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Evaluation of Anti-Hepatotoxicity of Cassia Tora Flower as Compared to *Cassia Tora* Leaves.

Antima Mansore* *, *Anant Kumar Patel, Dr. P. k Dubey

Swami Vivekanand College of Pharmacy, Indore
Email id: mansoreantima@gmail.com

ABSTRACT:

In the present study assessment of liver damage by carbon tetrachloride, the determination of enzyme activities such as aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) is largely used. Serum activities of AST, ALT are the most frequently utilized indicators of hepatocellular injury. Necrosis or membrane damage releases the enzymes into circulation; and therefore, they can be measured in serum. ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage.In the present study, the CCl_4 treated rats showed a significant elevation in the serum activities of ALT, significantly decreasing the levels of total protein and albumin as compared to the normal control rats, thereby indicating liver damage. Administration of Cassia tora leaf at doses of 5 g/kg, significantly prevented the rise in the levels of the marker enzymes as well as it significantly prevented the decrease in the serum levels of total protein and albumin. The diminished rise of serum enzymes, together with the diminished fall in the levels of total protein and albumin in the extract treated groups, is a clear manifestation of the hepatoprotective effect of the extract. All the variables tested as GSH, ALP, SGOT and SGPT recorded a significant alteration observed in CCl_4 treated rats. However treatment with herbal extract restored the level to near normal was observed. The potential hepatoprotective activity of Cassia toraleaf and flower is due to the presence of phytochemical constitution present in plant. Some of these phytochemicals have possessed hepatoprotective activity.

Keywords : Cassia toraleaf ,liver damage, GSH, ALP, SGOT, Extract.

Introduction :

Hepatotoxin is a toxic chemical substance which damages the liver. Toxic liver injuryproduced by drugs and chemicals may virtually mimic any form of naturally occurring liver disease In some physiological condition mitochondrial membrane can lose its structure and functional integrity by the opening of mitochondrial permeability transitional pore (MPTP) and this can disturb ATP synthesis and storage. Impaired ATP synthesis cause sudden increase in Intracellular calcium level by the activation of plasma membrane calcium ATPase. Sometime MPTP opening also allow release of preapoptotic protein, such as caspases, Cytochrom c and Apoptosis inducing factor. . Large amount of lipid accumulation in hepatocytes can cause macrovaculaor steatosis (Steatohepatitis) which also known as Fatty liver. Therefore a large number of plants and formulations have been claimed to have hepatoprotective activity so the development of plant based hepato protective drugs has been given importance in the global market.

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The liver is a vital organ of vertebrates and some other animals. In the human it is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification various metabolites, protein synthesis, and the production of bio chemicals necessary for digestion. The liver's main job is to filter the blood from the digestive tract, before passing it to the rest of the body. The liver also detoxifies chemicals and metabolizes drugs. As it does so, the liver secretes bile that ends up back in the intestines. The liver also makes proteins important for blood clotting and other functions.

Material and method

The leaf and flowers of the plant Cassia Tora was collected from Local market Indore**.** The plant materials were air dried at room temperature and powdered.

Chemical-chloroform,methanol.ethaylacetate, petroleum ether

Collection &authenticafication of plant material:The fresh leaves and flower was collected by indore ,Madhya Pradesh ,India.the plant was authenticated by Dr.S.NDewedi,Department of Botany,Janta PG College,A.P.SUniversity,Rewa.MadhyaPradesh,India as Cassia tora Leaves(family Fabaceae) in the provided Voucher Specimen Number:J/Bot./2021-0147CSWP;29/12/2021'

EXTRACTION: The shade dried Leaves and Flowers were ground by mechanical grinder into coarse particles using the sieve number 2000µm. The 2 gm ground material was extracted with 250ml of chloroform in a Soxhlet apparatus at 35-40°C until the extract was clear or colourless. Controlled conditions temperatures were maintained to avoid loss of heat sensitive phytochemicals. Extracts were filtered through Whatman No.1 filter and clarified extracts were concentrated in a rotary evaporator under reduced pressure at 40°C. Dried extracts were weighed in an analytical balance. The extracted materials were stored at 4°C until use.

