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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF HIBISCUSSABDARIFFA PETALS

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ABSTRACT

This study aimed at Hepatoprotective Activity of Hibiscus Sabdariffa Petals was evaluationin alcohol induced Liver Necrosis in rats. The Hepatoprotective Activity of aqueous extract of petals of Hibiscus Sabdariffa was evaluated at different dosage of 100 mg/kg and 200 mg/kg. Administration of this extract showed significantly decreased serum levels of AST, ALT, ALP, and bilirubin in a dose-dependent manner. These are indications of Hepatoprotective Activity of aqueous extract of petals of Hibiscus Sabdariffa proving hepatoprotective Activity by comparing favorably well with Silymarin, a standard hepatoprotective drug. The aqueous extract of petals of Hibiscus Sabdariffa restored liver function and hematological parameters to normal control levelsagainst hepatic damage induced by ethanol.

KEYWORDS: Hepatoprotective Activity, Alcohol induced liver toxicity, Hibiscus Sabdariffa,Hepatotoxicity.

INTRODUCTION

The liver is the heaviest gland of the body, weighing about 1.4 kg (about 3 lb) in an averageadult. Among the organs of the body, it is second only to the skin in size. It is located inferior to the diaphragm and occupies most of the right hypochondriac and part of the epigastric regions of the abdominopelvic cavity.

Beside its main function such as storage and distribution of nutrients, liver also function asdetoxifying organ. The liver takes up chemical substances ingested through the food and absorbed through the gastrointestinal tract. Doing so, however, harbours the danger that the substances which are degraded and/or eliminated by liver lead to tissue damage. Most of these substances are compatible with cellular metabolic processing thus utilized by body intact or can be transformed further into components of cells and tissues. But substances which are not processed further or metabolized completely enter into portal circulation and processed by hepatocyte so that they canbe excreted out of body.

Being the main detoxifying and metabolizing organ, liver is prone to a number of diseases. Severity of diseases depends on the onset and duration of pathological conditions. On the bases ofit diseases can be categorised into acute and chronic. They are characterised into, hepatic steatosis, jaundice, hepatitis, fibrosis, cirrhosis, cholestasis and cancer (hepatocellular carcinoma and cholangiocarcinoma).

Hepatotoxicity: The toxic liver injury produced by drugs and chemicals may virtually mimicany form of naturally-occurring liver disease in fact, any patient presenting with liver disease or unexplained jaundice is thoroughly questioned about history of drug intake or exposure to chemicals. Severity of hepatotoxicity is greatly increased if the drug is continued after symptomsdevelop.

II. MATERIALS AND METHOD

Animals

Wistar rats weighing 150-200 gm obtained from Authentic venders were used. The animals received standard pellet diet and allowed free access to food and water ad libitum and were maintained under standard environmental conditions were approved by the Institutional Animal Ethics committee of Anuradha College of pharmacy, Chikhali. Project proposal no: (751/PO/Re/S/03/CPCSEA/2023/1-1)

Chemicals and standard drug

Silymarin, ethanol, normal saline solution was procured and used.

Collection and Authentication of the drugs

The plant material procured locally and petals of Hibiscus sabdariffa identified and authenticated by Sahakar Maharshi Late Bhaskarrao Shingne Junior Arts Science and CommerceCollege, Khamgaon.

Preparation of extract

After authentication, Petals of Hibiscus Sabdariffa washed and sun dried and was ground in a mechanical grinder to obtain coarse powder. For preparation of water extraction, 25 g sample of powder was added to 250 ml distilled water and the mixture was boiled for 10 min while stirring with a magnetic stirrer. Then, the extract was filtered from filter paper.

Preliminary Phytochemical Evaluation of Hibiscus sabdariffa petals Extract

Test for saponins

1. **Foam test** - Drug extract or dry powder was wobbled vigorously with water. If persistentfoam observed, shows presence of saponins.
2. **Haemolytic test** -Added drug extract or dry powder to one drop of blood placed on glassslide. If hemolytic zone is found indicates presence of saponins.

