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Hepatoprotective Activity of Polyherbal Extract

Sharukh A. Khan, Aref S. Shekh, Aslamkha C. Pathan
Anuradha College of Pharmacy, Chikhli, Buldhana, Maharastra, India
sharukh6785@gmail.com

Abstract: *The largest solid organ in the body, situated in the upper part of the abdomen on the right side. The liver has a multitude of important and complex functions, Including to manufacture proteins, Including albumin (to help maintain the volume Of blood) and blood clotting factors; to Synthesize, store, and process fats, Including fatty acids (used for energy) and cholesterol to Metabolize and store Carbohydrates (used as the source for the sugar in blood) to form and Secrete bile. That contains bile acids to aid in the intestinal absorption of fats and the fat- soluble Vitamins A, D, E, and K; to eliminate, by metabolizing or secreting, the potentially Harmful Biochemical products produced by the body, such as bilirubin, from the Breakdown of old red Blood cells and ammonia from the breakdown of proteins; And to detoxify, by metabolizing And/or secreting, drugs, alcohol, and Environmental toxins. Liver disease remains one of the serious health problems.Polyherbal extract was found to be much effective in protecting hepatocytes at selected dose. Recovery against paracetamol induced necrosis in their compact arrangement of hepatic cells was observed; whereas the section of liver of animals treated with Silymarin showed that extent of liver damage was lesser in magnitude as compared to the paracetamol treated animals.*

Keywords: Heoatotoxicity, Paracetamol, Silymarin, Polyherbal extract.

I. INTRODUCTION

Liver

The largest solid organ in the body, situated in the upper part of the abdomen on the right side. The liver has a multitude of important and complex functions, Including to manufacture proteins, Including albumin (to help maintain the volume Of blood) and blood clotting factors; to Synthesize, store, and process fats, Including fatty acids (used for energy) and cholesterol to Metabolize and store Carbohydrates (used as the source for the sugar in blood) to form and Secrete bile [1]. That contains bile acids to aid in the intestinal absorption of fats and the fat- soluble Vitamins A, D, E, and K; to eliminate, by metabolizing or secreting, the potentially Harmful Biochemical products produced by the body, such as bilirubin, from the Breakdown of old red Blood cells and ammonia from the breakdown of proteins; And to detoxify, by metabolizing And/or secreting, drugs, alcohol, and Environmental toxins. Liver disease remains one of the serious health problems [2].

Current scinerio

Millions of people suffer and die from liver diseases every year. He incidence of different Kinds of liver disease like hepatitis, liver cirrhosis, liver cancer and other related diseases are Very common in Bangladesh. Most common liver diseases in Bangladesh are di ˊ و erent types of viral hepatitis. Virus related liver diseases are important causes of morbidity in Bangladesh. It has been reported that about 7percent to 10 percent (9.1 million to 13 million) of the Population have hepatitis B and at least 2 percent to 3 percent (2.6 million to 3.9million) have Hepatitis C infection [3]. Some have multiple viralinfections. Another study showed that about 3.5 Percent of pregnantmothers have hepatitis B infection.About 90 percent of mothers infected with Hepatitis B and are 'e'antigen (HBeAg) positive may transmit this virus to their children. He HBsAg is positive in 7.5 percent of healthy adult jobseekers So,it is easily understood that Only hepatitis B may produce a disastrous health situation in Bangladesh and throughout the World if is nottimely controlled. Hisa problem of grave concern and needs reliablescientific Based management [4]. Management options for common liver diseases such as cirrhosis, fatty liver And chronic hepatitis are problematic etcej. But medicinal herbs play a vital role in the management of variousliver disorders and there are Number of medicinal preparations inAyurveda recommended for the treatment of liver disorders. Medicinal plants are widely used all over the world including Bangladesh for production of both Traditional and modern drugs, and development of new drugs. Here are

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more than 300 Traditional medicine manufacturers, including Unani, Ayurvedic and Homeopathy in Bangladesh. Maximum companies produce their patent drugs according to National Formulary Hey claim that some of their drugs are effective in various hepatic disorders. Most of these Claims are based on old literature, folk sayings, occasional experiences, and traditional uses, but Not on any significant clinical or pharmacological studies and statistical data. So, the present Study was undertaken to investigate the e ́ ects of one polyherbal formulation named Rohitakarista on the status of hepatoprotection and release of marker enzymes in the serum Dier Inducing hepatotoxicity by administering carbon tetrachloride (CCl4) in experimental rat [5].

