



HEPATOPROTECTIVE ACTIVITY OF CAJANUS CAJAN IN SODIUM FLUORIDE TREATED SWISS ALBINO (BALB/C) MICE

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Article Received on
24 July 2015,

Revised on 14 Aug 2015,
Accepted on 05 Sep 2015

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ABSTRACT

The present study is an attempt to demonstrate the role of hepatoprotective activity of aqueous extract of *Cajanus cajan* in sodium fluoride induced hepatotoxicity in mice. Aqueous extract of *Cajanus cajan* leaves is prepared. Normal control rats receive distilled water only while rats in Sodium fluoride control group receive Sodium fluoride (250ppm) only for 30 days. Another group rats receive *Cajanus cajan* extract along with 250ppm Sodium fluoride for 30 days. 4th Group rats receive commercial drug (Liv 52) along with 250ppm Sodium fluoride for 15 days. Sodium fluoride produced significant changes in biochemical (increase in AST, ALT, cholesterol, TBAR level), histological (hepatocytes damage). Treatment with *C. Cajan*

extract and Liv 52 significantly prevented the biochemical and histological changes induced by Sodium fluoride. Extract of *C.cajan* exhibited significant anti oxidant and hepatoprotective activity.

KEYWORDS: Sodium Fluoride, Hepatoprotective activity, *Cajanus cajan*, BAL B/C Mice.

INTRODUCTION

Liver

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion and it is a highly sensitive organ (Patel *et al.*, 2010). It has a surprising role in the maintenance, performance and regulating homeostasis of the body (Ahsan *et al.*, 2009). The major functions of the liver are carbohydrate, protein, and fat metabolism, detoxification,

secretion of bile and storage of vitamin. In order to maintain good health, liver is a crucial factor (Ahsan *et al.*, 2009).

Fluoride

Fluoride is a cumulative poison (Ranjan *et al.*, 2009) and it is highly electronegative anion causing cumulative toxic effects from prolonged ingestion (Khalil *et al.*, 2010). Fluoride is a well-known soil, water, and air contaminant, and its toxicity in humans has been widely studied. Intake of excess of fluoride through drinking water (Rao *et al.*, 2011) is the major source of fluorosis due to geological crust contamination. As a very active site of metabolism, the liver is especially susceptible to fluoride intoxication (Shashi and Thapar, 2000).

Cajanus cajan

Cajanus cajan which is synonymous to *Cajanus indicus* is given different names like red gram, pigeon pea Congo pea and “Tur” by the local people of India. The leaves are pinnately trifoliate and the leaflets are oblong-lanceolate and entire (Patil *et al.*, 2011). *Cajanus cajan* is well known for its hepatoprotective action (Manna *et al.*, 2007). The leaves are traditionally used as tringent, diuretic, laxative, anti-inflammatory and oral ulcers.

MATERIALS AND METHODS

Preparation of Sample

Cajanus cajan leaves were dried in shade and powdered well using blender. Aqueous extract of *Cajanus cajan* was prepared by Soxhlet apparatus and it is used for further estimations.

Animals

Swiss albino male mice were used in the experiments. Mice were maintained under standard husbandry conditions. The study was conducted in conformity with standard experimental animals study ethical protocols.

Experimental design

20 mice were used in the experiments, divided into four groups each containing five mice. The normal control group I received only distilled water for 30 days. The Sodium fluoride control group received only 250ppm NaF (group II) for 30 days. Group III received 250ppm NaF along with plant extract for 30 days. Group IV received 250ppm NaF along with commercial drug (Liv 52) for 15 days.

Biochemical tests**Estimation of Protein**

The protein content was determined by the method of Lowry *et al.*,

Estimation of Blood Glucose

Blood glucose was estimated by the method Dinitrosalicylic Acid by Miller (1959).

Estimation of Serum AST (SGOT)

AST (SGOT) activity was determined by the method of Reitman and Frankel, 1957

Estimation of ALT (SGPT)

ALT (SGPT) activity was determined using 2, 4 dinitro phenyl hydrazine Jhon, 1980.

Estimation of Total Cholesterol

Total cholesterol was estimated by the method of Zak (Zak, 1957).

Estimation of Lipid Peroxidation

Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS).

Estimation of Plasma TBARS

Lipid peroxides in plasma were assayed by the method of *Yagi* (Yagi, 1978).

Estimation of Tissue TBARS

Lipidperoxidation (TBARS) in tissue was estimated by the method of *Ohkawa et al.*, (Ohkawa *et al.*, 1979).

Estimation of Erythrocyte TBARS

TBARS in erythrocytes was estimated by the method of Donnan (Donnan, 1950).

