more potent than the standard ketoprofen. whereas low dose as shown equipotent activity.

By observating anti inflammatory activity result is revealed that at both tested dose level inhibitory activity on Carrageenan – Induced hind oedema method was found to be significant but less potent than standard Diclofenac.

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