



ANTI-INFLAMMATORY, ANALGESIC AND SKELETAL MUSCLE RELAXANT ACTIVITIES OF *CASSIA FISTULA* LEAVES EXTRACTS

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Abstract

Bioprospecting is the systematic and organized search for the products derived from bio resources like plants, microorganisms and animals. These products can be further improved or commercialized for the welfare of the society. Phytotherapy is the use of natural extracts from medicinal plants as medicines or as to promote health. It involves the methodical inspections of the plant extracts to scout the molecules that are potent in their actions against various diseases to cure or alleviate the symptoms. The studies included the pharmacological activities like, anti-inflammatory, analgesic and skeletal muscle relaxant activities for the alkaloid fraction prepared using chloroform, methanol and water separately for the extraction from the leaves of *Cassia fistula*. For all these studies eight groups of animals comprising six in each group were set. First group was normal control, second group was the group treated with standard drug and the animals in groups from third to eight were treated with extracted alkaloid fractions using chloroform, methanol and water. The doses were 100mg/kg and 200mg/kg body weights for each type of extract. The anti-inflammatory activity and analgesic effects of the alkaloid fractions from the *Cassia fistula* leaves support the efficacies of the extracts as anticancer in action. Of the three alkaloid extracts, the one prepared using methanol for extraction had relatively higher anti-inflammatory (55.93% and 59.32%), analgesic (70.08% and 71.50%) and skeletal muscle relaxant activities (76.83% and 78.51%) at both the doses (100mg/kg and 200mg/kg) respectively compared to chloroform and water fractions. Also these results were significantly closer to the standard drugs used in the respective studies.

Keywords: Anti-inflammatory, Analgesic, Skeletal muscle relaxant, Diclofenac, DMSO

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Introduction

The fact that inflammation and pain are closely linked to cancer has been well accepted by the studies and research. Above all the risk of cancer is increased in chronic inflammation and hence elimination of inflammation and pain are considered to be important strategies in cancer treatments (Balkwill and Mantovani, 2001). Various malignancies are demonstrated to be significantly related to molecular mediators that induce inflammation like various cytokines (Kany et al., 2019). Hence the study on cancer treatment and drug development include the studies on anti-inflammatory and analgesic substances that reduce

the inflammation and pain respectively (Won Ho and Keyong Ho, 2017).

This work comprises the secondary studies of *Cassia fistula* leaves extracts. By using TLC, alkaloids were partially from chloroform, methanol and water extracts of *Cassia fistula*. These fractions were evaluated for anti-inflammatory, analgesic and skeletal muscle relaxant activities in animal models using Swiss albino mice and rats of either sex. These alkaloid extracts were injected at two different concentrations 100mg/kg and 200mg/kg body weights.



Carragenan induced edema was reduced significantly in mice injected with the alkaloid fraction of methanol extract than chloroform and water extracts at all the doses studied. The same fraction in addition showed analgesic and skeletal muscle relaxant activities. The maximum inhibition of acetic acid induced writhing responses closer to diclofenac sodium used as a reference drug. The skeletal muscle relaxant activity was 14% less than standard diazepam. Hence in all these *in vivo* studies the alkaloids from methanol extract, showed promising role of alkaloids in the conducted pharmacological studies.

Materials and Methods

Plant Material

Leaves were separated from the *Cassia fistula* plants and sprayed with ethanol to avoid the contamination. Then shade dried at room temperature for few days. The dried leaves were crunched, crushed and powdered with the help of mixer grinder and used for alkaloid extraction in different solvents (Haleshappa et al., 2020, Patil et al., 2014).

Chemicals and Reagents

All the chemicals were of the standard grades and procured from Sigma Aldrich.

Compound Preparation

Stock solutions were prepared by dissolving 200mg/ml of the crude extracts in DMSO (0.5% by volume). The stock solution was then diluted to the required concentration during treatment.

In Vivo Studies

Animals

The Swiss albino mice and rats of either sex weighing 20-25g and 150-220g respectively were used for the experiment. The animals were reared in polypropylene cages and maintained under standard laboratory conditions throughout the study. The standard conditions were 12h light/dark cycle, with a controlled temperature of 24±2°C. The animals were fed with standard rodent pellet feed and water *ad libitum* in an animal house approved by the committee for the purpose of control and supervision on experiments on animals. All the study groups include 6 animals (Patil and Patil, 2011, 2012; Kamble et al., 2017).

Ethics Statement

All the methodologies of animal experiments and their handling were performed in accordance to the guidelines of animal use and care. The experiments had the efforts to minimize the total number of animals and their suffering (Simon and Robin, 2007).

