CCl_4 Induced Hepatotoxicity and It's Recovery by Leaves and Seed Extract of *Cassia Fistula*

***Anil Kumar and R.K. Singh**

Department of Medical Microbiology and Medical Lab. Technology, Sardar Bhagwan Singh University, Balawala, Dehradun, India.

***Email: anilpoojagupta@gmail.com**

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Abstract-The present communication is based on herb, namely, *Cassia fistula*. belonging to family Fabaceae (Caesalpiniaceae) for the prevention of intoxication induced with a sublethal dose of Carbon tetrachloride (CCL_4). Male mice with a dose weighing around 6.5 mg/kg body weight were exposed to CCl_4, a common toxic agent used for hepatotoxicity. Seed and leaves extracts of *Cassia fistula* were given thereafter to the male mice to check their effect on the toxicity of CCl_4. After several weeks of exposure to these herbal extracts, there was a significant change observed in the biochemical parameters with a significant increase in values of serum oxaloacetatate transferase, serum glutamate pyruvate transferase, gamma-glutamyl transferase, alkaline phosphatase, creatine kinase, creatinine, glucose, and cholesterol. In contrast, no significant statistical difference was perceived in the values of all biochemical parameters in mice treated with leave and seed extracts, except the value of serum cholesterol in mice treated with seed extract which decreased as compared to the control group. These findings suggest the importance of the seeds and leaves and their combined usage against CCl_4 toxicity, thus promising these plant products to be promising therapeutic agents against hepatotoxicity, cardiotoxicity, nephrotoxicity, and metabolic disorders due to CCl_4 toxicity. This also makes the safety of herbal products an important public health issue.

Keywords: Seeds and leave extract of *Cassia fistula*, CCl_4, Biochemical Parameters, Hepototoxicity.

Introduction

India is one of the richest countries among the world in resources of medicinal plants in various sytems like Ayurveda, Siddha, Unani, etc[1,2]. The treatment of patients with these medicinal plants is increasing from previous to till dates[3]. The all medicinal plants contain several biochemical or chemical active substances that show a definite physiological action on human or animal body while applying as Treatment[4]. Some literature show the value and emerging effect of medicinal plant in Ayurveda. In Ayurvedic medicine, the golden shower tree is known as aragvadha, meaning disease killer[5]. The plant aragvadha all species are native to the Indian subcontinent and adjacent regions of Asia, South Africa, Mexico, East Africa and Brazil. It is found in worldwide but it is known as national tree of Thailand and its flower is national flower of Thailand. In India, it is the also state flower of Kerala and of immense importance amongst the Malayali population[6]. *Cassia fistula* is widely grown as ornamental profusely yellow flowering tree in tropical area which blooms in late spring but many times no leaf being seen. A tree 6-9 m high; trunk straight; bark smooth and pale grey when young, rough and dark brown when old; branches spreading, slender. The leaves are deciduous and pinnate with three to eight pairs of leaflets. The flowers are pendulous racemes, slender, pubescent and glaborous 47 cm diameter[7]. Calyx long divided to the base, pubescent; segments oblong, obtuse, corolla yellow, stamens all antheriferous. The fruit is legume with a pungent odor and containing several seeds. The long pods green pods turn black on ripening after flowers shed[8,9]. Pulp is dark brown in colour, sticky, sweet and mucilaginous, odour characteristic, and somewhat disagreeable[10,11]. Drug occurs in flat or curved thick pieces; outer surface smooth to rough with warty patches; greenish grey to red; inner surface rough, reddish with parallel striations; fracture, laminate; odour, sweet and characteristic; taste, astringent[12]. The pods are pendulous, with numerous (40-100) horizontal seeds immersed in a dark coloured sweetish pulp. Seeds are broadly ovate, slightly less in breadth and thick[13]. The plant is widely planted as tree and has a number of common names in various languages from its native range surrounding regions such as in English (golden shower cassia, Indian Laburnum, golden shower, pudding pipe tree, purging cassia, purging