Experimental Design

Body weights of the animals were recorded and they were divided into **4** groups of 6animals each as follows.

Sn.	**Group**	**Treatment**	**Dose**
1.	**Group 1**	Salimariyan	200 mg/kg
2.	**Group 2**	Carbon tetrachloride (CCl_4) in olive oil	1:2,v/v, 1ml of CCl4 i.p./kg
3.	**Group 3**	Cassia tora Leaves	5 g/kg (orally through intragastric tube)
4.	**Group 4**	Cassia tora Flower	5 g/kg (orally through intragastric tube)

Preliminary Phytochemical Screening

The obtained extracts were subjected to chemical investigation using qualitative chemical test in order to identify various phytoconstituents.

Test for Triterpenoids and Steroids

a. **Salkowski's Test** The extract was treated with chloroform, filtered separately and the filtrate was added with little amount of concentrated sulphuric acid. The mixture was then allowed to stand for nearly two minutes after gentle shaking. The formation of red color at the inferior part indicates the occurrence of sterols while golden yellow color may be attributed the existence of triterpenes.

b. Libermann-Burchard Test

Extract was added with few drops of acetic anhydride and boiled. Then the cooled solution was added with sulfuric acid along the side of the reaction vessel. Formation of brown ring occurred at junction of two layers. If the upper layer turned green, it may indicate presences of steroid while development of deep red color would indicate the positive test for triterpenoids.

Tests for glycosides

The test solution was prepared by dissolving the leaf extract in alcohol (90%) or aqueous alcoholic solution.

a. Baljet's test

The test solution treated with sodium picrate gave yellow to orange colour.

b. Legal's test

Alcoholic solution of leaf extract was treated with 1ml of pyridine and 1 ml. of sodium nitroprusside was added to it. Pink to red colour develops.

c. Keller-Killiani test

The test solution was treated with a small amount of ferric chloride solution, mixed and sulphuric acid (containing ferric chloride solution) was added. It formed two layers, lower layer showed reddish brown colour while upper layer might turned bluish green.

d. Borntrager test

To 3ml. extract, added dill. H_2SO_4, boiled and filtered. To the cold filtrate, equal volume of benzene or chloroform was added. The organic solvent was shaken and ammonia was added. Ammoniacal layer might turn pink or red.

Tests for saponins

The test solution was prepared by dissolving the leaf extract in the water.

a. Foam test .

Test solution on shaking showed formation of foam, which was stable at least for 15 min.

b. Haemolysis test

Two test tubes containing 2 ml of 18% sodium chloride were taken and to one test tube added distilled water and to the other, 2 ml test solution. Little amount of blood was added to both the test tubes, mixed and observed for haemolysis under microscope.

Tests for carbohydrates

The test solution was prepared by dissolving test extract with water, hydrolysed with 2N hydrochloric acid and subjected to following tests:

a. Molisch's Test

Approximately 2-3 drops of alcoholic α-naphthol solution was added to the extract and concentrated sulphuric acid (2 ml) was added carefully along the wall of the test tubes. Existence of carbohydrates is confirmed by development of violet ring at the junction of two liquids.

b. Barfoed's Test

1 ml of extract was heated with 1 ml Barfoed's reagent on water bath. Red colour due to development of cupric oxide indicates the occurrence of monosaccharide.

c. Benedict's test

1 ml of extract was heated with 1 ml Benedict's reagent on water bath. Reddish brown colour indicates the presence of monosaccharide.

d. Fehling's test

1ml each of Fehling solution A & Fehling solution B were mixed and boiled for one min. The solution was then added with 1 ml of test solution and heated on boiling water bath for 15 min. Primarily, a yellow followed by brick red colour might be observed.