Acute Toxicity studies

OECD guidelines (Guideline no. 425) were followed to acute toxicity studies. Three animals of same sex were used in each group. *Cassia fistula* leaf extract was administered to each group at 10, 50, 300, 2000mg/Kg body weight respectively. The animals were fasted overnight before the administration of extract. Animals were observed regularly for 14 days for any sign and symptoms of toxicity. These drug dosages are as per the OECD guidelines (No. 425) (OECD, 2008; Patil et al., 2010, 2011). An acute toxicity study of leaf extract did not show any mortality of animals at the dose of 1000mg/Kg body weight. Hence doses of 100mg/Kg and 200mg/kg body weights were selected for investigation.

Anti-inflammatory Activity

To evaluate the inhibition of inflammation, edema 11136 was induced to all the eight groups by injecting 0.1ml of 1% carrageenan into the sub planter tissue of the right hind paw after measuring the initial right hind paw volume of each rat (Manjith et al., 2010).

The animals were divided into eight different groups as follows:

Group 1: Served as control and received 5% DMSO

Group 2: Received diclofenac,
Dose: 40mg/kg, served as a standard

Group 3: Received the alkaloid fraction from chloroform extract,
Dose: 100mg/kg body weight

Group 4: Received the alkaloid fraction from chloroform extract,
Dose: 200mg/kg body weight

Group 5: Received the alkaloid fraction from methanol extract,
Dose: 100mg/kg body weight

Group 6: Received the alkaloid fraction from methanol extract,
Dose: 200mg/kg body weight

Group 7: Received the alkaloid fraction from water extract,
Dose: 100mg/kg body weight

Group 8: Received the alkaloid fraction from water extract,
Dose: 200mg/kg body weight

$$\% \text{ Inhibition} = \left(1 - \frac{V_t}{V_c}\right) 100$$

Where V_t = Volume of test, V_c = Volume of control

Analgesic Activity

In mice (weighing 25-30g) the abdominal constriction was induced by intraperitoneal injection of 1%v/v acetic acid (2.3ml/kg), 30 minutes before acetic acid dispense (Asie et al., 2016). Animals were pretreated with two different doses (100mg/kg and 200mg/kg) of the alkaloid fractions of chloroform, methanol and water

$$\% \text{ Analgesic activity} = \left(1 - \frac{\text{No. of writhing's (drug/standard)}}{\text{No. writhing control}}\right) 100$$

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Skeletal Muscle Relaxant Activity

Rota rod is a horizontal rubber coated metal rod of about 50 cm, has 3cm diameter, put at a rotation of 25 rpm. The metal rod is above the surface to prevent the animal from jumping off the roller. On the revolving rod the mice were placed. The initial basal reading of the number of rotations covered by each animal before falling from the rota rod was recorded. 1hr before the test and standard compound was administered placing the rats on the rod. The number of animals falling from the rod during this period was counted. The animals falling from the rota rod within the test period was calculated for every test and standard drug concentrations and compared (Jayasree et al., 2015).

Mice were divided into 8 groups consisting of 6 animals each.

1st group received normal saline (1ml/kg body weight), 2nd group received diazepam (4mg/kg) which is reference standard. Group 3rd to 8th sequentially received the alkaloids from chloroform, methanol and water extracts at the concentrations of 100mg/kg and 200mg/kg body weights.

Statistical Analysis

Values for anti-inflammatory activity were expressed as 'Mean increase in paw volume \pm SEM'. For analgesic activity the values were expressed as 'Mean increase in writhing response \pm SEM'. For

Using plethysmometre at 0h, 1h and 3h paw volumes were measured for each group. The % inhibition of inflammation was calculated using the formula,

extracts. Control group received 5% DMSO and reference analgesic drug diclofenac sodium (10ml/kg) to positive control group. For a period of 20 minutes, numbers of abdominal constrictions were cumulatively counted and % of analgesic activity was found out using the formula,

skeletal muscle relaxant activity the values are expressed as 'Mean number of differences for fall in time before and treatment with the drug \pm SEM'. ANOVA followed by Tukey-Kramer Multiple Comparisons. In all the groups n=6. The significance of difference between means was determined by student's t-test value of $P < 0.05$ as significant and $P < 0.01$ and $P < 0.001$ as highly significant. For *in vitro* pharmacological studies, all the values are expressed as Mean \pm SEM. The experiments were conducted in triplicates. One way ANOVA was done using Graphpad Prism 5.1. Differences were regarded as significant when p value was less than 0.05.