Blood supply of the liver

The liver receives blood from two sources. From hepatic artery it receives Oxygenated blood and From the hepatic portal vein it receives deoxygenated blood Containing newly absorbed Nutrients, drugs and possibly microbes and toxins from Gastrointestinal tract. Branches of the Hepatic artery and the hepatic portal vein Carry blood into liver sinusoids, where oxygen, most Of nutrients and certain toxic Substance are taken up by the hepatocytes [6].

Anatomy of liver

The liver is a large, solid, gland situated in the right upper quadrant of the Abdominal cavity. Liver is reddish brown in Colour,soft in consistency and very Fraible. It weighs about 1600 gm In males and about 1300 gm in females. The liver Occupies the whole of the right Hypocondrium, the greater part of the epigastrium And extends into the left hypocondrium Reaching up to the left lateral line. The liver is the largest gland in the body. It secretes bile and performs various Other metabolic Functions. The liver is also called the 'heaper' from which we have The adjective hepatic [7].

Function of liver

Carbohydrate metabolism

The liver is especially important in maintaining a normal blood glucose level. When blood Glucose is low, the liver can break down glycogen to glucose and Realease glucose into the Bloodstream, when blood glucose is high it converts Glucose to glycogen and triglycerides for Storage liver can also convert certain Amino acid and lactic acid to glucose [8].

Lipid metabolism

Hepatocytes store some triglycerides, breakdown fatty acid to generate ATP, Synthesize Cholesterol and use cholesterol to make bile salt [9].

Protein metabolism

Hepatocytes deaminate amino acid so that the amino acid can be used for ATP Production or Converted to carbohydrates or fats, resulting toxic ammonia is Converted to less toxic urea, Which is excreted in urine. Hepatocyte synthesizes Most plasma proteins, such as alpha and beta Globulin, albumin, prothrombin and Fibrinogen [10].

Processing of drugs and hormones

The liver can detoxify substances such as alcohol or excreted drug such as Penicillin, Erythromycin and sulfonamides into bile [11].

Storage

In addition to glycogen, the liver is a prime storage site of certain vitamins (A, B12, D, E and K) And minerals (iron and copper), which are released from liver [12].

Basic hepatic histopathology

Hepatocellular changes

Hydropic change is a descriptive term applied to the hepatocytes with pale watery Cytoplasm And a normal nucleus. A wide variety of conditions produce this Relative lack of cytoplasmic Staining. Increased eosinophilia may occur with drug Relatedhydroperplasia of the smooth Endoplasmic reticulum. Active regeneration Of hepatocytes after neurosis as in several viral Hepatitis or recovery phase of fatty Liver, produce a widespread hepatocellular hydronic change As well as a Cobblestone pattern of liver cords. Hydronic change is also an indicator of Hepatocellular damage and is noted in acute viral hepatitis and drug induce hepatic Injury Including alcohol injury. Hepatocellular fat accumulations may be either large cytoplasmic bodies of foamy Fat. Fatty Liver occurs because of [13]

➢Sudden increase in mobilization of fat from the periphery to the liver

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➢ Relative lack of protein necessary for hepatocellular fat release

➢ Increased hepatocellular fat formation by metabolic changes and

➢ Decrease hepatocellular fat degradation.

