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Hepato-protective and Antioxidant Activity of Extract of *Coccania Grandis* (Roots) induced by CCl_4 in Rats

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Abstract

In the present study an attempt has been made to find out Heptoprotective and Antioxidant Activity of Extract of Coccania Grandis (Roots) Induced By Cc4 In Rats. There are many chemical agents that cause hepatotoxicity and these agents called Hepatotoxins. These cause hepatotoxicity by the generation of free radicals and damage the liver cells and cause of m any liver diseases. Further works are being carried out to isolate and identify the active principle involved in the hepatoprotective and antioxidant activities of this plant extract. The present study has demonstrated that(EECT)(100 and 200 mg/kg) exhibited significant dose-dependent hepatoprotective activity against liver injury induced by CCl4. Carbon tetrachloride induces hepato-toxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi- normal metabolic function.

Carbon tetrachloride is metabolically activated by the cytochrome P450 in the endoplasmic reticulum to form a trichloromethyl free radical (ccl3.) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation, which leads to change in the structures of endoplasmic reticulum and other membrane, loss of metabolic enzymes activation, reduction of protein synthesis and elevation of serum transaminases leading toliver damage. Amino transferases contribute a group of enzymes that catalyze the interconversion of amino acids and α-ketocids by the transfer of amino group. Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease. The assessment of liver function can be made by estimating the activities of serum enzymes such as ALT, AST and ALP. During hepatic damage, there may be increase in these enzyme levels in serum with the extent of liver damage. The altered levels of these enzymes in CCl4-treated rats in the present study corresponded to the extensive liver damage induced by the toxin.

Keywords: Heptoprotective, Antioxidant Activity, Extract, Coccania Grandis, Roots, CCl_4, Rats.

Introduction

Liver is the largest and the only human internal organ that actually can regenerate itself. In fact, the liver is capable of natural regeneration of lost tissue as little as 25% of remaining liver can regenerate into whole liver again.

Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins, lipids and excretion of waste metabolites.

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Additionally, it is also handling the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other things, plays an important role in digestion. Hepatic disease (Liver disease) is a term that affects the cells, tissues, structures, or functions of the liver. Liver has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion and synthesis as well as breakdown of small and complex molecules, many of which are necessary for normal vital functions. The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Therefore, maintenance of a healthy liver is essential for the overall well-being of an individual. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide etc., chronic alcohol consumption and microbes is well-studied [(1)].

Hepatotoxicity:

Hepatotoxicity implies chemical-driven liver injured. Convinced medicinal agent when in use in overdoses and sometimes even when introduced within beneficial ranges, may possibly damage the organ. Additional chemical agent, such as those used in laboratories (e.g. CCl4, paracetamol, alcohol) and industries (e.g. lead, arsenic), natural chemicals (e.g., microcystins, aflatoxins) and herbal remedies (Cascara sagrada) can also induce hepatotoxicity. Chemical that effect liver injury is known as hepatotoxins. Further 900 drugs have been occupied in causing liver injury.

Chemicals that cause liver injury are called hepatotoxins. These agents be renovate in chemically immediate metabolities in liver, which have the ability to interconnect with cellular macromolecules such as protein, lipids and nucleic acids, leading to protein dysfunction, lipid per oxidation, DNA damage and oxidative stress. This damage of cellular function can dismiss in cell death and likely liver failure [(2)]

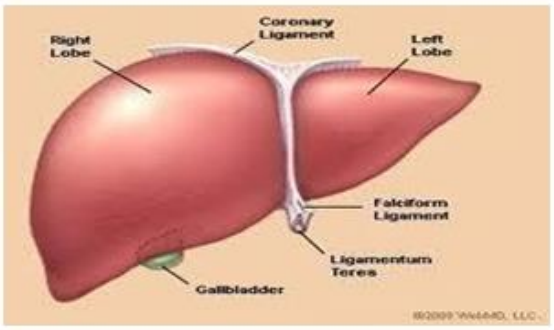

Fig 1: Histopathological alteration in hepatic damage

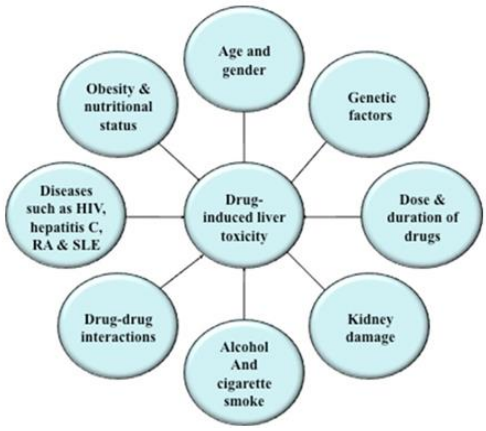

Fig 2: Factors affecting drug-induced hepatic toxicity

Various factors, such as , advancing age, gender, lifestyle factors, obesity, nutritional status, genetic background, dose, and duration of drugs may increase the risk of drug mediated hepatotoxic reactions.

