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Evaluation of *Cassia tora* Seeds for their Antioxidant and Antiulcer Activity

[1]N.S. Gill, [1]A. Sharma, [1]R. Arora and [2]Dr. M. Bali

The present investigation was carried out to evaluate the antioxidant potential using various *in vitro* methods by 1, 1-diphenyl-2-picrylhydrazyl and Hydrogen peroxide. Antiulcerogenic effect of methanolic *Cassia tora* seed extract was evaluated by pyloric ligation and non-steroidal anti inflammatory induced mucosal damage on Wistar Albino rats. The phytochemical screening shows the presence of various phytoconstituents belonging to class of alkaloids, flavonoids, triterpenoids and steroids. The extract showed maximum antioxidant activity i.e., 76.35±0.52 and 70.17±0.14 by 1, 1-diphenyl-2-picrylhydrazyl and Hydrogen peroxide at the dose of 200 μg mL^{-1}, respectively as compared to the standard (ascorbic acid). Further, antiulcer activity was assessed in which the experimental animals were divided into five groups categorized as control, standard, normal and two extract treated groups. Various biochemical parameters such as gastric volume, free and total acidity was recorded from all groups. The reduction of ulcer index as well as gastric acid output in extract treated animals was found to be statistically significant with respect to control animals. The extract exhibited optimum percentage inhibition of 75% by pyloric ligation and 70.31% by non-steroidal anti inflammatory induced ulcer method at concentration of 200 μg mL^{-1}, respectively which was comparable to standard (ranitidine). *Cassia tora* Linn. is well known traditional plant used in Chinese and Ayurvedic medicine. The present study suggests that *Cassia tora* seed extract possessed potent anti-ulcerogenic properties that might be afforded via cytoprotective mechanism by virtue of its antioxidant properties. These results supported the ethnomedical uses of *Cassia tora* seeds in the treatment of antiulcer.

Key words: *Cassia tora*, antioxidant, antiulcer, pyloric ligation, non steroidal anti inflammatory induced ulcer

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For further information about this article or if you need reprints, please contact:

Naresh Singh Gill
Rayat School of Pharmacy,
SBS Nagar,
Punjab Technical University,
Ropar-144533, India

Tel: +91-8146991679

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[1]Rayat School of Pharmacy, S.B.S. Nagar, Punjab Technical University, Ropar-144533, India
[2]Quest Group of Institutes, Jhanjeri, Punjab Technical University, Mohali, India

INTRODUCTION

Number of active constituents is being isolated from plants as 80% of population still relies upon plant based products for their primary health care (Ahmad *et al*., 2002). Antioxidants present in fruits and vegetables are the main factors to decrease the incidence of chronic diseases (Liu, 2003). Free radicals are produced either from normal metabolic processes in the body or external sources such as exposure to radiations etc. (Lobo *et al*., 2010). The imbalance between reactive oxygen species and antioxidant defense system may increase the oxidative burden and damages macromolecules (Jaikumar *et al*., 2010). The nutrients that slow down or retard the oxidation process caused by free radicals are known as antioxidants (Percival, 1998). The oxidative stress leads to the formation of oxygen derived free radicals which are directly involved in ulcerogenesis (Kayode *et al*., 2009).

Several factors are responsible for ulceration which includes increased acid pepsin, inefficient neutralization of bicarbonate. Peptic ulcer is the most common disorder characterized by ulcer formation in stomach and duodenum (Al-Attar, 2011). It occurs due to imbalance between offensive and defensive factors (Kumar *et al*., 2004). Different antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase control the accumulation. The imbalance in enzyme activity leads to accumulation of free radicals which are not properly removed from the body (Tandon *et al*., 2004). The synthetic drugs exhibit different side effects like dizziness, pain, muscular fatigue. Thus, natural products and plant extracts are considered as safer approach for treatment of ulceration (Sood *et al*., 2010). Several plants have already been identified for antiulcer activity like *A. serratus, O. sanctum, D. gagenticum, C. azadirachta* (Dharmani and Palit, 2006).

The genus *Cassia* was widely used in traditional medicine and it has been evaluated for antifungal and antimicrobial activity (Oladunmoye *et al*., 2007). *Cassia tora*, family *Caesalpinaceae* was considered to be potent medicinal agent in Ayurvedic Medicine (Ledwani and Oberoi, 2010). The root and leaves extract has laxative effect. The plant can be employed for various skin diseases against fungal infection, psoriasis (Roopashree *et al*., 2008). The decoction of parts of *Cassia tora* is used as an anticonvulsant, antipyretic, antifungal, anthelmintic (Deore *et al*., 2009). The *Cassia tora* seed oil has been used in folk medicine for many years as analgesic, anti-inflammatory and anti-contractive medicaments. Different species of this family has been explored for antimicrobial activity and main attention was given to roots, stem and leaves but seed part has not yet unexplored. The objective of the present study was to evaluate *Cassia tora* seeds for their antioxidant and antiulcer activity.