Hepatocellular Necrosis

Necrosis may be classified in many ways, including locations (zonal, periportal, Perivenular and Soon), mechanism (lytic, coagulative), amount (submassive versus Focal), cellular type (Lymphocytotoxicveruseshyline necrosis) and various Patterns are associated with different Etiologics factors. Zonal necrosis is a Common pattern of injury after an acute hepatic injury. Sharply demarcated Perivenular (Zone 3) coagulation necrosis is typical of several anoxic injury Or Acetaminophen injury and may be explained by differences in oxygenation and Activity of Drug metabolizing enzyme. Periportal necrosis (Zone 1) is not common And is noted in Eclampsia. Midzonal injury is reported for yellow fever [14]. Hepatocellular necrosis (hepatocytolysis) of the lytic type, with the associated Macriphage Activity that is so complete and rapid that dead hepatocytes are rarely Noted, such necrosis is common in viral. Hepatitis, alcoholic liver disease and many hepatotoxic reaction, and the type of Inflammatory Reaction varies in these conditions. Coagulative necrosis in the liver Is characterized by dying Hepatocyte that retain some staining of the cytoplasm and The nuclei basophilia and gradually Disappear. The Cells become shrunken and slowly disappear because of the action of Inflammatory cells. Acidophillic injury usually occurs in an isolated hepatocyte and is similar to Coagulative Necrosis except that the cytoplasm becomes more eosinophilic and Waxy and the nucleus may Be retained and be a dark. These bodies are common in Acute viral hepatitis, chronic active Hepatitis, severe burns and and other liver Disorders. Confluent necrosis is attributable to fusion Of focal or zonal necrosis And may result from intensive necrosis that bridge between different Zones. Submissive hepatic necrosis is recognized by Confluent necrosis that usually Involves Many perivenular areas and occurs most commonly in severe acute viral Hepatitis, drug injury Etc. Massive hepatic necrosis is distinguished from from Submissive hepatic necrosis by the Presence of a thin rim of viable appearing Around each portal tract [15].

Cirrhosis

The currently accepted definition of cirrhosis may be applied to a liver with a Diffuse (that is Entire liver and not focal) and contain regenerative nodules, which Are masses of hepatocytes Lacking the normal blood flow because of the lack of Terminal hepatic venules. Regenerative or Hyperplastic nodules are required for the Identification of cirrhosis because altered blood flow And portal hypertension [16]. In Contrast hepatic fibrosis which is usually a precursor to cirrhosis, is Not associated. As frequently with portal hypertension. The development of hepatocellular Carcinoma is relatively common in cirrhosis with the required regenerative Nodules and is not as Frequent as it is in fibrosis.Cirrhosis is classified as micronodular cirrhosis applies to the liver in which nearly. All the Nodules are less then 3 mm diameter through some have used 1.5 mm as the Minimum diameter Because that is the diameter of a normal lobule. Examples Include alcohol cirrhosis, biliary tract Obstruction and hepatic venous obstruction. Macronodular cirrhosis applies if most of the Nodules are greater then 3 mm Diameter, and it occurs in two forms. The more common from Has nodules divided In thin Septa that are incomplete and have linking to portal tract [17]. This Pattern is Common in so called post hepatic cirrhosis to Macronodulartype is 2.25 years and Majority of patients have such progression. Alcoholic cirrhosis often contains fat Within the Hepatocytes and the parenchyma is increased in weight. Etiological Factors related to cirrhosis Include alcoholism, hepatitis B virus, various metabolic Diseases such as hemochromatosis, Wilson,s disease etc. Cirrhosis without Recognizable cause is called as "crytogenic cirrhosis". The irreversiblity of Cirrhosis has been emphasized in several experimental and clinical studies. If Cirrhosis is reversible, it must be very rare, through in the most convincing patient Having Biliary obstruction has been corrected surgically. Functionally more Important than nodular size In cirrhosis is the size of the entire hepatic mass, which Can be estimated with radioisotope Scans [18].

Enzyme that detect hepatocellular necrosis

The liver contains thousands of enzymes, some of which are also present in serum In very low Concentrations. These enzymes have no known function in serum and Behave like other serum Proteins. They are distributed in plasma and intestinal Fluid and have characteristics half_lives of Disappearance, usually measured in Days. The elevation of a

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given enzyme activity in serum is Thought to primarily Reflect its increased rate of entrance into serum from damaged liver cells. Serum Enzyme tests can be grouped into two categories [19] :-

1) Enzymes whose elevation in serum reflects generalized damage to hepatocytes.

2) Enzymes whose elevation in serum primarily reflects cholestasis.

Alanine aminotransferase (ALT)

Liver cells produce the ALT level increase when liver cells are damaged or are Dying. The Higher the ALT levels, the more cell death or inflammation of the liver Is occurring. However ALTs are not always good indication of how well the liver Is functioning only a liver biopsy can Reveal that ALT level can remain low even. When the liver is inflamed or is developing scar Tissue, or during a child's immune Tolerant stage of disease [20].