Results

Anti-inflammatory Activity

In edema test induced by carrageenan, there was reduction in paw volume at all concentrations of the alkaloid from chloroform, methanol and water extracts. As against the standard anti-inflammatory drug, which showed the highest inflammation reduction (76.7%), the extracts showed a significant reduction in inflammation. Of the three extracts, methanol at 200mg/kg, had the highest reduction in inflammation (59.32%). Methanol extract at 100mg/kg showed 55.93%, chloroform extract at 100mg/kg showed 47.45% and at 200mg/kg showed 49.15%, water extract at 100mg/kg showed 22.03% and at 200mg/kg showed 23.72% reductions in inflammation (Table 1).



Treatment	Dose	Paw volume			Paw volume difference after 3h (in ml)	% Inhibition
		0h	1h	3h		
Control 5% DMSO	5ml/kg	0.70 ± 0.61	1.18 ± 0.01	1.29 ± 0.01	0.58 ± 0.02	---
Diclofenac	40mg/kg	0.65 ± .01 ^{***}	0.83 ± 0.01 ^{***}	0.79 ± 0.01	0.14 ± 0.01	76.27
Chloroform extract	100mg/kg	0.65 ± 0.01	1.02 ± 0.01	1.01 ± 0.01	0.31 ± 0.01	47.45
Chloroform extract	200mg/kg	0.65 ± 0.01 ^{***}	1.03 ± 0.01 ^{***}	1.00 ± 0.01 ^{***}	0.30 ± 0.01	49.15
Methanol extract	100mg/kg	0.60 ± 0.01	0.98 ± 0.01	0.86 ± 0.02	0.26 ± 0.02	55.93
Methanol extract	200mg/kg	0.60 ± 0.01 ^{***}	0.97 ± 0.01 ^{***}	0.84 ± 0.01	0.24 ± 0.03	59.32
Water extract	100mg/kg	0.68 ± 0.01	1.12 ± 0.01	1.10 ± 0.01	0.46 ± 0.01	22.03
Water extract	200mg/kg	0.69 ± .01 ^{***}	1.10 ± .01 ^{***}	1.12 ± 0.01 ^{***}	0.45 ± 0.01	23.72

Data were analyzed using student's t-test, n=6. All the values are expressed as mean ± SEM.
^{***} p<0.001 when compared with normal control.

Table 1: Anti-inflammatory activity of alkaloids fractions from chloroform, methanol and water extracts compared with controls (DMSO and Diclofenac)

Analgesic Activity

5% DMSO injected to mice served as the control. The standard drug diclofenac showed 76.92% reduction in pain at 40mg/kg body weights. The study on analgesic activity of *Cassia fistula* alkaloid fractions from different extracts when compared with standard analgesic drug diclofenac indicated the close values of reduction in pain with reference the analgesic activity of 76.92%. The alkaloids fraction of methanol extract had 70.08% and 71.50% analgesic activity at 100mg/kg and

200mg/kg body weights respectively and was relatively highest among all. The chloroform extract had 65.01% and 67.23% analgesic activity and the water extract showed 64.38% and 63.67% analgesic activity at 100mg/kg and 200mg/kg body weights respectively. These data suggested that the methanol extract showed highest analgesic activity of all extracts whose value is close and comparably significant to that of standard drug diclofenac (Table 2).

Skeletal Muscle Relaxant Activity

The muscle relaxant activity as evaluated using the extracts and compared to standard muscle relaxant drug diazepam, revealed that the methanol extract had highest activities of all the extracts though relatively less than standard drug. At 100mg/kg and 200mg/kg concentrations, methanol extract had 76.83% and 78.51% relaxant activity respectively, whereas chloroform extract had 77.14% and 77.20% and water extract had 75.57 % and 75.55% relaxant activities at same concentrations respectively (Table 3).

Discussion

A common symptom associated with the progression of cancer is inflammation. The cells that exhibit inflammation are genetically stable. There are other extrinsic factors which stimulate inflammation like alcohol intake, tobacco smoking but these factors in turn are connected to cancer triggering. Inflammation is systemic and local tissue response (Aggrawal et al., 2006; Patil et al., 2013). This was observed by Rudolf Virchow, in 19th century who hypothesized that presence of leukocytes in tumours are the possible links connecting inflammation and cancer.



Group	Treatment	Dose	Number of Writhing responses	% Analgesic effect
Group 1	Control 5% DMSO	5ml/kg	7.8± 0.514	---
Group 2	Diclofenac	40mg/kg	1.8± 0.26	76.92
Group 3	Chloroform extract	100mg/kg	4.9± 0.515	65.09
Group 4	Chloroform extract	200mg/kg	4.6± 0.56 ^{***}	67.23
Group 5	Methanol extract	100mg/kg	4.2± 0.895	70.08
Group 6	Methanol extract	200mg/kg	4.0± 0.80 ^{***}	71.50
Group 7	Water extract	100mg/kg	5.0± 0.96	64.38
Group 8	Water extract	200mg/kg	5.1± 0.90 ^{***}	63.67

Data were analyzed using student's t-test, n=6. All the values for the number of writhing responses are expressed as mean ± SEM. ^{***}p<0.001 when compared with a normal control.