MATERIALS AND METHODS

Plant material: The seeds were collected from Bindraban district of Palampur in the month of August 2010. The healthy seeds were authenticated by Department of Botanical and Environmental Science, Guru Nanak Dev University, Amritsar with voucher no-0393/Herb. The seeds were cleaned, washed and powdered by mechanical grinder.

Drugs and chemicals: The drugs and chemicals used were DPPH (1, 1-diphenyl-2-picrylhydrazyl) obtained from Sigma Chemical Co. Methanol, chloroform were purchased from SD Fine, Chem. Ltd, Mumbai. Ranitidine was obtained as free sample from Jackson Laboratories, Amritsar.

Preparation of extract: Extraction was carried out with methanol by cold maceration process for 24 h at room temperature. The solvent was recovered under reduced pressure at rotary evaporator. The crude extract was defatted with hexane and further investigation was done (Yen *et al*., 1998).

Experimental animals: Wistar albino rats of either sex weighing around 150-250 g were purchased from Punjabi Agricultural University, Ludhiana. Animals were maintained under proper conditions and had free access to feed and water *ad libitum*. The food was withdrawn 18 h before the experiment but allowed free access of water. Animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

The experimental study was carried from 6th September 2010 to 21st March 2011.

Phytochemical screening: The phytochemical screening of seed extract was carried out for various constituents like alkaloids, triterpenes, saponins, flavonoids, tannins, carbohydrates amino acids and proteins (Harborne, 1973).

Free radical scavenging activity

1, 1-diphenyl-2-picrylhydrazyl radical: Methanolic seed extract (1.0 mL) of *Cassia tora* (25-200 μg mL^{-1}) was added to 1.5 mL of 1, 1-diphenyl-2-picrylhydrazyl solution (0.05 mM). Absorbance was measured at 517 nm by a spectrophotometer (Shimadzu UV-1700 Pharma spec) after 30 min against ascorbic acid (Gill *et al*., 2011).

Hydroxyl peroxide method: The methanolic extract of different concentration (25-200 μg mL^{-1}) was added to 2.4 mL of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 mL of 43 mM solution of hydrogen peroxide. The absorbance was recorded at 230 nm after 10 min. A blank sample was performed. Ascorbic acid was used as standard drug.

Percentage (%) scavenging of hydrogen peroxide was calculated as follows:

$$\text{Percentage scavenged } (H_2O_2) = \{\frac{(A_c - A_s)}{A_c}\} \times 100$$

Where:
A_c = Absorbance of the control
A_s = Absorbance of the sample

Antiulcer activity

Experimental design for pyloric ligation induced gastric ulcer: Animals were divided into 6 groups, each comprising of 6 rats.

- **Group I:** Administered vehicle (normal saline 0.9% w/v, p.o.) 1h before pyloric ligation on the day of the experiment
- **Group II:** Rats are subjected to pyloric ligation for the induction of ulcer
- **Group III:** Administered standard (ranitidine 50 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of the experiment
- **Group IV:** Administered methanolic extract (150 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment
- **Group V:** Administered methanolic extract (200 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

After 18 h of fasting, ulcers were induced (Roy *et al*., 2010) and the experiment was carried out according to Manoharan *et al.* (2010).

Experimental design for NSAID induced ulcer model: Healthy Wister albino rats of weighting between 160-200 g were taken for the studies. The animals were divided in to four groups, each containing six rats:

- **Group I:** Administered vehicle (normal saline 0.9% w/v, p.o.) 30 min before Indomethacin induced ulcers
- **Group II:** Disease control group administered Indomethacin (25 mg kg^{-1}, p.o.) for the induction of gastric ulcers
- **Group III:** Administered standard (ranitidine 50 mg kg^{-1}, p.o.) 30 min before Indomethacin induced ulcers
- **Group IV:** Administered methanolic extract (150 mg kg^{-1}, p.o.) 30 min before Indomethacin induced ulcers
- **Group V:** Administered methanolic extract (200 mg kg^{-1}, p.o.) 30 min before Indomethacin induced ulcers

The experimental design for NSAID induced ulcer model was performed according to Patidar (2011).

Estimation of gastric volume, free and total acidity, ulcer index in PL model

Gastric volume: Four hours after ligation, stomachs were dissected out and contents were collected into measuring cylinder to measure the volume of gastric content.