Causative Agents of Hepatotoxicity

Causative Agent	Product	Type of Hepatotoxicity

Antibiotic Antibiotic	Amoxicillinclavulanate Macrolidesketolides erythromycin	hepatocellular, cholestatic or mixed hepatocellular-cholestatic hepatitis.[3,4] cholestatic pattern of injury with evidence of portal and bullous inflammation, eosinophilia and mild hepatocellular necrosis
Antibiotic	Pyrazinamide	Centrolobular cirrhosis and cholestasis.
Antibiotic	Rifampicin	Cholestatic hepatitis
Industrial Toxin	Carbon tetrachloride	Centrilobular necrosis.
Industrial Toxin	Mercury	Interference of bile excretion and destruction of hemoglobin
Medicinal Plant	*Larreatridntata*	Fulminant hepatitis, subacute hepatic necrosis, cholestatic hepatitis, acute liver failure
Pharmaceutical Drug	Corticosteroids or glucocorticoids and anabolic androgenic steroids	Glyogen storage in liver, enlarged liver.
Pharmaceutical Drug	Non-steroidal anti-inflammatory drugs	Acute, cytolytic, cholestatic or mixed hepatitis[5-9]

Mechanism of hepatotoxicity

Liver is the main organ for metabolism and elimination of drugs. Majority of drugs (oral) and xenobiotics are lipophilic which enables their easy absorption across intestinal membranes. They are rendered hydrophilic *via* hepatic metabolism and are easily excreted. Exogenous products are metabolized in the liver mainly through phase I and II reactions.[10] The hepatic metabolism involves Phase I and Phase II reactions. Phase I involves oxidative, reductive, hydroxylation and de-methylation pathways, primarily by way of the cytochrome P-450, the most important family of metabolizing enzymes system which is located in the endoplasmic reticulum in the liver. Phase I reactions mostly produce toxic intermediates which are transformed to non-toxic compounds by phase II reactions, usually considered as detoxification pathways. Phase II reactions involve the conjugation of chemicals with hydrophilic moieties such as glucuronide, sulfate or amino acids and lead to the formation of more water- soluble metabolite which can be excreted easily.[11] Further Phase II reaction implicates glutathione which can covalently bind to toxic intermediates by glutathione-S- transferase[12]. However, this phase can also lead to the formation of unstable precursors of reactive species that can cause hepatotoxicity.

The main role of liver is to metabolize xenobiotics and is the common link with gastrointestinal tract. Liver is highly vulnerable to damage from drugs and some other substances. Nearly 75% blood that reaches liver comes from gastrointestinal organ and spleen *via* portal veins, therefore the drugs and xenobiotic are in concentrated form. The liver injury occurs due to activation of drugs to chemically active metabolites. They have a tendency to react with cellular macromolecules like proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage, and oxidative stress.

Mechanism of liver injury can be classified as pathophysiological or through chemical induced mechanism.[13-14]

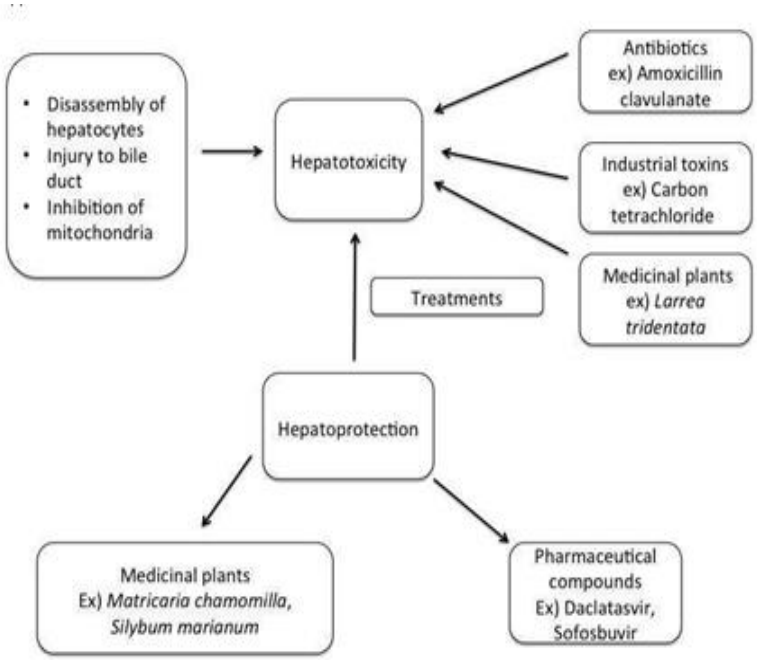

Fig 3: Mechanisms and treatments for hepatoprotection and hepatotoxicity

Material and Methods

Materials Used in Experimental work

Chemical/Kit	**Drugs**
Chloroform	CCl4
Methanol	Olive Oil
Petroleum ether	Silymarin
Kits	Methanolic plant extract
Biuretic Kit for protein estimation (Span diagonistic kit)	
Serum glutamate oxaloacetate transaminase (SGOT)	
Serum glutamate pyruvate transaminase (SGPT)	
Serum alkaline phosphates	
Serum Bilirubin	

Collection and Authentication

The plant coccania grandis (roots) were collected from local area of SRK University (Bhopal).

Physico-Chemical Analysis

A. Determination of Ash

The ash remaining after the ignition of medicinal plant material is determined by 3 different methods which measure total ash, acid insoluble ash and water soluble ash.