Test for alkaloids

1. **Dragendorff's test: -** the extract was treated with Dragendorff's reagent (potassium bismuth iodine solution), formation of reddish brown precipitate indicates the presence of alkaloids
2. **Mayer's test: -** the extract was treated with Mayer's reagent (potassium mercuric iodide solution) formation of creamy colour precipitate, indicate the presence of alkaloids.
3. **Wagner's test: -** the extract was treated with Wagner's reagent (iodine potassium iodide solution) reddish brown precipitate indicate the presence of alkaloids
4. **Hager's test: -** the extract was treated with Hager's reagent (saturated solution of picric acid) formation of yellow precipitate indicate the presence of alkaloids.

Test for flavonoids

1. **Shinoda test: -** to the extract was add few magnesium turnings and concentrated Hcl dropwise, pink scarlet, crimson red, or occasionally green to blue appears after few minutes.
2. **Alkaline reagent test: -** to the extract was add few drops of NaOH solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acidindicate the presence of flavonoids.
3. **Zinchydrochloride test: -** to the extract add a mixture of zinc dust and concentrated Hcl. It gives red colour after few minutes.

Test for phenols

2ml of extract was added to 2ml of ferric chloride solution a deep bluish green colour indicate the presence of phenol.

Test for Triterpenoids

1. **Libermann-Burchard test: -** treat the extract with few drops of acetic anhydride, boil and cool the extract, then add 1ml of conc. H_2SO_4 along the side of test tube. Formation of deep red colour ring indicates the presence of triterpenoids
2. **Sulfur powder test: -** to the extract small amounts of Sulphur powder, it sinks at the bottom, indicate the presence of triterpenoids.

Test for lignin's

Extract is treated with concentrated HCL and phloroglucinol solution, pink colour is indicate the presence of lignin

Thionine test for lignin

Extract was treated with thionine solution, after 15 min. wash with alcohol, bluish violet colour isformed

Determination of Acute toxicity (LD50)Preparation of dose

The extract of the Hibiscus sabdariffa petals was suspended in Tween-80 to prepare a dose of 2000 mg/kg b.w. of animal and administered 1ml/100 g b.w. of the animal. Acute toxicity study of the extract was done according to acute toxic classic method (OECD guideline 425, 2006) using albino male rats to determine the safe dose.

Table 6.1: Experimental Design

The groups are as follows

Group 1: Serves as Normal control received 10ml/kg normal saline orally for 28 days.

Group 2: Serves as toxic control received 5ml/kg Ethanol orally for 28 days once daily.

Group 3: Serves as Standard control received 100mg/kg Silymarin and Ethanol 5ml/kg orally for 28 days once daily.

Group 4: Serves as treatment control received 200mg/kg of Hibiscus sabdariffa petals extract and Ethanol 5ml/kg orally for 28 days once daily.

Group 5: Serves as treatment control received 400mg/kg of Hibiscus sabdariffa petals extract and Ethanol 5ml/kg orally for 28 days once daily

III. RESULTS

Phytochemical investigation

Table 7.1: The phytochemical investigation for various chemical constituents in *Hibiscus sabdariffa* petals Extract.

Phytochemical Constituent	Test Result
Alkaloids	Absent
Flavonoids	Present
Phenols	Present
Saponins	Present
Tannins	Present
Terpenoids	Present
Anthocyanins	Present
Glycosides	Absent
Coumarins	Absent

Acute oral Toxicity study (425) observations

The extract of the *Hibiscus sabdariffa* petals was studied for acute toxicity at the dose level of 2000 mg/kg, p.o. according to OECD guideline Annexure 425. It was found to be safe up to 2000 mg/kg body wt. by oral route. There was no mortality and no signs of toxicity and extract were found to be safe. So two dose levels i.e., 200 mg/kg ($1/10^{th}$), and 400 mg/kg ($1/5^{th}$) body weight were selected for the present study.