Determination of free acidity and total acidity: The gastric contents were centrifuged and subjected to titration for estimation of free and total acidity. The supernatant liquid (1 mL) was pipette out and diluted to distilled water (10 mL). The solution was titrated against 0.01 N of sodium hydroxide using Topfer's reagent (Srikanth and Muralidharan, 2009) as indicator to the end point when solution turned to orange color. Titration was further carried out by adding 1% solution of phenolphthalein till the solution gained pink color. The volume of sodium hydroxide required was noted and was taken as corresponding to total acidity. The sum of two titrations was total acidity (Rajkapoor *et al*., 2002).

Ulcer index: The number of ulcers was counted and scoring was undertaken according to the reported method (Desai *et al*., 1995).

Percentage ulcer protection was calculated using the formula (Takagi *et al*., 1969).

Statistical analysis: All the biochemical results were expressed as Mean±Standard Error of Means (SEM). Data were analyzed by Tukey's multiple range tests using Sigma Stat Version -3.5 software. A probability value of $p<0.05$ was considered to be statistically significant.

RESULTS

Phytochemical screening: The preliminary phytochemical investigation of *Cassia tora* methanolic seed extract showed presence of flavonoids, triterpenoids, proteins, tannins and amino acid (Table 1). Thus, it was further analyzed for various activities i.e., antioxidant and antiulcer activity.

Antioxidant activity: The antioxidant activity of *Cassia tora* methanolic seed extract was carried by 1, 1-diphenyl-2-picrylhydrazyl radical and Hydrogen peroxide methods in different concentrations ranging from 25 to 200 μg mL^{-1} by *in vitro* models. The percentage inhibition of DPPH at 200 μg mL^{-1} was 76.35±0.52 and Fig. 1 illustrates a significant ($p<0.05$) decrease in the concentration of 1, 1-diphenyl-2-picrylhydrazyl radical due to the scavenging ability of methanolic seed extract of *Cassia tora* as compared to standard. In case of H_2O_2 method the maximum scavenging ability was 70.17±0.14 at 200 μg mL^{-1} as shown in Fig. 1. The comparison of both methods showed that *Cassia tora* had significant ($p<0.05$) higher antioxidant activity.

Antiulcer activity: *Cassia tora* seeds methanolic extract was analyzed for its ulcerogenic effect by two models i.e., pyloric ligation and non-steroidal anti inflammatory induced ulcer. In case of pylorus ligation, there was increase in gastric volume, total acidity, free acidity and a decrease in pH in case of control group animals. The extracts produced significant ($p<0.001$) decrease in the ulcer score as compared to control (Table 2). The ulcer index was reduced with ranitidine followed by *Cassia tora* methanolic seed extract (150 and 200 μg mL^{-1}, respectively). There was a significant ($p<0.001$) reduction in gastric volume, free acidity and total acidity in ranitidine (standard) and extract-treated groups as compared to control. The methanolic seed extract of *Cassia tora* showed significant ($p<0.001$) percentage inhibition i.e., 60.40 and 75% at 150 and 200 μg mL^{-1} as shown in Table 3.

In case of Non-steroidal anti inflammatory induced ulcer model, the methanolic seed extract showed a maximum reduction in gastric volume when compared to standard. The extract produced decrease in the ulcer score with inhibition of 58.41 and 70.31% at dose range 150 and 200 mg kg^{-1} as compared to standard. The effect of

Table 1: Phytochemical screening of *Cassia tora* methanolic seed extract

Chemical constituents	Methanol
Flavonoids	+
Amino acids	+
Triterpenoids	++
Sterols	++
Alkaloids	++
Tannins	++
Coumarin glycosides	++

+: Presence of chemical constituent, ++: Maximum presence of chemical constituents

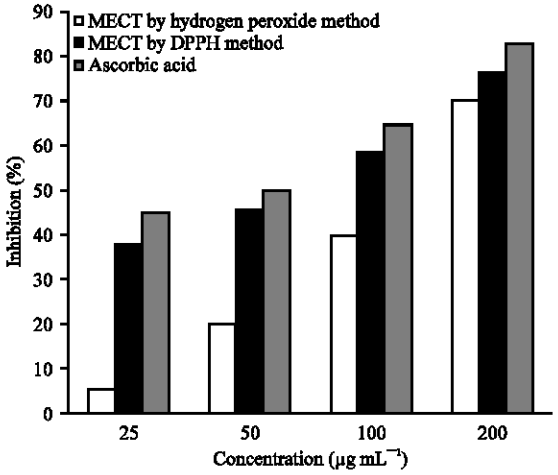

Fig. 1: Comparison of percentage inhibition of *Cassia tora* methanolic seed extract by DPPH method, hydrogen peroxide with the standard (ascorbic acid)

Table 2: Effect on gastric volume, free acidity and total acidity in PL induced ulcers

