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Reconnaissance of Hepatoprotective Activity of Polyherbal Formulation in Paracetamol and Ethanol Induced Hepatotoxicity Models of Albino Wistar Rats

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Abstract:-

A. *Objectives*

The present study was undertaken for investigating the hepatoprotective effect of ethanolic extract of Annona squamosa, Cassia fistula and Illicium verum in paracetamol and ethanol induced hepatotoxicity models in albino wistar rats.

B. *Method*

Paracetamol and ethanol were used to induce hepatotoxicity in rats. Silymarin was used as standard drug (100 mg/kg p. o.). Ethanolic extract of Annona squamosa, Cassia fistula and Illicium verum (200 mg/kg p. o. & 400 mg/kg p. o.) was administered along with one hour before administration of paracetamol and ethanol once daily for 21 days.

C. *Results*

Liver biomarkers such as ALT, AST, ALP, Serum Bilirubin & Total Protein were elevated in paracetamol and ethanol administration. The treatments of ethanolic extract of Annona squamosa, Cassia fistula and Illicium verum at 200 mg/kg and 400 mg/kg were significantly reduced liver biomarker enzymes. Histopathological reports reveal that administration of paracetamol and ethanol caused degeneration of fatty cysts, infiltration of lymphocytes, proliferation of kupffer cells and congestion of liver sinusoids. Upon treatment with polyherbal plant extract, the histopathological observations showed normal hepatic globular architecture, less lymphatic infiltration and normal kupffer cells proliferation. This observation suggests that polyherbal plant extract protect the liver from adverse conditions. Hence, polyherbal plant extract of Annona squamosa, Cassia fistula and Illicium verum possesses hepatoprotective activity against paracetamol and ethanol induced hepatotoxicity at a dose of 200 mg/kg and 400 mg/kg.

D. Conclusion

The result of the present study indicated that polyherbal plant extract of Annona squamosa, Cassia fistula and Illicium verum showed protective effect on hepatotoxicity induced by paracetamol and ethanol.

Keywords:-Hepatoprotective activity, Paracetamol, Ethanol, Liver Biomarkers, Fatty degeneration, Annona squamosa, Cassia fistula, Illicium verum, Histological parameters.

I. INTRODUCTION

➢ *LIVER*

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It performs the biochemical pathways like growth, fight against disease, nutrient supply and energy provision. The liver performs more than 500 vital metabolic functions. It is involved in the synthesis of plasma proteins, clotting factors and urea. It regulates blood levels of amino acids. Liver parenchyma serves as a storage organ for glycogen, fat and fat soluble vitamins. Thus, to maintain a healthy, liver is a crucial factor for the overall health and well-being. Histologically, the liver is composed of several components:

- *Hepatocytes*

  Hepatocytes are the major functional cells which form complex threedimensional branched, irregular arrangements called hepaticlaminae. Grooves in the cell membranes provide spaces for canaliculi into which the hepatocytes secrete bile.

- *Bile Canaliculi*

  These are small ducts between hepatocytes that collect bile produced byϖ the hepatocytes. The bile ducts unite and exit the liver as the common hepatic duct which joins the cystic duct from the gallbladder to form the common bile duct.

- *Hepatic Sinusoids*

  These are highly permeable blood capillaries between rows ofϖ hepatocytes that receive oxygenated blood from branches of the hepatic artery and nutrient rich deoxygenated blood from branches of the hepatic portal vein. Stellate reticulo- endothelial (Kupffer) cells are also present in hepatic sinusoids, which destroy worn-out white and red blood cells and bacteria.

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➢ *Functions of Liver*

Each day, hepatocytes secrete 800–1000 mL of bile a yellow, brownish or olive-green liquid. It has a pH of 7.6–8.6 and consists mostly of water, bile salts, cholesterol, and phospholipid called lecithin, bile pigments, and several ions. In addition, the liver performs many other vital functions such as Carbohydrate Metabolism, Lipid Metabolism, Protein Metabolism, Processing of Drugs and Hormones, Excretion of Bilirubin, Synthesis of Bile Salts, Storage, Phagocytosis and Activation of Vitamin D.