- The total ash method is to measure the total amount of material remaining after ignition.
- Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the insoluble matter. This measure the amount of silica present, especially as sand and siliceous earth.
- Water soluble ash is the difference in weight between the total ash and residues after treatment of the total ash with water.

Total ash

Place about 2-4g of the ground dried material, accurately weighed, in a previously ignited and tared crucible. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600^{0}C until it become white, indiacating the absence of carbon. Cool in a desicator and weigh. If carbon free ash cannot be obtained in this manner, cool the crucible and the residue with about 2 ml of water or a saturated solution of Ammonium nitrate. Dry on water bath, then on hot plate and ignite to constant weight. Allow the residue to cool in a suitable desicator for 30 min, and then weigh about without delay.

Calculate the total ash in mg per g of air dried material.

Calculation:

$$\text{Total ash \%} = \frac{(W_2 - W_1)}{W} \times 100$$

Where W = Weight of sample, W_1 = Weight of empty crucible, W_2 = Final weight of crucible

a) Acid insoluble ash

To the crucible containing the total ash, 25 ml of dil. HCl will be added and covered it with watch glass and boiled gently for 5 min. then the watch glass will be rinsed with hot water and this liquid will be added to the crucible. The insoluble matter on ash less filter paper will be collected and filter will be washed until the filtrate become neutral. The filter paper containing insoluble matter will be transferred to the original crucible, will be dried on hot plate and ignited to constant weigh. The residue will be allowed to cool in a suitable desiccator for 30 min and weighed without delayed. The content of acid insoluble ash is calculated in mg per gm of dried material.

Calculation:

$$\text{Acid Insoluble ash \%} = \frac{(W_2 - W_1)}{W} \times 100$$

Where W = Weight of sample, W_1 = Weight of empty crucible, W_2 = Final weight of crucible

B) Water soluble ash

To the crucible containing the total ash, 25 ml of dil. HCl will be added and boiled for 5 minutes. The insoluble matter in a sintered glass crucible will be collected or on ash less filter paper. Then it will be washed with hot water and crucible is ignited for 5 min at temperature not exceeding 450C. Subtract the weight of the residue in mg from the weight of total ash. The content of water soluble ash will be calculated in mg per g of air dried material.[54]

Determination of Moisture content:

Sample preparation: Seeds will be broken into smaller size and the use of high speed mills will be avoided in the preparation of sample.

Procedure : About 10 gm of drug will be taken in a evaporating dish and dried in the hot air oven at 105^{0}C for 5 hour and weighed , continue the drying and weighed after 1 hour interval until difference between two successive weighing correspond to not more than 0.25% constant weight. Constant weight is reached when two consecutive weightings after drying for 30 min, in a desicator, show not more than 0.01 gm difference. Then the percentage of loss on drying will be calculated with reference to the air dried drug.

Calculation:

$$\text{Loss on drying \%} = \frac{W - (W_2 - W_1)}{W} \times 100$$

Where W = Weight of sample, W2= Weight of empty dish, W3= Weight of sample after drying

Evaluations of crude drug

S. No.	Parameter of crude drug	Result%(w/w)
1	Total ash	4.44
2	Water insoluble ash	0.89
3	Water soluble ash	1.32
4	Moisture content	3.9

Extraction method:

Petroleum ether: The shade dried coarse powder of the leaves will be packed in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with petroleum ether for 18 hours or till the clear extraction will be obtained. The extract will be filtered while hot and the resultant extract will be distilled in vacuum under reduced pressure in order to remove the solvent completely. It will be dried and kept in a desiccators till experimentation. Obtained extract will be weighed and percentage yield will be calculated in terms of air-dried.
powdered crude material.

Chloroform extract: Merck left after petroleum ether dried below 50^0C (hot-air oven) . and then packed well in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with chloroform for 18 hours or till clear extraction obtained. The extract filtered while hot and the resultant extract will be distilled in vacuum under reduced pressure in order to remove the solvent completely. It will be dried and kept in a desiccators till experimentation. Obtained extract will be weighed and percentage yield will be calculated in terms of air-dried powdered crude material.

Methanol extract: Mark left after chloroform dried below 50^0C (hot-air oven).and then packed well in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with methanol for 18 hours or till clear extraction obtained. The extract filtered while hot and the resultant extract will be distilled in vacuum under reduced pressure in order to remove the solvent completely. It will be dried and kept in a desiccators till experimentation.

Aqueous extract: The marc left after methanol extraction will be dried completely in hot- air oven below 50^0C. The dried material was packed in a percolator. The aqueous extract was obtained by cold maceration of the successive marc in water. The mare was dip in distilled water for three days. The extract was then collected through tape of the percolation. The excess water will be evaporated on water bath. The resultant extract will be distilled in vacuum under reduced pressure in order to remove the aqueous solvent completely. It will be dried and kept in a desiccators till experimentation. Obtained extract will be weight and percentage yield will be calculated in terms of air- dried powdered crude material [(55)].

Procedure for Plant Extraction:-

- Coarse plant leaf used for extraction with petroleum ether (60-80^{o}C) for 18hrs
- Extract filtered while hot and distilled in vacuum to remove solvent and dried
- Weight and calculate % yield
- Mark left after petroleum ether dried below 50^{o}C (hot air oven)
- Continuous extraction by methanol (18hrs)
- Extract filtered and dried
- Weight and calculate % yield

Phytochemical Analysis

Tests for Alkaloids

0.5 gm of extract will be dissolved in 10 ml of dilute Hydrochloric acid (0.1 N) and filtered and this filterate will be used to test the presence of alkaloids.