Table 7.2: Acute Toxicity study observations.

RESPIRATORY BLOCKAGE IN NOSTRIL	
Dyspnea	Nil
Apnoea	Nil
Tachypnea	Nil
Nostril discharge	Nil
MOTOR ACTIVITIES	
Locomotion	Normal
Loss of righting reflex	Nil
Anaesthesia	Nil
Ataxia	Nil
Toe walking	Nil
Fasciculation	Nil
Tremor	Nil
CONVULSION (INVOLUNTRAY CONTRACTION)	
Clonic / tonic / tonic - clonic convulsion	Nil
Asphyxial convulsion	Nil
Opistotones (titanic spasm)	Nil
REFLEXES	
Corneal	Normal
Eyelid closure	Normal
Righting	Normal
Light	Normal
Auditory and sensory	Normal
OCULAR SIGNS	
Lacrimation	Nil
Miosis	Nil
Mydriasis	Nil
Ptosis	Nil
Conjunctivitis	Nil
SALIVATION	
Saliva secretion	Nil
PILOERECTION	
Contraction of erectile tissue	Nil
ANALGESIA	
Decrease in reaction to induced pain	Nil
MUSCLE TONE	
Hypo or hypertonia	Nil
GIT SIGN	
Solid dried / watery stool	Nil
Emesis	Nil
Red urine	Nil
SKIN	
Oedema	Nil
Erythema	Nil

Pharmacological Activities

Effect of *Hibiscus sabdariffa* petals Extract is on alcohol induced Liver Necrosis in rats

In the present study, the hepatotoxicity was successfully produced by administration of 5ml/kg Ethanol P.O. and the hepatoprotective activity of *Hibiscus sabdariffa* was determined from the serum parameters AST, ALT, ALP and bilirubin.

AST (Serum aspartate transaminase)

Effect of *Hibiscus sabdariffa* petals Extract on Serum level of AST are shown in table 7.3 and fig 7.1.It was

observed that ethanol administration resulted in a significant elevation in the Serum level of AST compared to control group. The standard (Silymarin) treatment and treatment with 200mg/kg and 400mg/kg of *Hibiscus sabdariffa* petals extract showed extremely significant ($p<0.001$) decrease in serum AST level.

Table 7.3: Serum levels of AST values of means of 6 rats in each group.

Groups	Treatment	AST (IU/L)
Group I	Normal control- 10ml/kg normal saline	85.31±0.26
Group II	Toxic control - received 5ml/kg Ethanol	337.53±0.4
GroupIII	Standard control - 100mg/kg Silymarin+ Ethanol 5ml/kg	95.53±0.4***
GroupIV	Treatment control (Low dose) - 200mg/kg of *Hibiscussabdariffa* petals extract +Ethanol 5ml/kg	123.32±1.21***
GroupV	Treatment control (High dose)- 400mg/kg of *Hibiscussabdariffa* petals extract+ Ethanol 5ml/kg	109.41±1.9***

One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6.p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

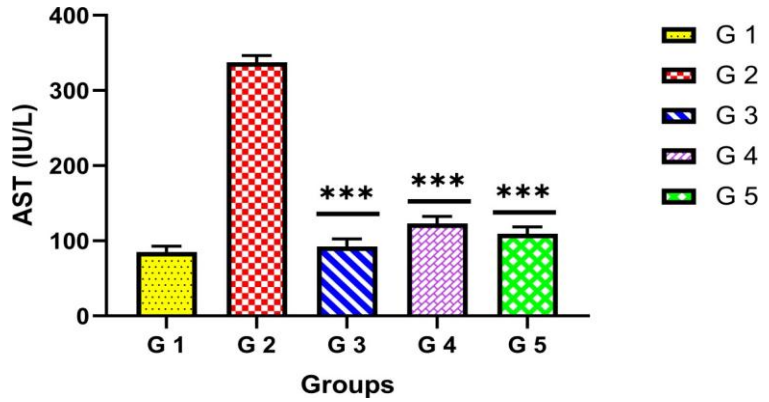


Fig 7.1: Graphical representation of Serum levels of AST values of means of 6 rats in each group.