II. HEPATOTOXICITY

Hepatotoxicity is an injury to the liver that is associated with impaired liver function caused by exposure to a drug or any other noninfectious agent. Hepatotoxicity implies chemical-driven liver damage. The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. More than 900 drugs are implicated as hepatotoxins. Injury to hepatic cells and bile ducts lead to accumulation of bile acid inside the liver. Damage to hepatocytes results in the activation of immune system cells like kupffer cells (KC), natural killer (NK) cells, and natural killer T (NKT) cells that result in producing proinflammatory mediators such as tumor necrosis factor-α (TNF), interferon-γ (IFN) and interleukion-β (IL) which produces liver injury. Hepatotoxicity can be characterized into two main groups, each with a different mechanism of injury: Hepatocellular and Cholestatic.

- *Hepatocellular or cytolytic injury*

Involves predominantly initial serum aminotransferase level elevations, usually preceding increases in total bilirubin.

- *Cholestatic injury*

Is characterized by predominantly initial alkaline phosphatase level elevations and increase in the levels of serum aminotransferases.

III. DIAGNOSIS

- *Pathology*

Liver pathology serves as an important tool for identifying diseases such as zonal necrosis, hepatitis, cholestasis, steatosis, granuloma, vascular lesions and neoplasm. Liver injury caused by hepatotoxicity can also be determined with X-rays, computerized tomography [CT] scan and endoscopic retrograde cholangiopancreatography (ERCP).

- *Biochemical Markers (Clinical Biochemistry)*

Liver injury can be diagnosed by certain biochemical markers. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity. Biochemical markers such as Alanine Aminotransferases (ALT), Aspartate Aminotransferases (AST), Alkaline Phosphatase (ALP), γ-Glutamyl Transferase, Total Bilirubin Levels, Urobilinogen Level, Bile Acids, Lactate Dehydrogenase, Glutamate Dehydrogenase, Albumin and Total Proteins.

IV. SIGNIFICANCE OF PLANTS

Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of year as a result of man's inquisitive nature. Today a vast store of knowledge concerning therapeutic properties of different plants has accumulated. The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids etc. Plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. India is one of the countries contains more than 45,000 plant species, out of that 15,000-20,000 plants are showing good medicinal properties, but currently 7,000-7500 plants only used for medicinal purposes. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But, there is meager drug available for the treatment of liver disorders. Hence, there is an increasing need for safe hepatoprotective agent.

The present study was carried out to screen the hepatoprotective effect of polyherbal formulation of the ethanolic extracts of Annona squamosa, Cassia fistula and Illicium verum in paracetamol and ethanol induced hepatotoxicity models of albino wistar rats.

V. AIMS & OBJECTIVES

The aim of the present study was "Appraisal of Hepatoprotective activity of Polyherbal Formulation of the Plants, Annona squamosa, Cassia fistula and Illicium verum in paracetamol and ethanol induced hepatotoxicity models of albino wistar rats".

➢ *Objectives*

- Collection and authentication of the plants Annona squamosa, Cassia fistula and Illicium verum.
- Preparation of extracts of leaves of Annona squamosa, leaves of Cassia fistula and fruitsϖ of Illicium verum using maceration process.
- To investigate preliminary phytochemical constituents of polyherbal plant extract ofϖ Annona squamosa, Cassia fistula and Illicium verum.
- Preparation of suitable formulationϖ & dosage form of above polyherbal plant extracts for administration into test animal.
- To evaluate the pharmacological profile of prepared polyherbal plant extracts for itsϖ hepatoprotective activity using paracetamol and ethanol induced hepatotoxicity models of albino wistar rats.

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- Studying the effect of above polyherbal plant extract on SGPT, SGOT, ALP, Serumϖ Bilirubin and Total Protein.
- To obtain histopathological studies of the test and control
- animals.ϖ Statistical Analysis.ϖ

VI. METHODOLOGY

➢ *Collection and Authentication*

The three plants were collected from different places according to their geographical and habitual locations.

- The leaves of Annona squamosa were collected along the coast of Osman Sagar Dam,ϖ Hyderabad in the month of December, 2016.

- The leaves of Cassia fistula were collected from a local herbal garden, Hyderabad in theϖ month of January, 2017.
- The fruits of Illicium verum were collected from a local herbal garden, Hyderabad in theϖ month of January, 2017.

The above three plants were authenticated by Dr. L. Rasingam, Scientist In-charge, Botanical Survey of India, Hyderabad. The identification was made on botanical and morphological basis. The macroscopical and organoleptic characters were observed and the plants were confirmed on the basis of literature description.