Mayer's test: Filtrate will be treated with Mayer's reagent. Formation of yellow cream coloured precipitate will indicate the presence of alkaloids.

Mayer's reagent:

a) 1.36 gm of mercuric chloride dissolved in 60ml of distilled water.

b) 5 mg of potassium iodide dissolved in 20 ml of distilled water, and (a) & (b) will mixed together and volume will be adjusted to 100ml with distilled water.

Dragendroff's test: filtrate will be treated with Dragndroff's reagent. Formation of reddish brown coloured precipitate will indicate the presence of alkaloids.

Dragendroff's reagent:

a) 8 gm of bismuth nitrate dissolve in 20 ml of nitric acid.

27.2gm of Potassium iodide dissolved in 50 ml distilled water, and (a) & (b) will mixed together and volume will be adjusted to 100ml with distilled water.

Wagner's test: Filtrate will be treated with Wagner's reagent. Formation of reddish brown coloured precipitate indicate the presence of alkaloids

Wagner's reagent: 1.27 g of iodine and 2 g of Potassium Iodide dissolved in 5ml of water and this solution will be diluted to 100ml with water.

Hager's test: Filtrate will be treated with Hager's reagent. Formation of yellow coloured precipitate will indicate the presence of alkaloids.

Hager's reagent: Saturated solution of picric acid in distilled water.

Tests for carbohydrates

Molisch's test: Few drops of Molisch's reagent will added to the filterate, followed by addition H2SO4 by the side of the test tube. The mixture will be then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Molisch' reagent: 10gm of α-napthnol dissolved in 100 ml of 95% alcohol.

Barfoed's test: It is the general tests for monosaccharide**.** About 1ml of extract dissolved in distilled water and filtered. 1 ml of the filtrate was then mixed with 1 ml of Barfoed's reagent in a test tube and then heated on a water bath for a period of 2 minutes. Reddish precipitate of cuprous oxide was considered as a positive test.

Barfoed reagent: 13.3g of crystalline neutral copper acetate dissolved in 200ml of 1% acetic acid solution.

Fehling's test: The 1 ml of filtrate will be heated with 5 ml of equal volumes of Fehling's solution A (Copper sulphate in distill water) and Fehling's solution B(.Potassium tartarate and sodium hydroxide in distilled water). Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Benedict's test: Filtrate will be treated with few drops of Benedicts reagent, (alkaline solution containing cupric citrate complex) and boil on water bath ,reddish brown precipitate formation will indicate the presence of reducing sugars.

Tests for Glycosides:

0.5 gm of corn seed extract will hydrolyzed with 20ml of dil. HCl (0.1N) and filtered. The filtrate will be used to test the presence of glycosides.

Modified Borntrager's test: The filtrate will treated with Ferric chloride solution and heated on a boiling water bath for 5 min. the mixture will be cooled and shaken with equal volume of benzene. The benzene layer will be separated and will treated with half its volume of ammonia solution. The formation of rose pink or cherry red colour in the ammonical layer will be indicated the presence of anthraquinones.

Keller killiani's test: Small portion of the filtrate will be shaken with 1 ml of glacial acetic containing a trace amount of ferric chloride solution. These contents are transferred to 2ml conc.sulphuric acid. A reddish brown layer acquires bluish green coplor appears due to presence of digitoxose.

Legal test: To the corn seed extract in pyridine, sodium nitroprusside is added to make it alkaline. Pink or red color is produced which shows the presence of cardiac glycosides.

Baljet test: 1 ml of filtrate solution will be treated with few drops of sodium picrate reagent. Formation of yellow to orange color will indicate the presence of cardiac glycosides.

4. Tests for Protein and Amino acids:

Millon's test : 2 ml of filtrate will be treated with 2ml of Millon's reagent in a test tube and heated in a water bath for 5 minutes, cooled, and few drop of $NaNO_2$ solution will be added. Formation of white precipitate, which will turns into red colour upon heating. This indicates the presence of proteins and amino acids.

Millon's reagent: It consists of mercury dissolved in nitric acid (forming a mixture of mercuric & mercurous nitrates).

Ninhydrin test: 2 ml of filtrate, 0.25% Ninhydrine reagent will be added in a test tube and boiled for 2 minutes. Formation of blue colour indicates the presence of amino acids.

Ninhydrin's reagent: 0.25% solution of ninhydrin in n-butanol.

5. Tests for phytosterols and Triterpenoids

Liebermann's Test: 2 ml of filtrate will be taken in a test tube and 2-3 drops of acetic anhydride was added and gently heated. The contents of the test tube were cooled. Few drops of concentrated

sulphuric acid were added from the side of the test tube. A blue colour appears indicated the presence of sterols.

Libermann-Burchard test: 2 ml of filtrate will be dissolved in chloroform and few drops of acetic anhydride will be added to it, followed by concentrated sulphuric acid from the side of the tube. Formation of brown ring at the junction of two layers and the upper layer turns green which shows the presence of sterols and formation of deep colour will indicate the presence of triterpenoids.