II. MATERIAL AND METHOD

Drug and chemicals:

Sr. No	Drug and Chemicals	Company
1	Paracetamol	Research Lab
2	Country Made Liquor	Marketed formulation
3	Silymarin	SILYBON, Microlabs Ltd. Mumbai
4	Biochemical Estimation Kits (AST, ALT, ALP, TB and TP)	Pathozyme Kit
5	Ether(Anaestetics)	Research Lab

Appratus and Equipment

Sr. No.	Equipment	Company	Specification
1	Analytical Weighing Balance	Shimadzu	Weighing capacity0.1-200g
2	Cooling Centrifuge	Remi	-
3	Auto analyzer		

Standard Drug: Silymarin

Priliminary Phytochemical Investigation:

The phytochemical estimation was performed

Sr. No.	Chemical Constituents	Test	Observation	Inference
1	Steroid	Salkowski Test	Chloroformed layer –red, acid layer–greenish	Steroid present
2	Saponin	Shinoda Test	Persistant Foam	Saponin present
3	Flavonoids	Saponins Foam Test	Slight pink colour	Flavonoid pres
4	Tannins & Phenolic compounds	Tannins Extract +FeCl3	Slight yellowcolour	Tannin present

Animals:

The Male wistar rats (150-180 g) were housed in the Animal House of Institute. Animal were maintained at standard environmental condition as per guidelines of CPCSEA with the free access of food and water before or during the experiment.

Acute Oral Toxicity

The testing of acute oral toxicity was performed by using OECD, Guidelines 423 (2001). The acute toxic class method set out in this Guideline is a stepwise procedure with the utilization of 3 animals of a single sex per step. Depending on

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the mortality and/or the moribund status of the animals, on average 2-4 steps may be required to permit judgment on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods (Test Guidelines 420 and 425). For the said purpose, male, young and healthy wistar rats were selected. Weight of each animal was recorded. Animals were divided into different groups containing three animals each. Solutions of test extract were prepared in distilled water in different concentrations of 5, 50, 300 and 2000 mg/kg body weight. The different doses of test drug extract solution (5, 50, 300 and 2000 mg/kg bw) were given orally. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily then after, for a total of 14 days. After testing different doses of test drug extract solution (5, 50, 300 and 2000 mg/kg body weight), appropriate maximum tolerated safe dose was considered. 1/8th & 1/4th of the maximum tolerated safe dose was selected as treatment dose for further studies of pharmacological activities according to OECD, Guideline 423 [21].

Experimental Procedure :

Experimental protocol approved by IAEC formed as per norms CPCSEA, Reg. No. (751/PO/abc/03/CPCSEA). In this study 36 albino wistar rat (150-180g) were used. These study based on the one screening animal model, and paracetamol induced hepatotoxicity (21days). Each screening model having six groups and each group having 6 animals.

Paracetamol Induced Hepatotoxicity

For the paracetamol induced hepatotoxicity studies, paracetamol (500mg/kg po) suspension was prepared using 1% gum acacia and was administered to all animals except the animals of the normal control group. Silymarin (100 mg/kg po) was used as standard. Animals were divided into five groups of six animals each as stated [22].

Group design forhepatoxicity

Sr.No.	Groups	Procedure(1to21days)
1	Control	Water(1ml/100g)
2	Negative contol	Paracetamol(1gm/kg body weight) for8days and water from 9to13days
3	Positive control	Paracetamol(1gm/kg body weight) +std.drug (silymarin100mg/kg p.o.)
4	Testdrug1	Paracetamol(1gm/kgbodyweight) +hydroalcoholic extract of*TestDrug* (100mg/kgp.o)
5	Testdrug2	Paracetamol(1gm/kgbodyweight) +hydroalcoholic extract of*TestDrug* (200mg/kgp.o)
6	Testdrug3	Paracetamol(1gm/kgbodyweight) +hydroalcoholic extract of*TestDrug* (400mg/kgp.o)

Determination of Biochemical Parameters

The biochemical parameters like SGOT, SGPT, ALP and bilirubin were estimated using respective assay kits according to the methods described.