➢ *Preparation of Extract*

The collected samples (leaves of Annona squamosa, leaves of Cassia fistula and fruits of Illicium verum) were washed, cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve number 60. The final uniform powder was used for the extraction of active constituents of the plant material. The powder was kept in dry, clean air tight glass jars. 2000g of each powdered plant was mixed with absolute ethanol (99.9%) for 48 hours with occasional shaking and was filtered. The filtrate was dried in Petri dishes and concentrated to brownish residue by evaporation at 4°C under reduced pressure in drying oven. The dried alcoholic extract is stored in refrigerator. The extract was dissolved in adequate amount of ethanol just before administration to respective group of rats.

➢ *Preliminary Phytochemical Screening*

Preliminary phytochemical analysis of polyherbal plant extract was carried out as per standard methods.

- *Detection of Alkaloids*

Wagner's Test: To the filtrate, add Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

- *Detection of Flavonoids*

Sulphuric Acid Test: To the extract, add few drops of sulphuric acid. Formation of orange color indicates the presence of flavonoids.

- *Detection of Steroids*

Liebermann Burchard's Test: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of sulphuric acid. The formation of green color indicates the presence of steroids.

- *Detection of Terpenoids*

Salkowski's Test: 0.2 g of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid (3ml) was carefully added. A reddish brown coloration of the interface indicates the presence of terpenoids.

- *Detection of Phenols*

Ferric Chloride Test: Extract was treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

- *Detection of Saponins*

About 0.2 g of the extract was shaken with 5 ml of distilled water. Formation of frothing shows the presence of saponins.

- *Detection of Tannins*

A small quantity of the extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

- *Detection of Carbohydrates*

Molisch Test: To 2 ml of extract, few drops of freshly prepared 20 % alcoholic solution of α- naphthol was added. 2 ml of concentrated sulphuric acid was added through the sides of the test tubes. Appearance of red violet ring indicates the presence of carbohydrates.

- *Detection of Triterpenoids*

Liebermann Burchard's Test: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of sulphuric acid. The formation of violet color indicates the presence of triterpenoids.

- *Detection of Proteins*

Biuret's Test: To 1 ml extract, 5 drops of 10 % w/v copper sulphate solution was added and heated. Formation of violet red color indicates the presence of proteins.

➢ *Animals used*

A total of 60 adult albino wistar rats of either sex (160-250gm) were used. The animals were housed in clean metabolic cages, maintained in controlled temperature (27 ±3°C), under 12 hours dark/light cycle. They were fed with standard pellet diet and water ad libitum. Fasting for 12hrs has

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done prior to experiment. The study was carried out for 21 days.

➢ *Acute Toxicity Studies*

- *Animals*:- Swiss albino mice weighing 20-25 gram were selected for the study. Animals were fed with standard rat pellet diet and water ad libitum and kept at 24-280C with 60-70% relative humidity and 12 hour day and night cycle. Animals were fasted for overnight, but had access to water freely.

- *Procedure*:- The acute toxicity study was conducted according to the Organisation for Economic Co-operation and Development (OECD) Test specifications 423 using female mice using the limit test procedure. Animals were grouped into four at the dose levels of 5, 50, 300, 2000 mg/kg, each group consisting of 30 animals; a single dose was received by animals through intragastric intubation with ethanolic plant extract (EPQ) of 5 mg/kg dissolved in absolute ethanol. After the dosing it was observed for any mortality, behavioral, autonomic and toxic profile changes for one to four hours and up to 14 days, at least once daily for the immediate and delayed acute toxicity as the parameters specified below during the monitoring period as per the method outlined by OECD Test specifications Motor activity, Grooming, Touch response, Tone response, Pain response, Tremors, Convulsion, Righting reflex, Gripping strength, Pinna reflex, Corneal reflex, Writhing, Pupils, Urination, Salivation, Skin colour, Lacrimation and Defaecation .

- *Research Attempt*:- A toxic dose or repeated doses of a known hepatotoxins (carbon tetrachloride, paracetamol, thioacetamide, alcohol, D-glucosamine, allylalohol) is administered, to induce liver damage in experimental animals. The test substance is administered after the toxin challenge then it is inferred the test substance is effective. Liver damage and recovery from damage are assessed by measuring serum marker's enzymes, bilirubin, histopathological changes in liver, biochemical changes in liver. When the liver is damaged, liver enzymes such as glutamate pyruvate aminase, glutamate oxaloacetate transaminase and alkaline phospahate enters into the circulation. An increase in the levels of these marker enzymes in the serum is an indication of liver damage.