Salkowaski Test: 2ml of filtrate will be taken in 2ml of chloroform and 2ml of conc. sulphuric acid will be added from the side of the test tube. The test tube will be shaken for few minutes. The development of red colour in the chloroform will indicate the presence of sterols and formation of yellow coloured lower layer indicates the presence of triterpenoids.

Test for Phenolic and Tannins

Ferric Chloride Test: Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

6.

Lead acetate Test: 2 ml of filtrate and few drops of lead acetate solution will bw added in attest tube. Formation of yellow precipitate will indicate the presence of tannins.

Test for flavonoids

Shinoda's test for flavonoids: About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. To the seed extract few pieces of magnesium chips will be added in the test tube followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

Alkaline reagent test: To the test solution 2 ml few drops of sodium hydroxide will be added, formation of yellow colour which turns to colourless on addition of few drops of dilute hydrochloric dication for the presence of flavonoids.

Tests for Saponins

Foam Test: Dilute 1ml of Corn seed extract with distilled water and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.[56]

Phytochemical tests of Methanolic extract of coccania grandis (roots)

S.No.	Chemical Test	Observation	Inference
1.	**Tests for Alkaloids**		
	Mayer’s reagent:	Yellow cream ppt	Positive
	Dragendroff’s reagent:	Reddish brown ppt	Positive
	Wagner’s reagent	Yellow ppt	Positive
	Hager’s reagent	Brown ppt	Positive
2.	**Tests for carbohydrates**		
	Molisch's test	Red-dull violet ppt	Positive
	Barfoed’s test	Reddish ppt	Positive
	Fehling's test	Reddish Brown ppt	Positive
	Benedict’s test	Red ppt	Positive
3.	**Tests for Glycosides:**		
	Modified Borntrager’s test	Red colour	Positive
	Keller killiani’s test	Bluish green colour	Positive
	Legal test	Pink colour	Positive
	Baljet test	Yellow colour	Positive
4.	**Tests for Protein**		
	Millon’s test	White ppt-red colour	Negative
5.	**Amino acids:**		
	Ninhydrin test	Precipated	Negative
6.	**Tests for phytosterols and Triterpenoids**		
	Liebermann’s Test	Blue colour	Positive

	Libermann-Burchard test	Ring at the junction	Positive
	Salkowaski Test		Negative
7.	**Test for Phenolic and Tannin**		
	Ferric Chloride Test	Yellow	Negative
	Lead acetate Test	Blue- Green ppt	Negative
8.	**Test for flavonoids**		
	Shinoda's test	Purple colour	Negative
	Alkaline reagent test	Yellow colour to colourless	Negative
9.	**Tests for Saponins**		
	Foam Test	Foam formation	Positive

TLC (Thin layer chromatography)

Thin layer chromatography (TLC) uses the same principles as extraction to accomplish the separation and purification of compounds: that is, the different separation of compounds between

Two phases based on differences in solubility of compounds in the two phases. In the case of TLC, one phase is a mobile liquid solvent phase and the other phase is a stationary solid phase with a high surface area. The stationary phase normally consists of a finely divided adsorbent, silica (SiO_2) or alumina (Al_2O_3) powder, used in the form of a thin layer (about 0.25 mm thick) on a supporting material. The support is usually a sheet of glass or metal foil. The mobile phase consists of a volatile organic solvent or mixture of solvents. The advantages of TLC are the very small quantities of sample required and the great ease and rapidity with which it is resolved. For these reasons, it is often used to monitor the progress of a reaction by running the crude sample beside the reaction sample on the same plate. It is also used to determine the best developing or eluting solvents for subsequent separation by column chromatography.

The following are some common uses of Thin-Layer Chromatography:

1. To determine the number of components in a mixture.
2. To determine the identity of two substances.
3. To monitor the progress of a reaction.
4. To determine the effectiveness of a purification.
5. To determine the appropriate conditions for a column chromatographic separation.
6. To monitor the progress and separation achieved by column chromatography.

Solvent system for Methanolic extract of coccania grandis (roots)

S.No.	Solvent system	No. of spots	Visualization agents	resolution
1	Glacial acetic acid:n-Hexane: Ethyl acetate	2	UV Light	Light
2	Toluene:acetone:formic acid	2	UV Light	Light
3	Glacial acetic acid: Chloroform: Formic acid	2	UV Light	Light
4	Methanol:ethyl acetate:water	4	UV Light	Dark

Stationary phase: Slica gel G254 nm TLC aluminium sheets with thickness 0.2mm. Solvent system: Methanol:ethyl acetate:water (40:50:10) Detecting agent: UV light 366nm Colour of spots: Yellow

Rf value A = Standard (Rf = 0.79)

B = Methanolic extract (0.78)

Pharmacological Activity

Selection of Dose: - The dose were selected on the basis of OECD guidelines 423, the dose were administered on the difference of 50 under 200, 500 and 1000 & 2000mg/kg. The significant dose was obtained at 1000 and 2000 mg/kg. So the 1/10 dose of these particular dose was 100,200 mg/kg according to OECD guidelines.

Description of groups:

Group 1 - Positive control (Vehicle treated)

Group 2 - Negative control (Disease induced)

Group 3 - Standard (Silymarin)
Group 4 - Test Group I (Methanol extract 100mg/kg)
Group 5 - Test Group II (Methanol extract 200mg/kg)
The study will performed on one models:

CCl4 induced model for Hepatotoxicity:

Model induced a number of CCl4 models are devised depending upon its dosage through different routes of administration.