Estimation of Serum Glutamic Pyruvic Transaminase (SGPT)

SGPT is a transaminase enzyme and is also called as Alanine transaminase (ALT) or Alanine Aminotransferase (ALAT). The enzyme is found in high concentration in the liver but is also present in a number of tissues, including the heart and skeletal muscles [Pratt et al., 2010].

The reversible transamination from alanine to α-ketoglutarate is catalyzed by the enzyme and the products of this reaction are pyruvate and glutamate. A coenzyme namely pyridoxal phosphate is responsible for this transamination reaction which is transformed into pyridoxamine in the first stage of the reaction, when an amino acid is transaminated into a keto acid. The reduction of pyruvate and simultaneous oxidation of NADH to NAD are catalyzed by Lactate dehydrogenase SGPT is a transaminase enzyme and is also called as Alanine transaminase (ALT) or Alanine

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Procedure

The method proposed by International Federation of Clinical Chemistry (IFCC) utilizing the LDH-NADH coupled assay, was used for ALT estimation. The reaction mixture (a mixture of 13mM α-Ketoglutaric Acid, 400 mM D-Alanine, 0.2mM NADH, 1200 U/L LDH and Tris Buffer, pH 7.5) was reconstituted by adding distilled water to it. One ml of compound under investigation was taken out into suitable tube and warmed at 37˚C for five minutes. About 0.10ml of sample was transferred to reagent to make total volume to 1.10 ml, mixed and incubated at 37˚C for one minute. After one minute absorbance was recorded and readings were repeated for next two minutes after regular interval of one minute. Average difference in absorbance per minute (Δabs./min.) was calculated. Results were expressed as IU/L (amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.) which was calculated by the formula [24].

$$\text{ALT (IU/L)} = \frac{\Delta\text{Abs./Min.} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \Delta\text{Abs./min.} \times 1768$$

Where,

ΔAbs./Min.	=	Average absorbance change per minute
1.10	=	Total reaction volume(ml)
1000	=	Conversion of IU/mltoIU/L
6.22	=	Millimolar absorptivity of NADH
0.10	=	Sample Volume(ml)
1.0	=	Light path in cm

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 hyperalkalinephosphatasemia, 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 [25].

Procedure

The method described by King (1965) was used to estimate the serum alkaline phosphatase. *p*- Nitrophenyl phosphate (pNPP) is employed in the modified technique which produces a yellow colored product upon hydrolysis by ALP. The maximum absorbance of the assay is achieved at 405 nm. The enzyme activity is said to be higher if reaction proceeds faster and vice versa. For each 96-well assay, 2 mL pNPP liquid substrate (10 mM), 5 mL Mg Acetate (5 mM) and 200 mL assay buffer. 200 mL distilled water (H2O) and 200 mL Tartrazine standard were mixed to form working solution and transferred into wells of a clear bottom 96-well plate. 5 mL samples were transferred into other wells and 195 mL working solution was also transferred to sample wells. This made a total volume of 200 mL into the sample wells. The

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plate was tapped to mix the solutions. Optical density was read at 405 nm immediately after mixing and again after 4 mins on a plate reader. Kinetics of changes in OD was measured [26].

The activity of ALP IU/L of the sample was calculated as:

$$= \frac{(ODSAMPLE4 - ODSAMPLE0) \times 1000 \times RV}{4 \times \varepsilon \times l \times SV}$$

Where,

ODSAMPLE4= OD (405nm) values of sample at 4 min

OD SAMPLE 0 = OD (405nm) values of sample at 0 min.1000 =Conversion of IU/ml to IU/L

RV =Total reaction volume(200mL)

4 = Incubation time(min)

ε =18.75mM^{-1}×cm^{-1}

l = (lightpath, cm)(ODTart–ODH2O)/(ε×c)=1.321

×ΔODSV=Samplevolume,i.e.,5mL

Estimation of Total Bilirubin

Bilirubin is a reddish-yellow pigment found in bile. The production of the pigment usually takes place during the fate of heme. As the pigment liberated from the origin, it is in —unconjugated‖ or —indirect state‖. The insoluble unconjugated form of the compound binds to albumin and travels to the liver where is transformed to—conjugated‖ or —direct‖ form by the treatment of. The conjugated form is water soluble and then moves into the bile. Maintenance and specific enzyme excretion of the pigment usually performed through the urine and stool and when the body cannot process the compound, unconjugated bilirubin can start to accumulate and a distinct yellow coloration of the eye and skin takes place. The person with hepatic disorders can develop dark yellow shade due to extreme hemolysis, biliary tract obstruction or buildup of the pigment in the body. [Annino, 1960]. Hyperbilirubinemia can also be coupled with pernicious anemia, hemolytic and hepatic jaundice, obstructive jaundice, and infectious hepatitis. Levels of this pigment can be evaluated with a blood test, and they may be checked as part of a workup [27].