VII. HEPATOPROTECTIVE SCREENING

➢ *Experimental Procedure*

Experimental animals were randomly divided into two models, each containing 30 rats. One is the paracetamol induced liver toxicity model and other ethanol induced liver toxicity model.

A. Paracetamol Induced Toxicity Model

In paracetamol induced liver toxicity model, the rats are divided into 5 sub-groups, each group containing 6 animals and the treatment scheduled for 21 days as follows,

- Group I : Normal (Normal saline 1ml/kg, p. o)ϖ
- Group II : Toxic (Paracetamol, 2g/kg b. w. p. o.)ϖ
- Group III : Standard (Silymarin, 100 mg/kg, p. o) + one hour before administrationϖ of paracetamol
- Group IV : Ethanolic Polyherbal Extract (200 mg/kg, p. o) + one hour beforeϖ administration of paracetamol
- Group V : Ethanolic Polyherbal Extract (400 mg/kg, p. o) + one hour beforeϖ administration of paracetamol.

GROUP	TREATMENT		DOSE
I	Normal		1 ml/kg b. w. Normal Saline p. o.
II	Paracetamol (2g/kg b. w.) p. o. for 21 days	Toxic (Untreated)	2 g/kg b. w. in Normal Saline p. o.
III		Standard (Silymarin)	100 mg/kg b. w. in Normal Saline p. o.
IV		Treatment 1 Ethanolic Polyherbal Plant Extract	200 mg/kg b. w. in Normal Saline p. o.
V		Treatment 2 Ethanolic Polyherbal Plant Extract	400 mg/kg b. w. in Normal Saline p. o.

B. Ethanol Induced Toxicity Model

In ethanol induced liver toxicity model, the rats are divided into 5 sub-groups, each group containing 6 animals and the treatment scheduled for 21 days as follows,

- Group I : Normal (Normal saline 1ml/kg, p. o)ϖ
- Group II : Toxic (Ethanol 40 % v/v, 2ml/100 gm b. w., p. o.)
- Group III : Standard (Silymarin, 100 mg/kg, p. o) + one hour before administrationϖ of ethanol
- Group IV : Ethanolic Polyherbal Extract (200 mg/kg, p. o) +one hour beforeϖ administration of ethanol
- Group V : Ethanolic Polyherbal Extract (400 mg/kg, p. o) + one hour beforeϖ administration of ethanol

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GROUP	TREATMENT		DOSE
I	Normal		1 ml/kg b. w. Normal Saline p. o.
II	Ethanol (40 % v/v, 2ml/100 gm b. w.) p. o. for 14 days	Toxic (Untreated)	2ml/100 gm b. w. p. o.
III		Standard (Silymarin)	100 mg/kg b. w. in Normal Saline p. o.
IV		Treatment 1 Ethanolic Polyherbal Plant Extract	200 mg/kg b. w. in Normal Saline p. o.
V		Treatment 2 Ethanolic Polyherbal Plant Extract	400 mg/kg b. w. in Normal Saline p. o.

On 22nd day, blood was collected by retro orbital puncture for estimation of liver biomarker enzymes and all animals were sacrificed by cervical decapitation for liver histopathological studies. Livers were removed, washed in normal saline and stored in 10% formalin. The livers and blood samples were sent to the pathology and biochemistry department for further evaluation.

VIII. HISTOPATHOLOGY

Livers were quickly removed, washed in normal saline water and immersed in 10 % formalin. These livers were sent to the laboratory of pathological studies for further processing and evaluation.