Acute hepatic damage: Acute liver damage is manifested as ischemia, hydropic degeneration and central necrosis which is caused by oral or subcutaneous administration of CCl4 (1.25ml/kg). The maximum elevation of biochemical parameters are found to be 24 hours after the CCl4administration normally administered as 50% v/v solution in liquid paraffin or olive oil.

Chronic reversible hepatic damage: Administration of CCl4 (1ml/kg S.C.) twice weekly for 8 weeks produces chronic, reversible liver damage

Chronic, irreversible hepatic damage: Administration of CCl4 (1ml/kg S.C.) twice weekly for 12 weeks simulates chronic, irreversible liver damage

Experimental design

The study was performed by using Sprague Dwaley/Wistar albino male & female rats were divided into various groups, each group had six animals. Hepatotoxicity was induced using CCl4 (0.5 ml/kg, s.c.) for 9 days. On the 10th day, all the animals were sacrificed under anesthesia and blood as well as liver samples were collected for biochemical and histopathological investigation.

Biochemical investigation: Blood samples were taken by retro orbital plexus and allowed to clot at room temperature for 45 min. Centrifugation was done at 1200–1500 rpm for 20 min for separation of serum. The serum was used for the estimation of biochemical parameters namely serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) by using auto analyzer[58,59].

Evaluation Parameter:

Biochemical estimations.
Serum glutamate oxaloacetate transaminase (SGOT)/ AST test:
Serum glutamate pyruvate transaminase (SGPT) /AST) test:
Serum alkaline phosphates test:
Serum total protein and albumin test:
Serum Bilirubin:

Serum glutamate oxaloacetate transaminase (SGOT)/AST test:

In different marked test tubes as Blank and Test, Pipetted 100 µl working reagent in both the tubes and10µl Serum to the tube marked as test. Mixed well, incubated for 5 minutes at 37 ºC and read the decrease in absorbance at 340 nm against Reagent blank for 180 seconds at an interval of 30 seconds using semi auto analyzer by kinetic method. The Units are expressed as IU/L.

$$\text{AST activity [IU/L]} = \Delta \text{ Absorbance / min.} \times \text{Factor}$$

Hepatoprotective activity of MECG on CCl4-treated rats are shown in fig. It is well documented that CCl4 is biotransformed under the action of microsomal cytochrome P- 450 of liver to reactive metabolites18. These metabolites attributed to damage structural integrity of liver and raise the levels of SGOT. In the present study, Methanolic extract of the of methanolic extract of coccania grandis (roots) (MECG) at a dose of 100, 200 mg/kg, p.o. caused a significant inhibition in the levels SGOT towards the respective normal range is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage.

Thus, the hepatic enzymes SGOT, in serum significantly ($P < 0.001$) increased in CCl4 treated animals when compared to control. The MECG treatment (100 mg/kg) did not have significant effect on the levels of hepatic enzymes when compared to CCl4- treated animals. The MECG treatments (200 mg/kg) significantly ($P < 0.05$, $P < 0.01$; respectively) reversed the levels of hepatic enzymes when compared to CCl4- treated animals. Silymarin (25 mg/kg)-treated animals also showed significant ($P < 0.01$) inverted the levels of hepatic enzymes when compared to CCl4-treated animals as compared to the control group, which was significantly ($P < 0.01$ and $P < 0.05$) reversed with the treatment of MECG (200 mg/kg) and MECG (100 mg/kg), respectively.

Serum glutamate pyruvate transaminase (SGPT)/ALT test:

Procedure: - Pipette into tube serum 100µl, working LT regent 1000µl. Mix well and aspirate immediately for measurement. Program the analyzer as per assay parameters. Blank the analyzer as per assay parameter. Read absorbance after 60 second. Repeat reading after every 30 sec, upto 120 second at 340 nm wavelength. Determine the mean absorbance change per min.

Hepatoprotective activity of MECG on CCl4-treated rats are shown in Fig. It is well documented that CCl4 is biotransformed under the action of microsomal cytochrome P- 450 of liver to reactive metabolites. These metabolites attributed to damage structural integrity of liver and raise the levels of SGPT. In the present study, SGPT level of positive control, Negative control, standard and different dose of plant extracts. Methanolic extract of the coccania grandis (roots) (MECG) at a dose of 100, 200 mg/kg, p.o. caused a significant inhibition in the levels SGPT towards the respective normal range is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage.

Thus, the present investigation revealed hepatic enzymes SGPT, in serum significantly ($P < 0.001$) increased in CCl4 treated animals when compared to control. The MECG treatment (100 mg/kg) did not have significant effect on the levels of hepatic enzymes when compared to CCl4-treated animals. The MECG treatments (200 mg/kg) significantly ($P < 0.05$, $P < 0.01$; respectively) reversed the levels of hepatic enzymes when compared to CCl4- treated animals. Silymarin (25 mg/kg)-treated animals also showed significant ($P < 0.01$) inverted the levels of hepatic enzymes when compared to CCl4-treated animals as compared to the control group, which was significantly ($P < 0.01$ and $P < 0.05$) reversed with the treatment of MECG (200 mg/kg) and MEET (100 mg/kg), respectively.