Fistula.The fruit and seed pulp and root have immense medicinal value. It is prescribed as emetics, purgatives, febrifuges and relievers of thoracic congestion[12,13,14]. It used in reliving asthma, leprosy, ringworm, fever and heart related diseases. In Ayurvedic medicine system, seed used as antibilious, aperitif, carminative and laxative, root for adenopathy, burning sensations, leprosy, skin diseases, syphilis, and tubercular glands, leaves for erysipelas, malaria, rheumatism and ulcers, the buds for biliousness, constipation, fever, leprosy and skin disease, the fruit for abdominal pain, constipation, fever, heart disease and leprosy [15]. In Unani system, leaves used for inflammation, flowers as purgative, the fruit as antiinflammatory, antipyretic, abortifacient, demulcent, purgative, refrigerant, good for chest complaints, eye ailments, flu, heart and liver ailments, and rheumatism, though suspected of inducing asthma[16] . The entire plant or plant parts are used by several ethnic groups for their tradation, rituals and to cure several diseases from their inherent knowledge. Plant has bright yellow flowers in spring and summer in northern India[17]. There was so many research documented of the hepatoprotective effect of *Cassia fistula*[18,19,20]. Some literature showed the value and emerging effect of hepatoprotective activity of leaf extracts of the *Cassia fistula* [19, 20]. In view of the above literature this study was planned to screen the hepatoprotective activity of both leaves and seed extract of *Cassia fistula.*

Meterial and Methods

Experimental animals

The experiments were done using male albino mice weighing 2431 g. The mice were obtained from the experimental animal house of the institute. The principles and rules of laboratory animal care were followed during all the experiments. The experimental animals were housed in standard polypropylene cages and maintained under controlled laboratory conditions of humidity (65%), temperature (25±1°C), and 12:12 h light:dark cycle, with balanced food and water. They were initially acclimatized for the study, and the study protocol was approved by the Institutional Animal Ethics Committee as per the requirements of Committee for the Purpose of Control and Supervision on Animals, New Delhi.

Leaves and seeds extraction

The new leaves normally appear during March-July and flowers in April to July in India. The long cylindrical pods develop rapidly and reach their full length by October and they ripen during December-March and ripe pods in May. The fresh young leaves and seeds of *Cassia fistula* were directly collected from some tree plantation farms in Hills of Dehradun, Uttarakhand. The leaves and seeds were thoroughly washed and dried at room temperature. The fine quality of dried leaves was kept in a dry plastic container until being used for extract preparation. The collected seeds were rendered free from soil and impurities manually were kept in a dry container until being used for extracts. The dried leaves (10 g) and seeds (30 g) were powdered separately and added to 500 ml cold water and mixed in an electric mixer for 10 min. Thereafter, the solution of leaves and seeds were filtered, and the filtrates were evaporated in an oven at 40°C to produce dried residues (active principles). With references to the powdered samples, the yield of the tea extract was 16.1%. Furthermore, the extracts were weekly prepared and stored in a refrigerator for subsequent experiments. Plant leaves and seeds were then authenticated by Mr. A. Sundriyal, Faculty at Department of Pharmacy, SBS University, Dehradun. While the identification completed, each sample was deposited in the SBS University, School of Pharmacy Dehradun, herbarium with individual voucher number.

Experimental treatments

A total of 48 mice were randomly divided into eight experimental groups of six mice in each group. The experimental groups were treated as follows [18].

1. Mice of Group 1 were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), 5 times weekly, for 7 weeks.

2. Mice of Group 2 were intraperitoneally given CCl_4 at the level of 6.5 mg/kg body weight (1/10 of LD 50), 5 times weekly, for 7 weeks.

3. Animals of Group 3 were orally supplemented with leaves extract of *Cassia fistula* (400 mg/kg body weight) and after 4 h received CCl_4 at the same dose given to Group 2, 2 times weekly, for 7 weeks.