IX. STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's t-test. P value 0.05 Not Significant.

X. RESULTS

S. No.	Response	Different Concentration of Polyherbal Extracts			
		5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
1	Motor Activity	N	N	N	N
2	Grooming	Ab	Ab	Ab	Ab
3	Touch Response	N	N	N	N
4	Pain Response	N	N	N	N
5	Tremors	Ab	Ab	Ab	Ab
6	Convulsions	Ab	Ab	Ab	Ab
7	Righting Reflux	N	N	N	N
8	Gripping Strength	N	N	N	N
9	Pinna Reflux	P	P	P	P
10	Corneal Reflux	P	P	P	P
11	Writhing	N	N	N	N
12	Pupils	N	N	N	N
13	Urinations	N	N	N	N
14	Salivation	N	N	N	N
15	Skin Color	N	N	N	N
16	Lacrimation	N	N	N	N

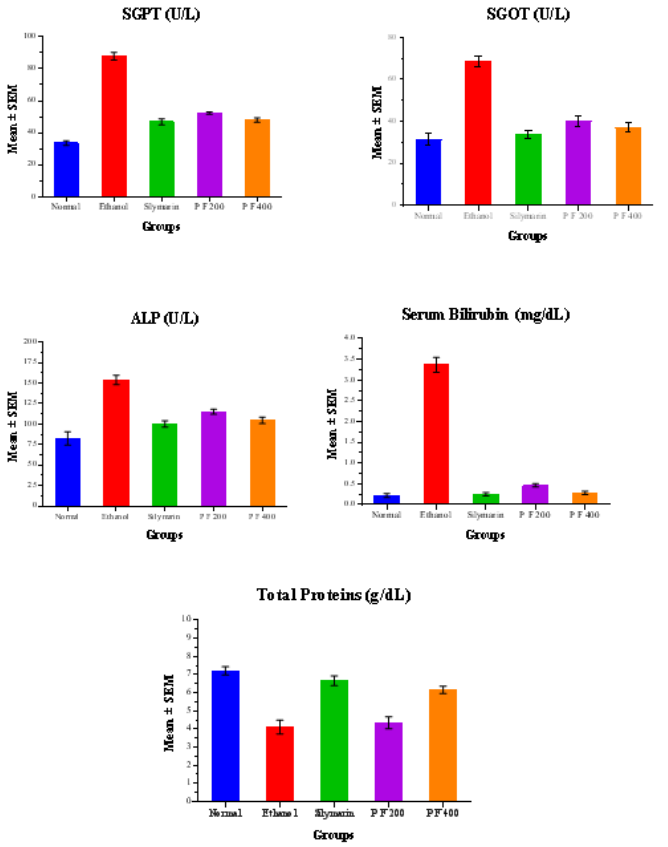
Acute Oral Toxicity Result

A. Paracetamol Induced Toxicity Model

Treatment	SGPT	SGOT	ALP	Serum Bilirubin	Total Proteins
Normal (1 ml/kg Normal Saline)	34.33 ± 1.17	26.5 ± 3.28	83 ± 7.58	0.23 ± 0.04	6.88 ± 0.28
Toxic (Paracetamol, 2g/kg b. w., p. o.)	82.83 ± 2.36	65 ± 3.55	150.33 ± 5.83	1.65 ± 0.27	4.83 ± 0.32
Standard (100 mg/kg b. w., p. o.)	40 ± 1.82	34.8 ± 1.97	86.83 ± 6.51	0.35 ± 0.04	6.95 ± 0.32
Treatment 1 (Polyherbal Plant Extract, 200 mg/kg b. w., p. o.)	52.33 ± 1.08	39 ± 2.62	97 ± 5.75	0.53 ± 0.07	7.52 ± 0.25
Treatment 2 (Polyherbal Plant Extract 400 mg/kg b. w.)	45.66 ± 1.49	34.5 ± 2.11	98.16 ± 3.59	0.43 ± 0.07	7.07 ± 0.22

Biochemical Parameters in Paracetamol Model

Values are the Mean ± SEM of six rats / treatment. Followed by ANOVA, Dunnett Multiple Comparisons Test. P <0.0001 Extremely Significant, P <0.01 Moderately Significant, P <0.05 Significant, P >0.05 Not Significant (NS)