Table 2: Analgesic activity of alkaloid fractions from chloroform, methanol and water extracts compared with controls (DMSO and Diclofenac)

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Group	Treatment	Dose	Fall in time (sec)		Difference	% of relaxant activity
			Before	After		
Group 1	Saline	5ml/kg	312.15 ± 0.01	---	---	---
Group 2	Diazepam	4mg/kg	312.15 ± 0.01	32.921 ± 0.85	279.22	89.45
Group 3	Chloroform extract	100mg/kg	314 ± 0.10	73.20 ± 0.20	240.80	77.14
Group 4	Chloroform extract	200mg/kg	312 ± 0.12	71.00 ± 0.15	241.00 ^{***}	77.20
Group 5	Methanol extract	100mg/kg	310.10 ± 0.02	70.15 ± 0.01	239.85	76.83
Group 6	Methanol extract	200mg/kg	312 ± 0.05	67.00 ± 0.01	245.10 ^{***}	78.51
Group 7	Water extract	100mg/kg	311 ± 0.16	75.10 ± 0.12	235.90	75.57
Group 8	Water extract	200mg/kg	312 ± 0.15	76.15 ± 0.20	235.85 ^{***}	75.55

Data was analyzed using one way ANOVA followed by pairwise comparison. Values are expressed as mean±SEM. n=6, ^{***}p<0.001 when compared with a normal control.

Table 3: Skeletal muscle relaxant activity of alkaloid fractions from chloroform, methanol and water extracts compared with controls (Saline and Diazepam)



Now we are evident with the above observation by Rudolf Virchow. The chronic inflammation which is a long term response is much harmful and may lead to death of the individual. The earlier studies show that presence of phytochemicals like saponins, alkaloids, polyphenols and steroids reverse the inflammation (Farina et al., 2014; Patil et al., 2013). These facts made us to take up the anti-inflammatory studies *in vivo*, of the *Cassia fistula* leaf extracts containing the partially purified alkaloids of three different extracts. The study model we used was carrageenan induced paw edema in rats. The standard anti-inflammatory drug was diclofenac. The results as evidenced indicated that alkaloid fraction in methanol extract had the highest inflammation reduction followed by chloroform and water extracts. Though the methanol extract has the anti-inflammatory activity less than diclofenac, this study was significant as these crude plant extracts need further purification to isolate and identify the active component responsible for anti-inflammatory activity.

The succeeding study was to evaluate the analgesic activity of the plant extracts. The pain was induced by injecting acetic acid to observe the writhing response and the standard drug used was diclofenac (an analgesic). The analgesic nature of the drugs in cancer therapy is to relieve the pain. The various studies from the plant extracts show that phytochemicals like alkaloids, terpenoids and polyphenols are the molecules that are analgesic and can be used to relieve the pain and also they exhibit antioxidant potential (Kolgi et al., 2021). Our study with the alkaloid fractions from chloroform, methanol and water extracts for anti-inflammatory, analgesic and skeletal muscle relaxant activities suggested that methanol extract had the highest effect. The remaining two fractions –chloroform and water extracts too exhibited the significant activities. In all these studies these extracts relatively had lower effects compared to the standard drugs which can be assigned to the fact that the samples are crude and further refining of the extract and recognition of the active component from *Cassia fistula* leaves could be carried out for the treatment of lowering the inflammation and acute pain in cancer and relaxing the skeletal muscle in general. Similar findings resulted by several researchers to reveal the alkaloid is a potential plant metabolite for various

biological studies (Madan and Kumar 2012; Kamble et al., 2017; Geetha and Surya Ramachandran, 2021; Amala and Sujatha, 2021).

These activities may be attributed to the presence of alkaloids in the plant extracts. Further assessment of the extract is advisable to take up to find out the quantitative phytochemical responsible for these activities and determine the exact biomolecule by the detailed spectroscopy.

Conclusion

By analyzing the above results and discussion we can conclude the *Cassia fistula* harbors the phytochemicals that can be anti-inflammatory, analgesic and muscle relaxant. The anti-inflammatory activity of the plant extracts shows that the further purification of the extracts needs to be taken up to find out the active principles responsible for these pharmacologically important activities. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. The statement has been justified in the current study where the methanol extract of *Cassia fistula* showed maximum total anti-inflammatory, analgesic and muscle relaxant activities compare to the two extracts. Chromatography and spectroscopy will reveal the more evidence for the further investigation to prove the novelty of the plant studies.

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Conflict of Interest

No conflict of interest

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