4. Mice of Group 4 were orally supplemented with seed extract of *Cassia fistula* (400 mg/kg body weight) and after 4 h received CCl_4 at the same dose given to Group 2, 5 times weekly, for 7 weeks.

5. Animals of Group 5 were orally supplemented with seed and leaves extract of *Cassia fistula* (200 mg/kg body weight) after 4 h received CCl_4 at the same dose given to Group 2, 5 times weekly, for 7 weeks.
6. Mice of Group 6 were intraperitoneally received saline solution at the same dose given to Group 1 and were orally supplemented with leaves extract of *Cassia fistula* at the same dose given to Group 3, 5 times weekly, for 7 weeks.
7. Mice of Group 7 were intraperitoneally received saline solution at the same dose given to Group 1 and were orally supplemented with seed extract of *Cassia fistula* at the same dose given to Group 4, 5 times weekly, for 7 weeks.
8. Animals of Group 8 were intraperitoneally received saline solution at the same dose given to Group 1 and were supplemented with tea and seed extracts of *Cassia fistula* at the same dose given to Group 5, 5 times weekly, for 7 weeks.

Biochemical analysis

After 7 weeks, mice were anesthetized with diethyl ether. Blood was collected from orbital venous plexus in plain gel tubes and centrifuged at 2500 rpm for 10 min, and serum were then collected and stored at 4°C till the determination time of serum glutamate oxalo transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), creatinine, glucose, total protein, triglycerides, and cholesterol by using Semi-Automated Clinical Chemistry Analysis System, ERBA chem-7.

Statistical analysis

Data were expressed as the mean±standard deviation and were analyzed by one-way analysis of variance using the Statistical Package for Social Sciences (SPSS for Windows, version 12.0). Multiple comparative analyses were conducted between all experimental groups using Tukey's test. Results were considered statistically significant at $p<0.05$.

Results and Discussion

Male albino mice exposed to sub-lethal doses of CCl_4 were treated with *Cassia fistula* leaves, and seed extracts, and their combination, which showed varied levels of biochemical markers in mice serum. Mice treated with CCl_4 (Group 2) showed a significant increase in the values of serum SGOT (84.21±4.21 IU/L), SGPT (106.61±12.10 IU/L), GGT (25.17±3.26 IU/L), ALP (235.80±44.9 IU/L), CK (620.01±25.11 IU/L), creatinine (2.83±1.14 mg/dl), glucose (162.17±36.44 mg/dl), total protein (4.83±0.26 g/dl) triglycerides (172.20±28.51 mg/dl), and cholesterol (201.8±28.1 mg/dl) when compared to control mice (Group 1). The leaves extract of *C. fistula* showed decrease in the values of serum SGOT (41.12±1.12 IU/L), SGPT (50.61±7.21 IU/L), GGT (13.11±2.22 IU/L), ALP (151.34±20.11 IU/L), CK (411.20±22.02 IU/L), creatinine (1.43±0.90 mg/dl), glucose (120.33±20.30 mg/dl), total protein (5.21±0.30 g/dl) triglycerides (98.67±14.6 mg/dl), and cholesterol (140.11±12.37 mg/dl). However the level of these all biochemical parameters are decreased in the group of mice treated with leaves extract of *C. fistula* while compare with treated group of mice with CCl_4 (Carbon tetrachloride) and treated with seed extract of *C. fistula* plus CCl_4. Other treated groups (Tables 1 and 2). However, the level of total serum protein statistically declined in Group 2 treated mice when compared to control and other treated group mice (Table 2). On comparison with the control mice, the mice treated with tea leaves extract plus CCl_4 (Group 3), seed extract plus CCl_4 (Group 4), and leaves and seed extracts of *C. fistula* plus CCl_4 (Group 5) showed a significant increase in values of serum ALT, AST, GGT, ALP, CK, creatinine, glucose, and cholesterol. In addition, some non-significant changes in serum total protein were observed in mice of Groups 3, 4, and 5. The triglycerides level statistically increased in mice treated with leaves extract plus CCl_4 (Group 3), seed extract plus CCl_4 (Group 4), while the levels remain unchanged in mice supplemented with leaves and seed extracts plus CCl_4 (Group 5). In addition, no significant statistical difference was perceived in the values of all biochemical parameters in mice treated with leave extract (Group 6), seed extract (Group 7), and leave and seed extracts (Group 8) except the value of serum cholesterol in mice treated with seed extract (Group 8) which decreased as compared to the control group. Together, the above results indicate that the toxic effect of CCl_4 was much decreased and attenuated with the combinatorial effect of leave and seed extracts of *C. Fistula*.