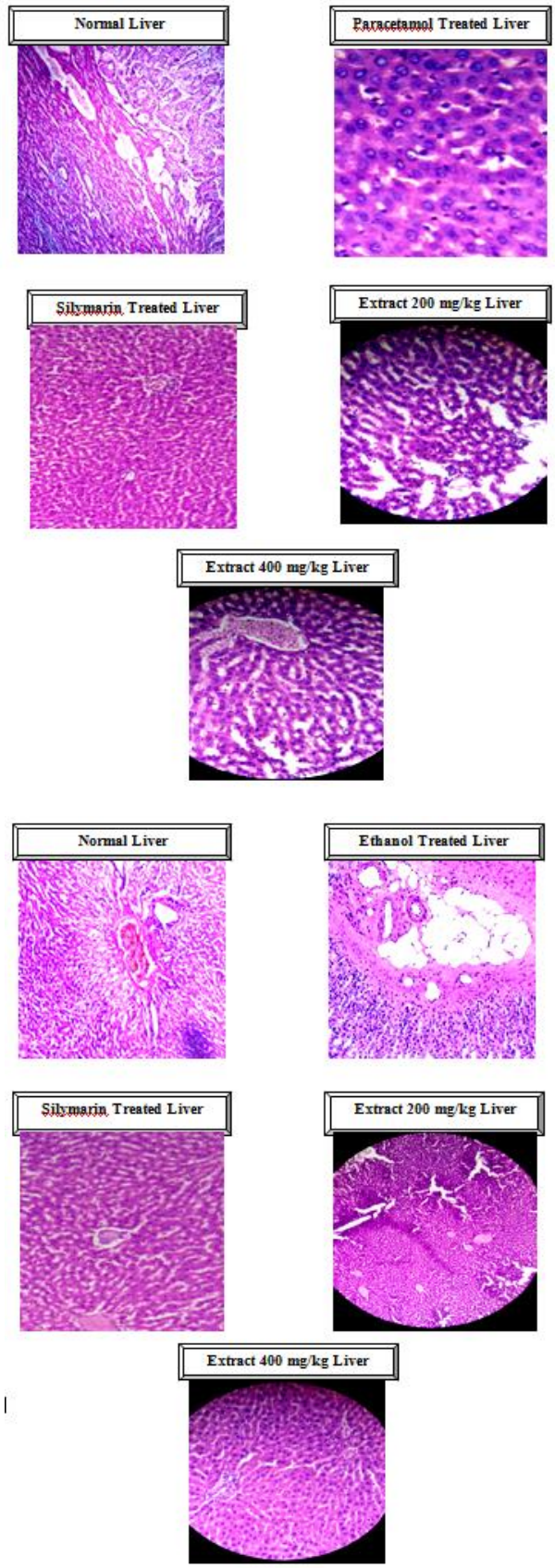


B. Ethanol Induced Toxicity Model Biochemical

Treatment	SGPT	SGOT	ALP	Serum Bilirubin	Total Proteins
Normal (1 ml/kg b. w. Normal Saline p. o.)	33.66 ± 1.43	31.5 ± 3.0	82.5 ± 20.09	0.22 ± 0.04	7.22 ± 0.22
Toxic (Ethanol 40 % v/v, 2ml/100 gm b. w., p. o.)	87.83 ± 2.36	68.33 ± 2.51	153.83 ± 14.27	3.36 ± 0.18	4.12 ± 0.38
Standard (100 mg/kg b. w., p. o.)	46.83 ± 1.9	33.66 ± 1.84	100.17 ± 9.72	0.25 ± 0.04	6.67 ± 0.27
Treatment 1 (Polyherbal Plant Extract, 200 mg/kg b. w., p. o.)	52.33 ± 0.72	40 ± 2.62	115 ± 7.72	0.47 ± 0.04	8.07 ± 0.12
Treatment 2 (Polyherbal Plant Extract, 400 mg/kg b. w., p. o.)	48 ± 1.37	37.17 ± 2.06	104.66 ± 9.42	0.28 ± 0.04	7.1 ± 0.27

Parameters in Ethanol Model

Vales are the Mean ± SEM of six rats / treatment. Followed by ANOVA, Dunnett Multiple Comparisons Test. P <0.0001 Extremely Significant, P <0.01 Moderately Significant, P <0.05 Significant, P >0.05 Not Significant (NS)