Histological Studies

All animals were sacrificed after 30days .the animal was dissected, Liver and Kidney sample were taken and fixed in 10%buffered formalin. The organs were processed in graded series of alcohol and embedded in paraffin wax. Serial sections of 5µm were cut and mounted on glass slides stained with hematoxylin-eosin stain.

Invitro Antioxidant Assay**Determination of DPPH Scavenging Activity**

Determination of DPPH radical scavenging activity of ethanolic extract was done followed by Blois, 1958.

Determination Of Total Antioxidant.

Ethanolic extract antioxidant activity was determined by Prieto *et al.*, 1999.

Determination of H₂O₂ Radical Scavenging Activity.

The H₂O₂ radical scavenging activity of ethanolic extract was determined by Ruch *et al.*, 1989.

RESULTS

The Hepatoprotective and antioxidant activity of *Cajanus cajan* leaf extract was analyzed in the present study.

Table 1: Level of protein.

GROUP	TREATMENTS	LEVEL OF PROTEIN (µg/ml)
1	Control	43.13±0.64
2	Sodium fluoride (NaF)	40.99±0.61
3	<i>Cajanus cajan</i> (Extract)	43.02±0.50
4	Drug (Liv.52)	42.48±0.51

Table 2: Level of Glucose.

GROUP	TREATMENTS	LEVEL OF GLUCOSE (mg/ml)
1	Control	52.98±0.40
2	Sodium fluoride (NaF)	156.1±0.49
3	<i>Cajanus cajan</i> (Extract)	69.68±0.54
4	Drug (Liv.52)	59.57±0.16

Table 3: Level of SGOT.

GROUP	TREATMENTS	SGOT LEVEL (IU/ml)
1	Control	201.4±0.12
2	Sodium fluoride (NaF)	349.1±0.57
3	<i>Cajanus cajan</i> (Extract)	223.6±0.48
4	Drug (Liv.52)	246.0±0.76

Table 4: Level of SGPT.

GROUP	TREATMENTS	SGPT LEVEL (IU/ml)
1	Control	111.9±0.91
2	Sodium fluoride (NaF)	184.6±0.72
3	<i>Cajanus cajan</i> (Extract)	109.7±0.58
4	Drug (Liv.52)	135.7±0.25

Table 5: Level of Cholesterol.

GROUP	TREATMENTS	LEVEL OF CHOLESTEROL (µg/ml)
1	Control	112.2±0.45
2	Sodium fluoride (NaF)	157.6±0.32
3	<i>Cajanus cajan</i> (Extract)	135.7±0.47
4	Drug (Liv.52)	145.9±1.04

Table 6: Level of Plasma TBAR.

GROUP	TREATMENTS	PLASMA (nmol/ml)
1	Control	105.8±0.55
2	Sodium fluoride (NaF)	111.6±0.51
3	<i>Cajanus cajan</i> (Extract)	107.5±0.45
4	Drug (Liv.52)	108.5±0.80

Table 7: Level of RBC TBAR.

GROUP	TREATMENTS	RBC (pmol/mg)
1	Control	44.35±0.25
2	Sodium fluoride (NaF)	51.45±0.45
3	<i>Cajanus cajan</i> (Extract)	48.84±0.35
4	Drug (Liv.52)	46.71±0.55

Table 8: Level of Tissue TBAR

GROUP	TREATMENTS	TISSUE (nmol/mg)
1	Control	7.701±0.40
2	Sodium fluoride (NaF)	14.60±0.42
3	<i>Cajanus cajan</i> (Extract)	10.65±0.50
4	Drug (Liv.52)	9.59±0.44

Table 9: DPPH scavenging activity

S.No	CONCENTRATION ($\mu\text{g/ml}$)	<i>Cajanus cajan</i> EXTRACT (%)
1	200	42.11
2	400	55.2
3	600	64.56
4	800	69.83