The values of routine biochemical markers such as

serum ALT, AST, GGT, ALP, CK, creatinine, glucose, triglycerides, and cholesterol were significantly higher, while low serum protein was observed with mice which were exposed only to CCl_4. These results were in concordance with observations of the previous studies which also showed severe alterations in physiological and Biochemical parameters in experimental animals[19,20]. The increase in the levels of ALP, ALT, AST, and GGT seen here indicated liver dysfunction, and these markers have been in use for biochemically monitoring chemical-induced tissue damage [21]. In addition, reports also confirm the release of these biomarkers in bloodstream when the hepatic parenchymal cells are exposed to toxic pesticides like CCl_4 (Carbon tetrachloride)[19,20,21]. These observations were supported by Anil *et al.* in different studies which also showed cardiotoxicity due to paracetamol exposure in experimental animals[18]. This increase indicates the inhibitory action of pesticide on cytochrome P450 enzymes. Furthermore, increased cholesterol concentration indicates liver disorders and cholestasis[19]. *C. fistula*, the traditional plant is proven to have a high content of flavonoids which are phenolic compounds of plants and have been attributed for several functions including many health benefits. These flavonoids are potent antioxidants, and metal chelators which protect cells from reactive oxygen species or free oxygen radicals are a few among Many other good studies which have shown the protective effect of green tea on pesticides and organophosphorus compounds [15,16,19,20,21]. In addition, this study also explored the use of seed extract which has also been studied in the prevention of decrease in antioxidant levels and having an ameliorative effect on hematological, biochemical, and histopathological alterations.

Conclusion

This study is an attempt toward showing the promising role of extracts of *C. fistula* in preventing hepatotoxicity, cardiotoxicity, nephrotoxicity and metabolic disorders induced due to CCl_4. Furthermore, there is a need of biochemical, histopathological, and physiological investigations using different dosages of these crude extracts to prove the plausible role of these extracts in preventing CCl_4 toxicity. In addition, the combinatorial effect of these extracts might also be explored to study their effect on other toxicants and pathogenic moieties.

Table-1 Serum of control and treated mice (with CCl_4, CCl_4+ leave extract of *C. fistula*, CCl_4+ seed estract of *C. fistula*, CCl_4+ leaves and seed extract of *C. fistula*, *C. fistula* leave extract alone, *C. fistula* seed extract alone, and combination of leaves and seed extract of *C. fistula*) showing different concentrations of SGOT, SGPT, GGT, ALP and CK.

S. No	Treatments	Parameters				
		SGOT (U/L)	SGPT (U/L)	G-GT (U/L)	ALP (U/L)	CK (U/L)
1	Control	22.66±1.12	33.33±2.11	6.82±4.21	102.00±4.32	300.5±7.12
2	CCl4	84.21±4.21^{ab}	106.61±12.10^{ab}	25.17±3.26^{ab}	235.80±44.9^{ab}	620.01±25.11^{ab}
3	leave extract of *C. fistula*+CCl_4	41.12±1.12^{a}	50.61±7.21^{a}	13.11+2.22^{a}	151.34±20.11^{a}	411.20±22.02^{a}
4	seed extract of *C. fistula*+CCl_4	44.32±1.21	60.22±12.26^{a}	14.12±2.22^{a}	158.26±38.20^{a}	442.67±16.21^{a}
5	leave extract and seed extract of *C. fistula*+CCl_4	33.20±2.01^{a}	46.60±6.22^{a}	7.00±1.21^{a}	144.61±22.71^{a}	401.22±17.60^{a}
6	leave extract extract of *C. fistula*	23.5±1.72	34.71±3.22	6.24±1.26	114.6±12.12	301.31±22.15
7	Seedextract of *C. fistula*	25.21±1.41	35.41±2.11	7.31±1.82	119.40±8.2	318.24±20.11
8	leave extract+ seed extract extract of *C.fistula*	24.33±1.21	36.42±3.26	6.30±0.56	110±8.4	292.31±14.12