One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6.p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Group 1: Serves as Normal control received 10ml/kg normal saline orally for 28 days.
Group 2: Serves as toxic control received 5ml/kg Ethanol orally for 28 days.
Group 3: Serves as Standard control received 100mg/kg Silymarin and Ethanol 5ml/kg orally for28 days.
Group 4: Serves as treatment control (Low dose) received 200mg/kg of *Hibiscus sabdariffa* petals extract and Ethanol 5ml/kg orally for 28 days.
Group 5: Serves as treatment control (High dose) received 400mg/kg of *Hibiscus sabdariffa* petals extract Ethanol 5ml/kg orally for 28 days.

ALT (Serum alanine transaminase)
Effect of *Hibiscus sabdariffa* petals Extract on Serum level of ALT are shown in table 7.4and fig 7.2.It was observed that ethanol administration resulted in a significant elevation in theSerum level of ALT compared to control group. The standard (Silymarin) treatment and treatment with 400mg/kg of *Hibiscus sabdariffa* petals extract showed extremely significant ($p<0.001$) decrease in serum ALT level.
Treatment with 200mg/kg of *Hibiscus sabdariffa* petals extract showed moderately significant ($p<0.01$) decrease in serum ALT level.

Table 7.4: Serum levels of ALT values of means of 6 rats in each group.

Groups	Treatment	ALT(IU/L)
Group I	Normal control- 10ml/kg normal saline	38.23±0.38
Group II	Toxic control - received 5ml/kg Ethanol	257.43±0.5
Group III	Standard control - 100mg/kg Silymarin+ Ethanol 5ml/kg	49.27±0.5***
Group IV	Treatment control (Low dose) - 200mg/kg of *Hibiscussabdariffa* petals extract +Ethanol 5ml/kg	186.54±2.1**
Group V	Treatment control (High dose)- 400mg/kg of *Hibiscussabdariffa* petals extract+ Ethanol 5ml/kg	70.23±0.77***

One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6.p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

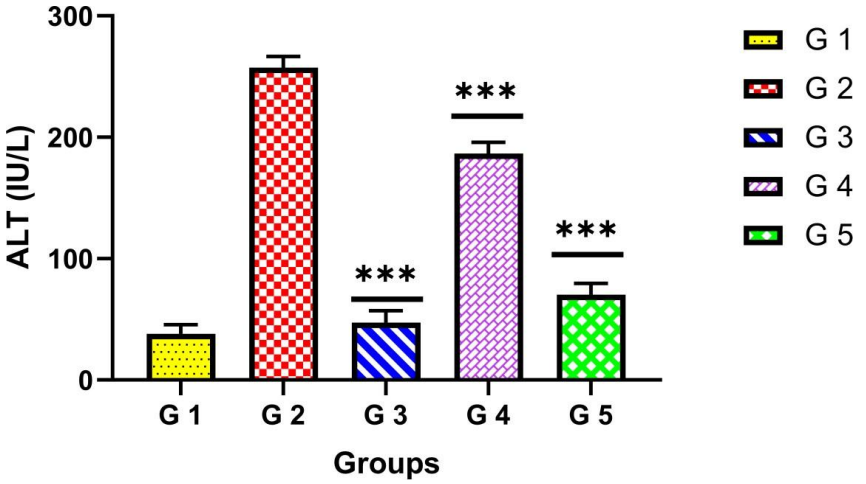


Fig 7.2: Serum levels of ALT values of means of 6 rats in each group.