Procedure

The procedure illustrated by Malloy et al., (1937) was exercised for total bilirubin estimation. Total bilirubin, both conjugated and free, were estimated by the technique. The process describes the use of stabilized diazonium salt of 3,5-dichloroaniline and bilirubin resulting in the formation of azobilirubin which shows its maximum absorbance at 540 nm. The concentration of bilirubin present is directly comparative to the absorbance of the azobilirubin measured spectrophotometrically at 540 nm. 1.0 mL of total Bilirubin Reagent (3,5- dichlorophenyldiazoniumtetrafluoroborate, 0.36 mmol/L) was dispensed in test tube and 0.05 mL sample was added to it. The solutions were properly mixed before their incubation for 5 mins at room temperature. The absorbance was measured at 540nm. The results were articulated as mg/dL bilirubin with the help of the formula given [28].

$$\text{Total Bilirubin} = \frac{\text{Absorbance of Unknown}}{\text{Calibrator Value Absorbance of Calibrator}} \times$$

III. RESULT

PHYTOCHEMICAL SCREENING

Qualitative tests for various phytochemical constituents were executed on the leaf extracts and the results thus obtained were as mentioned in respective tables

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TestName	Hydroalcoholic Extract
ForCarbohydrate Molish'stest	_
ForReducingSugar Fehling'stest	+
Benedict'stest	_
ForMonosaccharide Barfoedtest	_
TestForSteroids Salkowskitest	+
Liebermann'sburchard	+
Test For CardiacGlycosideBaljet'stest	_
Legal'stest	_
Keller'skillanitest	+
TestforsaponinsFoamtest	_
Heamolysistest	_
Test for AnthraquinoneGlycoside Borntrager'stest	_
Modified Borntrager's Test	+
Test For FlavonoidsShinodatest	_
NaOHTest	+
Alkaloids TestDragendorff'stest	+
Mayer'stest	_
Hager'stest	+
Wagner'stest	+
Tanninsandphenoliccompound5%Fecl3 test	_
Dil.Iodinetest	_
Dil.HNO3 test	+
Brominewatertest	+
TerpenoidsSalkowski'stest	-
Libermann-burchardTest	_

(+) Present; (-) Absent

HEPATOPROTECTIVE ACTIVITY

The effect of paracetamol and the hydro alcoholic extracts of plants on various biochemical parameters like AST, ALT, ALP & serum bilirubin as well as on marker enzymes for hepatic functions like SOD, have been investigated. It was found that administration of paracetamol caused significant alteration in the level of biochemical parameters as well as of enzymes when compared to the normal. There was a significant (P<0.001) restoration of these enzyme levels on administration of the leaf extract in a dose dependent manner and also by silymarin at a dose of 100 mg/kg. hydro alcoholic extracts , 1/8th i.e.250mg/kg & 1/4th i.e. 500 mg/kg of the dose was selected as treatment dose for further studies of pharmacological activities.

ACUTE TOXICITY STUDIES

Different doses of hydro alcoholic extracts solution (5, 50, 300 and 2000 mg/kg bw) were given orally. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily then after, for a total of 14 days. Since no mortality was observed upto 2000 mg/kg ofhydro alcoholic extracts , 1/8th i.e.250mg/kg & 1/4th i.e. 500 mg/kg of the dose was selected as treatment dose for further studies of pharmacological activities.

DETERMINATION OF BIOCHEMICAL PARAMETERS

The biochemical parameters like SGOT, SGPT, ALP and bilirubin were estimated using respective assay kits according to the methods described.