Data represents the means ±SD of 6 animals per group. [a]Indicates a significant difference between the control and treated groups. [b]Indicates a significant difference between mice exposed to CCL_4 and *C. fistula* leaves extract + CCl_4, *C. fistula* seed extract plus CCl_4, leaves and seeds extracts *C. fistula* plus CCl_4, *C. fistula* leave extract, *C. fistula* seed extract and leave and seed extracts *C. fistula* treated mice.

Table-2 Serum of control and treated mice (with CCl_4, CCl_4+ leave extract of *C. fistula*, CCl_4+ seed estract of *C. fistula*, CCl_4+ leaves and seed extract of *C. fistula*, *C. fistula* leave extract alone, *C. fistula* seed extract alone, and combination of leaves and seed extract of *C. fistula*) showing different concentrations of creatinine, glucose, total protein, triglyceride and cholesterol.

S. No	Treatments	Parameters				
		Creatinine **(mg/dl)**	Glucose **(mg/dl)**	Total protein **(g/dl)**	Triglyceride **(mg/dl)**	Cholesterol **(mg/dl)**
1	Control	0.67±0.14	82.12±8.3	5.18±0.43	82.24±4.65	94.80±4.22
2	CCl4	2.83±1.14^{ab}	162.17±36.44^{a}	4.83±0.26^{ab}	172.20±28.51^{ab}	201.81±28.1^{ab}
3	leave extract of *C. fistula*+CCl_4	1.43±0.90^{ab}	120.33±20.30^{a}	5.21±0.30	98.67±14.6^{a}	140.11±12.9^{a}
4	seed extract of *C. fistula*+CCl_4	1.53±0.33^{a}	124.50±11.2^{a}	5.42±0.22	108.60±16.8^{a}	150.01±12.37^{a}
5	leave extract and seed extract of *C. fistula*+CCl_4	1.34±0.48^{a}	148.67±24.5^{a}	5.13±0.29	94.00±5.9^{a}	116.66±8.46^{a}
6	leave extract extract of *C. fistula*	0.68±0.12	85.11±7.23	5.16±0.21	88.00±4.46	96.22±6.22
7	seed extract of *C. fistula*	0.76±0.18	94.93±6.99	5.15±0.32	77.81±4.1	98.46±6.2^{a}
8	leave extract+ seed extract extract of *C. fistula*	0.64±0.14	86.0±0.87	5.13±0.33	80.11±6.4	96.11±11.22

Data represents the means ±SD of 6 animals per group. [a]Indicates a significant difference between the control and treated groups. [b]Indicates a significant difference between mice exposed to CCL_4 and *C. fistula* leaves extract + CCl_4, *C. fistula* seed extract plus CCl_4, leaves and seeds extracts *C. fistula* plus CCl_4, *C. fistula* leave extract, *C. fistula* seed extract and leave and seed extracts *C. Fistula* treated mice. Data represents the means ±SD of 6 animals per group. [a]Indicates a significant difference between the control and treated groups. [b]Indicates a significant difference between mice exposed to CCL_4 and *C. fistula* leaves extract + CCl_4, *C. fistula* seed extract plus CCl_4, leaves and seeds extracts *C. fistula* plus CCl_4, *C. fistula* leave extract, *C. fistula* seed extract and leave and seed extracts *C. fistula* treated mice.

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