Tests for alkaloids

The test solution was prepared by dissolving extracts in dilute hydrochloric acid and filtered. Following tests were then performed on the above mentioned extract:

a. Mayer's Test

The extract was added with some drops of potassium mercuric iodide solution (Mayer's reagent). Formation of cream/white precipitate attributes to the presence of alkaloids.

b. Dragendroff's Test

Development of orange yellow precipitate authenticates the presence of alkaloids when the extract is subjected to the treatment of a few drops of potassium bismuth iodide solution (Dragendroff's reagent).

c. Hager's Test

Asaturated aqueous solution of picric acid (Hager's reagent) was added to the extract and allowed to stand for nearly two minutes. Development of yellow colored precipitate suggests the presence of alkaloids.

d. Wagner's Test

The extract was added with some drops of Wagner's reagent (solution of iodine in potassium iodide). Formation of reddish brown precipitates suggests the positive test for alkaloids.

Test for tannins and phenolic compounds

a. Ferric Chloride Test

Small amount of distilled water was added to the extract, shaken and warmed. Two ml. of 5% ferric chloride solution was added to the cool solution and monitored for the formation of green or blue color which may indicate the existence of phenols.

b. Lead acetate Test

A few milligrams of extract was separately stirred with about 2 ml distilled water and filtered. The mixture develops white precipitate when treated with few drops of 10% (w/v) lead acetate solution which may attribute to the occurrence of tannins.

c. Bromine water

A few milligrams of extract was separately stirred with about 2 ml distilled water and filtered. To the filtrate, few drops of bromine water were added andexamined for the decoloration of bromine water which may illustrate the presence of tannins.

d. Dilute iodine solution

A few milligrams of extract was separately stirred with about 2 ml distilled water and filtered. To the filtrate, few drops of dilute iodine solution were added and examined for the development of transient red color, which may assure the existence of tannins.

Isolation

The leaves were shade dried & coarsely powdered. The 1000 gm coarse powder was macerated with ethanol for four hours. Filtered the extract and the filtrate was partitioned with methanol. The methanol fraction was collected & concentrated under vacuum in a rotatory flash evaporator. The residue was dried in the desiccator to get an amorphous powder. This powder was subjected to Column chromatography and TLC studies.

Thin-layer Chromatography (TLC)

Thin-layer chromatography is a technique based on the adsorption of components on a solid support. Two phases namely stationary phase and mobile phase exist in the system. The former consist of a solid porous material which is applied on the solid support and the later is a liquid which runs through the stationary phase. Depending upon the nature of support and the combination of different solvents the technique may separate the mixture by partitioning or sometimes by a combined effect of adsorption and partitioning.

Rf Value

The distance of each spot from the point of its application was measured and RetentionFactor **(**Rf) values were calculated

$$\mathrm{Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Column Chromatography

The column chromatography is used for the separation of large quantity of sample. In this, the adsorbent is packed in a glass tube having glass wool or cotton at the bottom. The mixture of drug present in a solvent is poured on the top of this column. Then mixture of solvent is slowly added. The drug particles move down at different rates depending on their affinity for the adsorbent. If the drugs are colored, then different layers containing these drugs can be easily identified. The column can be extruded and each layer can be separated.

Biochemical Analysis

Estimation of Alkaline Phosphatase (ALP)

Eradication of the phosphate groups form macromolecules like proteins, nucleotides and alkaloids is termed as dephosphorylation and is executed by a hydrolase enzyme Alkaline phosphatase (ALP, ALKP). The hydrolysis of phosphate esters is catalyzed by ALP which generates an organic radical and an inorganic phosphate in an alkaline atmosphere. The enzyme is found principally in bones and liver in the mammals and its hyperactivity may lead to the disease hyper alkaline phosphatasemia, which is characterized by marked increase in serum ALP levels and may lead to several other complications including primary biliary cirrhosis, hepatic lymphoma, malignant billiary obstruction, sarcoidosis and primary sclerosing cholangitis. An elevated level of alkaline phosphate is observed in hepatic and bone disorders which may be considered as preliminary counsel of severe health troubles.