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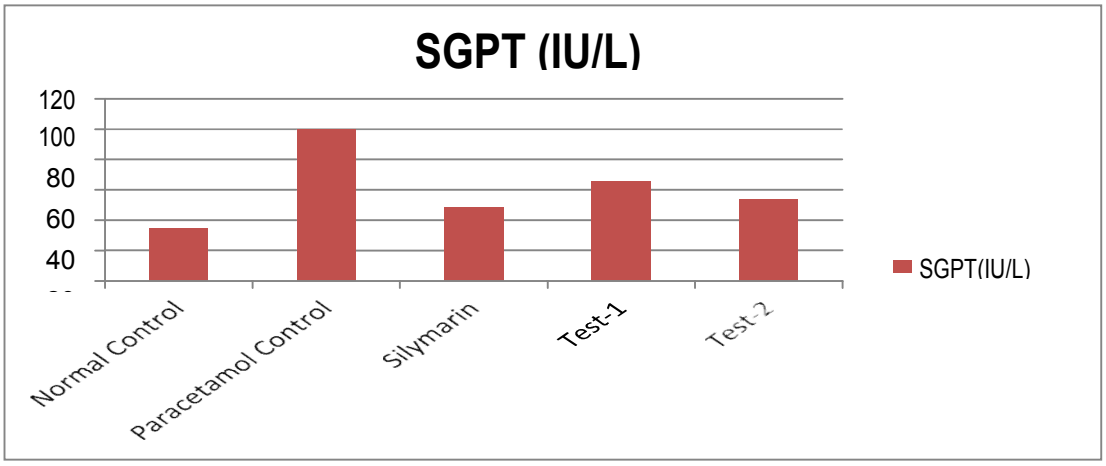
ESTIMATION OF SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

Effect of *Hydroalcoholic extract of test drug* and paracetamol on SGPT level of rats is shown in table. A single dose of paracetamol (500 mg/kg) caused significant elevation in the serum SGPT level when compared to normal group, while on treatment with *Hydroalcoholic extract of* a significant dose dependent reduction was observed in table.

Effect Hydroalcoholic extract of test drug on SGPT level.

Group	Treatment	Dose	No. of animals	SGPT(IU/L)
I	Normalcontrol	1 ml	6	34.74±2.283
II	Paracetamolcontrol	500mg/kg	6	99.85±0.7096[a]
III	Silymarin	100mg/kg	6	48.53±0.9488[a]
IV	Test-1200	200mg/kg	6	65.70±0.6678[a]
V	Test-2400	400mg/kg	6	53.53±0.3355[a]

ap<0.001; bp<0.01; cp<0.05; ns nonsignificant



Effect Hydroalcoholicextract of test drugon SGPT level

ESTIMATION OF SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT)

Paracetamol (500 mg/kg) caused significant elevation in the serum SGOT level when compared to normal group, while on treatment with *Hydroalcoholic extract of test drug* , a significant dose dependent reduction was observed.

Effect of Hydroalcoholic extract of test drug on SGOT level

Group	Treatment	Dose	No.ofanimals	SGOT (IU/L)
I	Normalcontrol	1 ml	6	44.01±0.187
II	Paracetamolcontrol	500mg/kg	6	107.7±0.378[a]
III	Silymarin	100mg/kg	6	53.43±1.06[a]
IV	TestDrug200	200mg/kg	6	76.32±1.148 [a]
V	TestDrug400	400mg/kg	6	57.10±0.523[a]

[a]p<0.001;[b]p<0.01;[c]p<0.05; [ns]non significant

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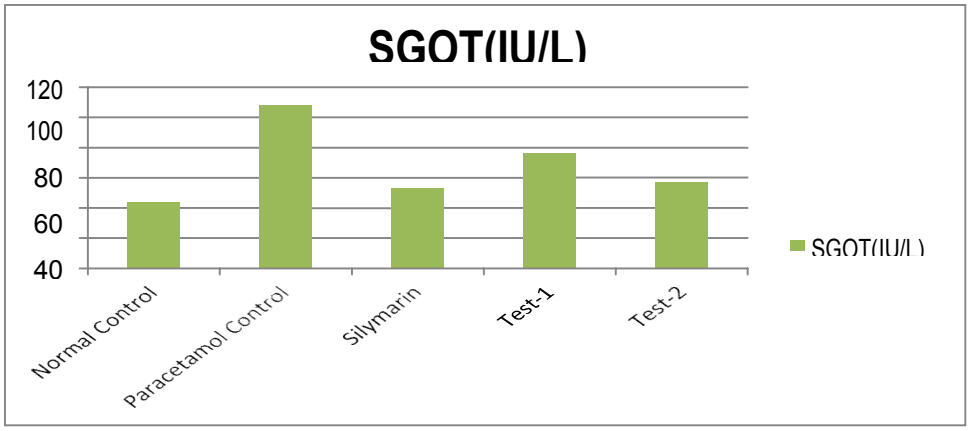