One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6.p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Group 1: Serves as Normal control received 10ml/kg normal saline orally for 28 days.
Group 2: Serves as toxic control received 5ml/kg Ethanol orally for 28 days.
Group 3: Serves as Standard control received 100mg/kg Silymarin and Ethanol 5ml/kg orally for28 days.
Group 4: Serves as treatment control (Low dose) received 200mg/kg of *Hibiscus sabdariffa* petals extract and Ethanol 5ml/kg orally for 28 days.
Group 5: Serves as treatment control (High dose) received 400mg/kg of *Hibiscus sabdariffa* petals extract Ethanol 5ml/kg orally for 28 days.

ALP (Serum alkaline phosphatase)
Effect of *Hibiscus sabdariffa* petals Extract on Serum level of ALP are shown in table 7.5 and fig 7.3.It was observed that ethanol administration resulted in a extremely significant elevationin the Serum level of ALP compared to control group. The standard (Silymarin) treatment and treatment with 200mg/kg and 400mg/kg of *Hibiscus sabdariffa* petals extract showed extremely significant (p<0.001) decrease in serum ALP level.

Table 7.5: Serum levels of ALP values of means of 6 rats in each group.

Groups	Treatment	ALP (IU/L)
Group I	Normal control- 10ml/kg normal saline	120.52±0.50
Group II	Toxic control - received 5ml/kg Ethanol	391.42±0.3
Group III	Standard control - 100mg/kg Silymarin+ Ethanol 5ml/kg	132.82±0.66***
Group IV	Treatment control (Low dose) - 200mg/kgof *Hibiscus sabdariffa* petals extract+Ethanol 5ml/kg	253.24±1.09***
Group V	Treatment control (High dose)- 400mg/kgof *Hibiscus sabdariffa* petals extract+Ethanol 5ml/kg	139.65±3.30***

One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6.p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05,*p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

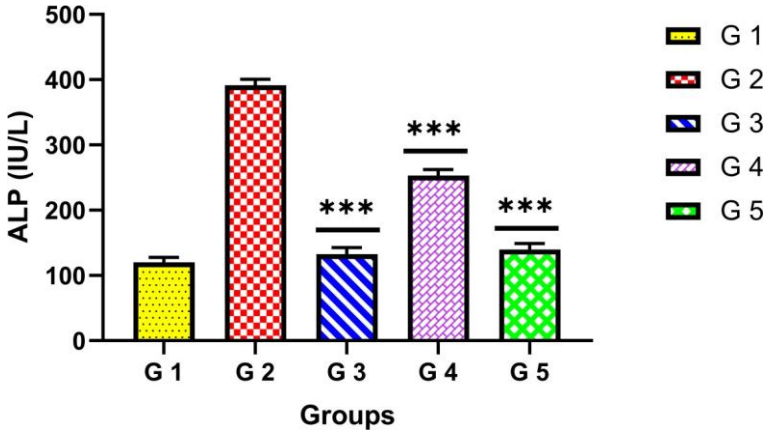


Fig 7.3: Serum levels of ALP values of means of 6 rats in each group.

One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6. p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Group 1: Serves as Normal control received 10ml/kg normal saline orally for 28 days.
Group 2: Serves as toxic control received 5ml/kg Ethanol orally for 28 days.
Group 3: Serves as Standard control received 100mg/kg Silymarin and Ethanol 5ml/kg orally for28 days.
Group 4: Serves as treatment control (Low dose) received 200mg/kg of *Hibiscus sabdariffa* petals extract and Ethanol 5ml/kg orally for 28 days.
Group 5: Serves as treatment control (High dose) received 400mg/kg of *Hibiscus sabdariffa* petals extract Ethanol 5ml/kg orally for 28 days.

Bilurubin

Effect of *Hibiscus sabdariffa* petals Extract on Serum level of BILURUBIN are shown intable 7.6 and fig 7.4.It was observed that ethanol administration resulted in a extremely significant elevation in the Serum level of BILURUBIN compared to control group. The standard (Silymarin) treatment showed extremely significant (p<0.001) decrease in serum BILURUBIN level. Treatment with 200mg/kg and 400mg/kg of Hibiscus sabdariffa petals extract showed Moderately significant (p<0.01) decrease in serum BILURUBIN level.