Result and Discussion

Solvent Extraction

Test Name	Cassia tora Leaves	Cassia tora Flower
For Carbohydrate Molish's test	–	+
For Reducing Sugar Fehling's test	+	–
Benedict's test	–	–
Barfoed test	–	–

Test For Steroids Salkowski test	+	+
Liebermann's burchard	+	+
Test For Cardiac Glycoside Baljet's test	+	+
Legal's test	+	–
Keller's killani test	–	–
Test for saponins Foam test	–	+
Heamolysis test	–	+
Test for Anthraquinone Glycoside Borntrager's test	–	–
Modified Borntrager's test	+	+
Test For Flavonoids Shinoda test	–	–
NaOH Test	+	+
Alkaloids Test Dragendorff's test	–	+
Mayer's test	–	–
Hager's test	+	+
Wagner's test	+	+
Tannins and phenolic compound 5% Fecl3 test	+	+
Dil. Iodine test	+	+
Dil. HNO3 test	+	+
Bromine water test	+	+
Terpenoids Salkowski's test	+	+
Libermann-burchard test	–	–

Chloroform Solvent Extraction

Sn.	Extract (200gm)	Colour	Weight (gm)	% yield
01.	Cassia tora Leaves	Yellowish Green	11.16	5.58 %
02.	Cassia tora Flower	Yellow	4.22	2.11 %

Phytochemical Screening

Qualitative tests for various phytochemical constituents were executed on the leaf and flower extracts and the results.

Isolation of compounds

In TLC three Rf value are obtained and The column chromatography showed 5 fraction both values shoved in table 5 and 6.

Table 5. TLC of Cassia tora leaves extraction.

Plant Extract	Solvent System Used	Spots	Rf value	Color	
				Iodine Chamber	UV light
Cassia Tora	Chloroform: Methanol	1	0.91	Dark Brown	Brown
		2	0.96	Dark Yellow	Yellow
		3	1.0	Dark Green	Green

Table 6. Column chromatography of Cassia tora leaves extraction.

Plant Extract	Solvent System Used	Fraction obtained	Fraction Obtained (g)	Color
Cassia Tora	Chloroform: Methanol	CT-1	0.75	Brown
		CT-2	0.80	Light Brown
		CT-3	0.44	Yellow
		CT-4	0.30	Light Green
		CT-5	0.10	Green

Hepatoprotective activity

The present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the Cassia torainvestigated a summarized in Table.

The present study was carried out to evaluate the Hepatoprotective activity of Cassia torain rats. The observations made on different groups of experimental animals were compared as follows.

Table represents the levels of protein in serum of normal and experimental rats. Group II CCl_4 intoxicated rats showed a significant decreased in the level of protein when compared toGroup I rats. Group III CCl_4 intoxicated rats treated with Cassia toraleaf significantly increased in the level of protein when compared to group II.

Effect of Cassia toraleaf and flower on protein in experimental rats

Parameters	Group I	Group II	Group III	Group IV
Cassia tora Leaves	7.43 ± 1.33	5.53 ± 0.96*	6.98 ± 0.90**	7.98 ± 0.90**
Cassia tora Flower	3.96 ± 0.64	2.39 ± 0.58*	3.42 ± 0.51**	4.42 ± 0.51**

table represent the Cassia toracaused significant elevation in the serum ALP level when compared to normal group, while on treatment with ethanolic extract of Cassia toraleaves extract.

Effect of Cassia toraleaf on ALP level of rats

Parameters	Group I	Group II	Group III	Group IV
ALP (IU/L) Leaves	41.70±1.952	114.8±0.735	57.02±0.681	55.02±0.681
ALP (IU/L) Flower	41.70±1.952	114.8±0.735	57.02±0.681	54.02±0.681

CONCLUSION

In the assessment of liver damage by carbon tetrachloride, the determination of enzyme activities such as aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) is largely used. Serum activities of AST, ALT are the most frequently utilized indicators of hepatocellular injury.. All the variables tested as GSH, ALP, SGOT and SGPT recorded a significant alteration observed in CCl_4 treated rats. However treatment with herbal extract restored the level to near normal was observed. The potential hepatoprotective activity of Cassia toraleaf and flower is due to the presence of phytochemical constitution present in plant. Some of these phytochemicals have possessed hepatoprotective activity.

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