Serum alkaline phosphates test (ALP):

a). Alkaline phosphatase reagent is used to measure alkaline phosphatase activity by a kinetic UV method using a 2-amino-2-methyl-1-propanol b). (AMP) buffer. In the reaction alkaline phosphatase catalyzes the hydrolysis of the c). colourless organic phosphate ester substrate, p-nitrophenyl phosphate to the yellow coloured product, p- nitrophenol and phosphate. The reaction occurs at an alkaline pH of 10.3. 5 µl of the sample reacted with 250 µl of the reagent.

The change in absorbance is monitored at 410 nm and this change is directly proportional to the activity of ALP. The activity is then calculated and expressed in IU/L. There action takes place at 37°C for three minutes.

The principal of the reaction is as follows:

ρ-Nitrophenylphosphate + H2O(pH 10.3, Mg) → ρ-Nitrophenol +Phosphate (Yellow)(Colourless)

Hepatoprotective activity of MEET on CCl4-treated rats are shown in Figs. It is well documented that CCl4 is biotransformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites18. These metabolites attributed to damage structural integrity of liver and raise the levels of SGPT. In the present study, Methanolic extract of the coccania grandis (roots) (MECG) at a dose of 100, 200 mg/kg, p.o. caused a significant inhibition in the levels SGPT towards the respective normal range is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage.

Thus, the present investigation revealed hepatic enzymes SGPT, in serum significantly ($P < 0.001$) increased in CCl4 treated animals when compared to control. The MEET treatment (100 mg/kg) did not have significant effect on the levels of hepatic enzymes when compared to CCl4-treated animals. The MECG treatments (200 mg/kg) significantly ($P < 0.05$, $P < 0.01$; respectively) reversed the levels of hepatic enzymes when compared to CCl4- treated animals. Silymarin (25 mg/kg)-treated animals also showed significant ($P < 0.01$) inverted the levels of hepatic enzymes when compared to CCl4-treated animals as compared to the control group, which was significantly ($P < 0.01$ and $P < 0.05$) reversed with the treatment of MECG (200 mg/kg) and MECG (100 mg/kg), respectively.

Serum total protein and albumin test:

5ml of Biuret reagent will pipette out into each of 7 test tubes.

Pipette 5ml of the Biuret blank reagent will pipette out into each of 7 test tubes.

Reagent-series will prepared by adding 100 µL of each of the protein standards to five separate test

tubes filled with the Biuret reagent. Reagent blank will prepared by adding 100 µL of water to a sixth different test tube filled with Biuret reagent. Serum unknown will prepared by adding 100 µL of serum to a seventh test tube filled with Biuret reagent. Each tube was mixed by placing a piece of a parafilm on the top and inverting several times

Blank-series will prepared by adding 100 µL of each of the protein standards to five separate test tubes filled with the Biuret blank reagent. Reagent blank was prepared by adding 100 µL of water to a sixth different test tube filled with Biuret blank reagent. Serum unknown will prepared by adding 100 µL of serum to a seventh test tube filled with Biuret blank reagent. Each tube was mixed by placing a piece of a parafilm on the top and inverting several times.

Cuvettes will allowed to stand at room temperature for 30 minutes.

Reagent-series blank will used, zero the Spec 20 at 540 nm and absorbance of the reagent series was measured including the serum unknown. All the test tube was inverted before that measurement.

Blank-series blank will used, re-zero the Spec 20 and absorbance of the blank series including the serum unknown will measured.

Blank subtraction will conducted by subtracting the absorbance of the blank-series from its reagent series counterpart. Concentration of the unknown will determined by the plotting of graph between absorbance vs. concentrations **(57)**.

Routinely estimated total proteins are in the normal range of 5.5 to 8 g/dl. The blood levels of plasma protein are decreased in extensive liver damage. Albumin (normal range 3.5 to 5.0 g/dl) synthesized in the liver constitutes a major part of the total proteins in the body and the other part being globulin. A low serum albumin concentration suggests chronic liver disease. Albuminaemia may occur in liver diseases caused by significant destruction of hepatocytes. Hyperglobulinaemia may be present in chronic inflammatory disorders such as in cirrhosis and in chronic hepatitis. In the present study, Methanolic extract of the coccania grandis (roots) (MECG) at a dose of 100, 200 mg/kg, p.o. caused a significant inhibition in the levels towards the respective normal range is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage.

Thus, the present investigation revealed hepatic enzymes SGPT, in serum significantly ($P < 0.001$) increased in CCl_4 treated animals when compared to control. The MECG treatment (100 mg/kg) did not have significant effect on the levels of hepatic enzymes when compared to CCl_4-treated animals. The MECG treatments (200 mg/kg) significantly ($P < 0.05$, $P < 0.01$; respectively) reversed the levels of hepatic enzymes when compared to CCl_4- treated animals. Silymarin (25 mg/kg)-treated animals also showed significant ($P < 0.01$) inverted the levels of hepatic enzymes when compared to CCl_4-treated animals as compared to the control group, which was significantly ($P < 0.01$ and $P < 0.05$) reversed with the treatment of MECG (200 mg/kg) and MECG (100 mg/kg), respectively.