Table 7.6: Serum levels of BILURUBIN values of means of 6 rats in each group.

Groups	Treatment	Bilurubin(mg/dL)
Group I	Normal control- 10ml/kg normal saline	0.58±0.42
Group II	Toxic control - received 5ml/kg Ethanol	3.48±1.20
Group III	Standard control - 100mg/kg Silymarin+Ethanol 5ml/kg	0.98±1.80***
Group IV	Treatment control (Low dose) - 200mg/kg of *Hibiscus sabdariffa* petals extract +Ethanol5ml/kg	2.15±0.65**
Group V	Treatment control (High dose)- 400mg/kg of *Hibiscus sabdariffa* petals extract+ Ethanol5ml/kg	1.60±1.2**

One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6. p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

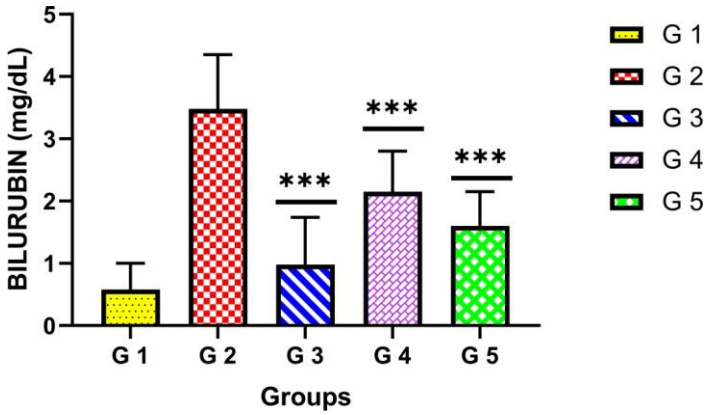


Fig 7.4: Serum levels of BILURUBIN values of means of 6 rats in each group.

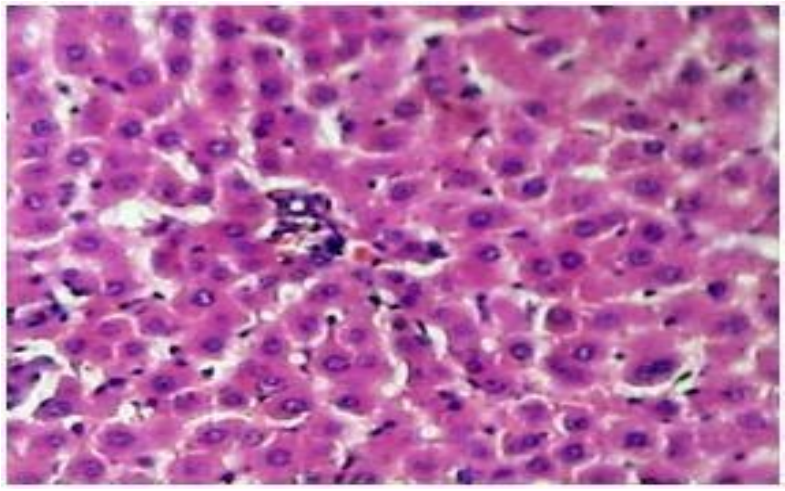
One-way ANOVA followed by Dunnett's t test. All the values are Mean± SEM, n=6. p< 0.001 when compared with vehicle treated control group with toxic control. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Group 1: Serves as Normal control received 10ml/kg normal saline orally for 28 days.
Group 2: Serves as toxic control received 5ml/kg Ethanol orally for 28 days.
Group 3: Serves as Standard control received 100mg/kg Silymarin and Ethanol 5ml/kg orally for28 days.
Group 4: Serves as treatment control (Low dose) received 200mg/kg of *Hibiscus sabdariffa* petals extract and Ethanol 5ml/kg orally for 28 days.
Group 5: Serves as treatment control (High dose) received 400mg/kg of *Hibiscus sabdariffa* petals extract Ethanol 5ml/kg orally for 28 days.