Histopathology of Ethanol Model

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XI. DISCUSSION

The liver is a major target organ for toxicity of xenobiotics and drugs, because most orally ingested xenobiotics and drugs pass through the liver. Hepatic cells participate in a variety of enzymatic metabolic activities and also actively involved in alcohol induced marked liver damage. The therapeutic activity of a hepatoprotective drug to reduce the injurious effects due to hepatotoxins and to preserve the normal hepatic physiological mechanisms.Paracetamol, when used at high doses, could cause acute liver injury most probably via formation of N-acetyl-p-benzoquinoneimine, a toxic metabolite, by cytochrome P4502E1 (CYP2E1).In the assessment of liver damage by paracetamol, the determination of enzyme activities such as ALT and AST is largely used. In the present study, the increase in serum activities of ALT, AST, ALP, Serum Bilirubin and Total Proteins in paracetamol treated rats had been attributed to the damaged structural integrity of the liver. Additionally, histological findings showed that paracetamol administration to rats revealed a remarkable centrilobular (zone III) necrosis, cytoplasmic changes, and sinusoidal narrowing around the central vein. Moreover, chronic administration of paracetamol statistically decreased serum albumin and increased serum globulin. Excess levels of these species can attack biological molecules such as DNA, protein and phospholipids which leads to lipid peroxidation, nitration of tyrosine and depletion of antioxidant enzymes.Alcohol treatment of rats is known to cause the translocation of fat from the peripheral adipose tissue to liver for accumulation. It has been reported that Kupffer cells are the major sources of hepatic cell necrosis during chronic alcohol consumption, and these are activated for enhanced formation of proinflammatory factors. The animals treated with alcohol had a significant hepatic damage as indicated by the elevated levels of serum enzyme markers like AST, ALT, ALP, Serum Bilirubin and Total Proteins.

The present study reveals that the effect of ethanolic polyherbal plant extract had been effective in offering treatment which was comparable to silymarin. This extract has shown liver protective actions.The paracetamol and ethanol induced hepatotoxicity is associated with variety of biochemical abnormalities and attributed to the release of intracellular constituents into circulation, such as serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transamiases (SGOT), serum alkaline phosphatase (ALP). ALP mainly arises from the lining of canaliculi in hepatocytes. Increased activity of alkaline phosphate occurs mainly due to hepatic dysfunction. Polyherbal plant extract of Annona squamosa, Cassia fistula and Illicium verum therapy has reversed the increased level of enzyme and caused a subsequent recovery towards normalization. Bilirubin is the conventional indicator of liver disease. Bilirubin concentration was elevated in paracetamol and ethanol treated rats. In the present study, in paracetamol and ethanol intoxicated rats, the levels of serum bilirubin was increased. It recovered by treatment with polyherbal plant extract ofAnnona squamosa, Cassia fistula and Illicium verumwhich showed decreased level of serum bilirubin.In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes, with an impaired protein secretion by hepatocytes. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass. In the present study this paracetamol and ethanol induced increased total liver weight was prevented by pretreatment with polyherbal plant extract of Annona squamosa, Cassia fistula and Illicium verum thus indicates a hepatoprotective effect.

Histopathological reports reveal that administration of paracetamol and ethanol caused degeneration of fatty cysts, infiltration of lymphocytes, and proliferation of kupffer cells and congestion of liver sinusoids. This further confirms that paracetamol and ethanol administration causes hepatotoxicity. Upon pretreatment with polyherbal plant extract of Annona squamosa, Cassia fistula and Illicium verum the histopathological observations showed that hepatic globular architecture was normalized, less lymphatic infiltration was seen and kupffer cells proliferation appeared to be normal. This observation suggests that polyherbal plant extract of Annona squamosa, Cassia fistula and Illicium verumpossesses hepatoprotective activity against paracetamol and ethanol induced hepatotoxicity.

XII. CONCLUSION

Paracetamol and Ethanol induced hepatotoxicity model was used to assess the hepatoprotective activity of polyherbal plant extract of *Annona squamosa*, *Cassia fistula* and *Illicium verum*. Hepatotoxicity was confirmed by the rise in biochemical markers of livers (SGPT, SGOT, ALP, Serum Bilirubin & Total Proteins). *Annona squamosa*, *Cassia fistula* and *Illicium verum* treatment effectively prevented hepatic damage, by reducing the elevated enzymes levels ofSGPT, SGOT, ALP, Serum bilirubin and Total Proteins. Treatment with polyherbal plant extract effectively cured hepatic damage, by restoring the normal hepatic metabolism and functions of hepatocytes. Similar observations were also observed with silymarin.Histopathological studies also showed that paracetamol and ethanol caused hepatic injury. Treatment with polyherbal plant extract exhibited hepatoprotective activity, which was comparable to that of silymarin.

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