Effect of coccania grandis (roots) extract on serum liver enzymes (ALT, AST, and ALP), total protein in CCl_4 induced liver damage in rats.

S. No.	Group	SGOT	SGPT	ALP	Protein
1	Positive	35.9±4.56	56.5±3.45	789.15 ± 4.456	10.45±1.456
2	Negative	139.8±17.61*	548.45±18.48*	285.45 ± 4.189	4.54±0.18
3	Standard	98.9±1.73***	452.78±14.89***	89.45 ± 2.447***	8.45±1.45***
4	Test (100mg/kg)	101.7±7.35*	289.45±12.58*	278.24 ± 5.456*	8.75±0.35**
5	Test 200(mg/kg)	89.5±7.52***	287±14.78***	145.78 ± 14.358***	9.66±0.45***

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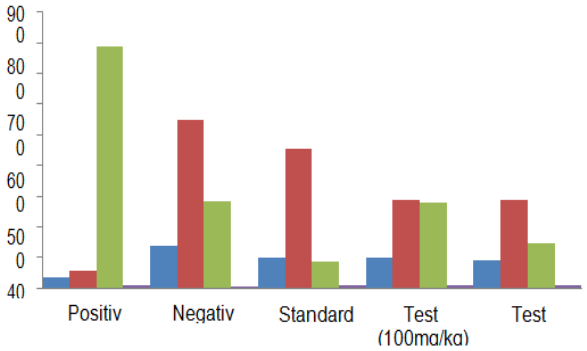

Fig 4: Graphical representation of methanolic extract of coccania grandis (roots) on SGOT,SGPT, ALP Protein in CCl4-induced hepatotoxicity rats after 7 days of treatment.

Statistical analysis

The Statistical analysis will be carried out as per standard method. All result will be expressed as MEAN±SEM. Groups of data were compared with the analysis of variance (ANOVA) followed by dunnett's t-test values for statistical significant.

Conclusion

Hepatotoxicity implies chemical-driven liver damage. There are many chemical agents that cause hepatotoxicity and these agents called Hepatotoxins. These cause hepatotoxicity by the generation of free radicals and damage the liver cells and cause of m any liver diseases. Further works are being carried out to isolate and identify the active principle involved in the hepatoprotective and antioxidant activities of this plant extract.

The present study has demonstrated that(EECT)(100 and 200 mg/kg) exhibited significant dose-dependent hepatoprotective activity against liver injury induced by CCl4. Carbon tetrachloride induces hepato-toxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi- normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P450 in the endoplasmic reticulum to form a trichloromethyl free radical (ccl3.) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation, which leads to change in the structures of endoplasmic reticulum and other membrane, loss of metabolic enzymes activation, reduction of protein synthesis and elevation of serum transaminases leading toliver damage. Amino transferases contribute a group of enzymes that catalyze the interconversion of amino acids and α-ketocids by the transfer of amino group.

Coccinia grandis Linn (Curcubitaceae) is a climber herb cultivated throughout India. In folklore medicine, the fruit is used to treat leprosy, fever, asthma, infective hepatitis, jaundice, and sore throats. It is also used as expectorant and astringent. In order to provide a better understanding of the possible role of the extract of Coccinia grandis fruits in the hepatoprotective effect observed in this study, we carried out a preliminary phytochemical screening of the extract of the fruit and found it to contain flavonoids and glycosides. Earlier report indicated that the flavonoids are phenolic compounds exert multiple biological effects, including antioxidant properties and free radical scavenging abilities. Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders in addition to other natural healing processes of the liver. Hepatotoxicity implies chemical-driven liver damage. There are many chemical agent that cause hepatotoxicity and these agents called Hepatotoxins. These cause hepatotoxicity by the generation of free radicals and damage the liver cells and cause of m any liver diseases. Further works are being carried out to isolate and identify the active principle involved in the hepatoprotective and antioxidant activities of this plant extract.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease. The assessment of liver function can be made by estimating the activities of serum enzymes such as ALT, AST and ALP. During hepatic damage, there may be increase in these enzyme levels in serum with the extent of liver damage. The altered levels of these enzymes in CCl4-treated rats in the present study corresponded to the extensive liver damage induced by the toxin.

These are liver special enzymes and are considered to be very sensitive and reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effect of various compounds. Both AST and ALT levels increase due to toxic compounds affecting the integrity of liver cells. Alkaline phosphatase is a membrane bound glycoprotein enzyme with a high concentration in sinusoids and endothelium.

This enzyme reaches the liver mainly from the bone. It is excreted into the bile; therefore, its elevation in serum occurs in hepatobiliary diseases. The results of the present study indicate that AET probably stabilize the hepatic plasma membrane from CCl4-induced damage. The liver is known to play a significant role in the serum protein synthesis, being the source of plasma albumin also the other important components like α and β- globulin. The liver is also concerned with the synthesis of γ-globulin. The serum albumin level is low in hepatic diseases. The result reveals that in the animals pretreated with hepatoprotective agents prior to the challenge with CCl4, the liver biosynthesis of protein continues to be unaffected. The metabolic transformation of amino acid in liver by synthesis, transamination, etc., may be impaired due to the escape of both non- proteins and protein nitrogenous substances from injured liver cells as mediated by raise in the serum enzyme levels of ALP, AST and ALT.

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