Table 10: Total Antioxidant assay

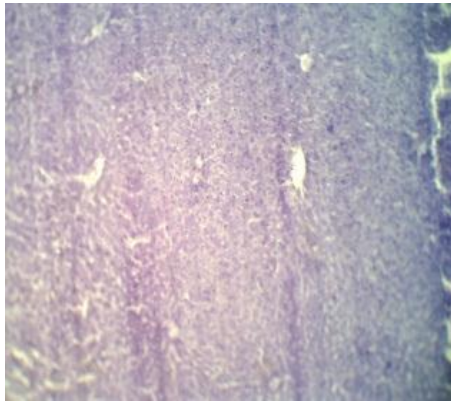
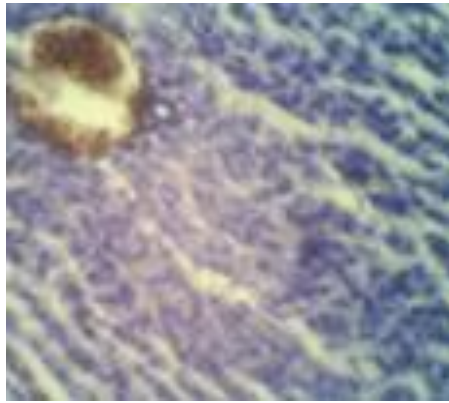
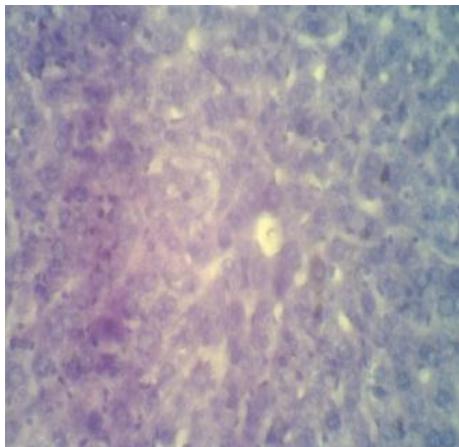
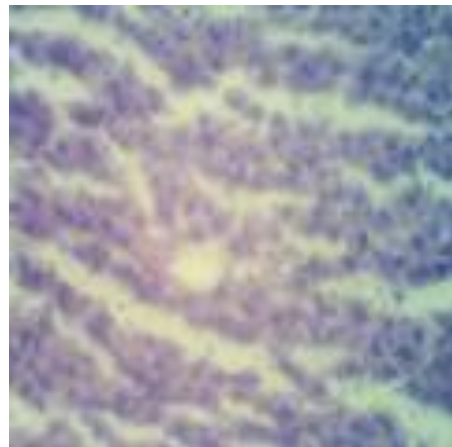
S.No	CONCENTRATION ($\mu\text{g/ml}$)	<i>Cajanus cajan</i> EXTRACT (%)
1	200	49.13
2	400	52.72
3	600	61.69
4	800	64.69

Table 11: H₂O₂ Radical scavenging activity

S.No	CONCENTRATION ($\mu\text{g/ml}$)	<i>Cajanus cajan</i> EXTRACT (%)
1	200	53.3
2	400	60.27
3	600	64.03
4	800	69.94

Table 12: Phytochemical Tests

PHYTOCHEMICALS	RESULTS	PHYTOCHEMICALS	RESULTS
Alkaloids	Present	Sterol	Present
Terpenoids	Present	Amino Acid	Present
Flavanoids	Present	Saponins	Absent
Phenolic compounds	Present	Reducing sugar	Present
Sugar	Absent	Tannins	Present

LIVER SECTIONS**Control****NaF****Extract****Liv 52****DISCUSSION**

The continuous ingestion of *Cajanus cajan* led to a reduction in hepatotoxicity. Due to the induction of hepatotoxicity in mice several changes could be found in the Serum protein level. Serum protein decreased in mice receiving sodium fluoride. There was no elevation in serum protein in mice receiving sodium fluoride along with *Cajanus cajan* and Liv 52. Glucose level was found to be elevated in mice receiving sodium fluoride whereas mice receiving Sodium fluoride along with *Cajanus cajan* and Sodium fluoride along with Liv 52 showed normal level of glucose.

ALT and AST are enzymes which act as biomarkers of liver injury. Increase in level of these enzyme results by destruction of liver cells. Therefore the presence of these enzymes in serum at high level indicates the liver damage in sodium fluoride induced hepatotoxic mice. In the present study, significant increase in these enzymes was found in mice receiving

sodium fluoride alone compared to mice receiving *Cajanus cajan* along with sodium fluoride and Liv 52.

Total cholesterol level showed an increase in mice receiving sodium fluoride but there was no rapid increase of cholesterol level in mice receiving *Cajanus cajan* along with sodium fluoride and Liv 52.

Similarly increase in plasma, RBC TBAR and liver TBAR level in mice receiving sodium fluoride was found. Determination of plasma TBARS is considered as the most reliable marker of tissue damage in various pathological conditions. The increased level indicates the over production or diffusion of lipid peroxidation from plasma, RBC and liver.

The histological studies indicate that the group of mice treated with sodium fluoride showed changes in hepatocytes (Ballooning degeneration). However there was not significant change in mice receiving *Cajanus cajan* and Liv 52. These histopathological findings conform the hepatoprotective activity of *Cajanus cajan*.

Cajanus cajan extract also showed to possess some phytochemical present in it similarly the extract proved to contain high anti-oxidant property.

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