Group	Treatment	Dose (mg kg^{-1})	Gastric volume (mL/100 g)	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)
I	Normal	--	4.52±0.61	40.56±0.88	75.80±0.38
II	Disease	--	7.21±0.28[a]	59.26±1.64[a]	99.23±3.20[a]
III	Ranitidine	50	1.43±0.15[b]	27.84±2.40[b]	54.34±1.40[b]
IV	MECT	150	2.40±0.18[c]	37.01±1.40[c]	67.83±1.14[c]
V	MECT	200	1.96±0.33[b]	32.75±1.38[b]	60.29±1.04[b]

Values are Mean±SEM, n = 6 animals in each group; [a]$p<0.001$ compared to control group, [b]$p<0.001$ compared to disease treated group, [c]$p<0.001$ compared to ranitidine treated group

Table 3: Effect of *Cassia tora* methanolic seed extract on ulcerative index and percentage inhibition in PL and NSAID

			PL	NSAID		
Group	Treatment	Dose (mg kg^{-1})	Ulcerative	Index	Percentage	Inhibition
I	Normal	--	0.00±0.00	0.00±0.00	0.0	0.0
II	Disease	--	4.80±2.20[a]	6.30±1.11[a]	0.0	0.0
III	Ranitidine	50	0.90±1.02[b]	1.44±0.26[b]	81.25	77.14
IV	MECT	150	1.90±0.60[c]	2.62±0.45[c]	60.40	58.41
V	MECT	200	1.20±0.41[b]	1.87±0.30[b]	75.00	70.31

Values are Mean±SEM, n = 6 animals in each group; [a]$p<0.001$compared to control group, [b]$p<0.001$ compared to disease treated group, [c]$p<0.001$ compared to ranitidine treated group

methanolic seed extract of *Cassia tora* in the gastric lesion induced by NSAID presented very significant ($p<0.001$) decreased values as can be observed in Table 3.

DISCUSSION

Previous researches gave evidence that the whole plant as well as specific parts such as roots, leaves, stem and bark of *Cassia tora* possess various pharmacological activities like antitumor (Rejiya *et al*., 2009), anti-inflammatory (Maity *et al*., 1998), antigenotoxic (Wu and Yen, 2004). The phytochemical studies showed that it contains chrysophanol as marker constituent, others include emodin, quercitin, isoquercitin and β-sitosterol (Chavan *et al*., 2011). The isolation of flavonoids *Cassia tora* by other researchers gave the basis to evaluate seeds for antioxidant activity. The mechanism of action involved in the protection of cellular components against the oxidative stress by methanolic seed extract of *Cassia tora* is not well known. Thus, the models (1, 1-diphenyl-2-picrylhydrazyl and Hydrogen peroxide) were employed to assess the antioxidant activity of *Cassia tora.* The free radical of 1, 1-diphenyl-2-picrylhydrazyl gives strong absorption maxima at 517 nm and is purple in color. When the odd electron of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical gets paired with hydrogen, it gets reduced to DPPH-H which leads to change in color from purple to yellow. In case of hydrogen peroxide method the maximum activity is observed at 200 μg mL^{-1}. The above results showed maximum antioxidant activity i.e., 76.35±0.52 and 70.17±0.14 by 1, 1-diphenyl-2-picrylhydrazyl and hydrogen peroxide method respectively which conclude that extract showed remarkable free radical scavenging activity. Therefore, it was further analyzed for antiulcer activity as the role of free radicals in gastric ulceration is well documented. The extract showed dose dependant antiulcer effect in both pyloric ligation and non-steroidal anti inflammatory method. Excessive production of free radicals degrades the epithelial membrane components and alters the cellular and genetic components (Demir *et al*., 2003). The extract showed decline in ulcer score with percentage inhibition of 75% by pyloric ligation and 70.31% by non-steroidal anti inflammatory method at 200 μg mL^{-1} dose. These results indicate that the extracts of *Cassia tora* seeds have effective anti-ulcer activity against induced stomach lesions. It can be inferred from the above study that methanolic seed extract of *Cassia tora* can be used as potent antioxidant and antiulcer agent.

CONCLUSION

The study has proved that *Cassia tora* seeds show significant scavenging activity and can be used as natural antioxidant. From the above results, it can be inferred that methanolic seed extract showed marked antioxidant activity. Therefore, anti ulcer activity was analyzed by pylorus ligation induced gastric ulcer and Non steroid anti-inflammatory induced ulcer which revealed potent antiulcer effect as compared to standard. These results give pharmacological evidence and support to *Cassia tora* seed as potent antioxidant and antiulcer agent. Further, studies can be carried out to assess its toxic and non-toxic nature of the drug.

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