Effect of Hydroalcoholic extract of test drug on SGOT level

ESTIMATION OF ALKALINE PHOSPHATASE (ALP)

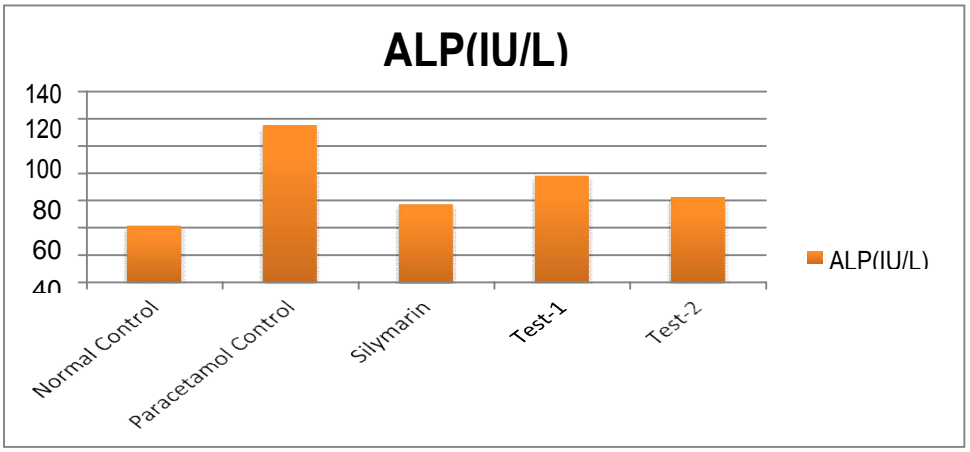
Effect of polyherbal extract and paracetamol on ALP level of rats is shown in table 4.38. A single dose of paracetamol (500 mg/kg) caused significant elevation in the serum ALP level when compared to normal group, while on treatment with hydroalcoholic extract of polyherbal

extract, a significant dose dependent reduction was observed. A graph of comparative ALP level of different groups is shown in fig

Effect of polyherbal extract on ALP level

Group	Treatment	Dose	No.ofanimals	ALP(IU/L)
I	Normalcontrol	1 ml	6	41.70±1.952
II	Paracetamolcontrol	500mg/kg	6	114.8±0.735[a]
III	Silymarin	100mg/kg	6	57.02±0.681[a]
IV	Test-1200	200mg/kg	6	78.04±0.496[a]
V	Test-2400	400mg/kg	6	62.57±0.602[a]

ap<0.001; bp<0.01; cp<0.05; ns non significant



Effect of polyherbal extract on ALP level.

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ESTIMATION OF TOTAL BILIRUBIN

Comparative effect of polyherbal extract and paracetamol on serum bilirubin level is presented in graph 4.30.There was a considerable increase in serum bilirubin level after paracetamol administration which was then reversed by polyherbal extract

Effect of *polyherbal*extract on bilirubin level

Group	Treatment	Dose	No.ofanimals	Totalbilirubin(mg/dl)
I	Normalcontrol	1 ml	6	1.232±0.081
II	Paracetamolcontrol	500mg/kg	6	5.915±0.494[a]
III	Silymarin	100mg/kg	6	2.307±0.148[b]
IV	Test-1200	200mg/kg	6	4.007±0.379[a]
V	Test-2400	400mg/kg	6	2.547±0.063[b]

[a]p<0.001;[b]p<0.01;[c]p<0.05; [ns]non significant

IV. CONCLUSION

Polyherbal extract was found to be much effective in protecting hepatocytes at selected dose. Recovery against paracetamol induced necrosis in their compact arrangement of hepatic cells was observed; whereas the section of liver of animals treated with Silymarin showed that extent of liver damage was lesser in magnitude as compared to the paracetamol treated animals.

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