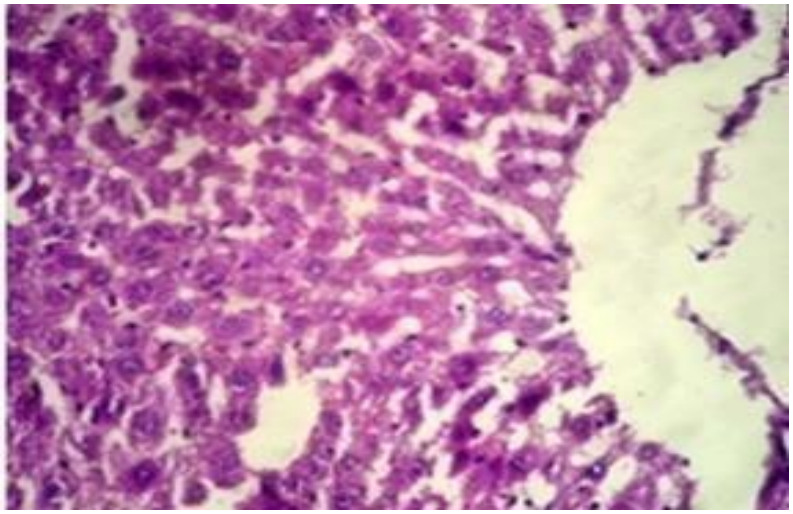
Histopathological studies of the liver

The histopathological evaluation of Ethanol toxicity in all the groups was examined. .Liversection of normal group shows liver parenchyma with intact architecture. Most hepatocytes appearnormal. In toxic control group shows inflammation, centrilobular degeneration and necrosis. Treatment with (200 & 400 mg/kg of *Hibiscus sabdariffa* petals extract) found to reduce inflammation, centrilobular and bridging necrosis. Liver section of this

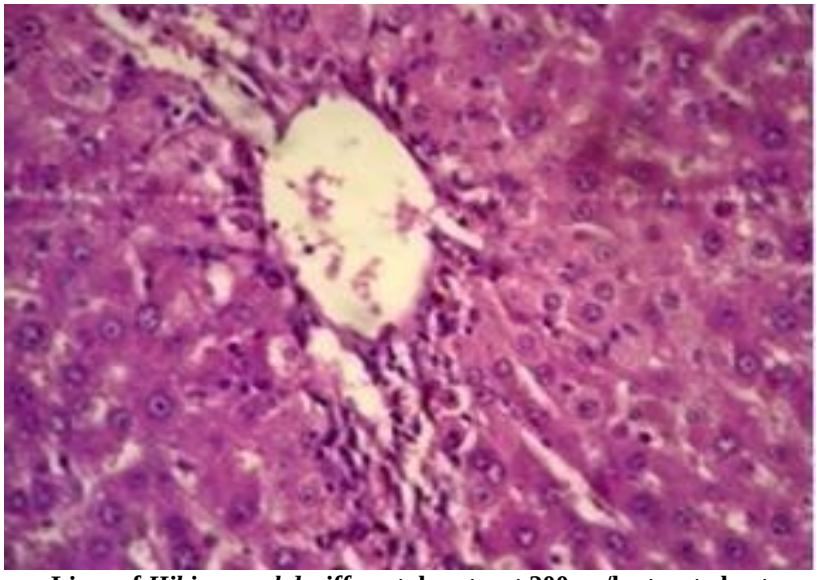
group shows normal hepatocytes with significant reduction in areas of necrosis when compared to toxic group. These changes show protective effect of the drug against hepatic damage induced by Ethanol.



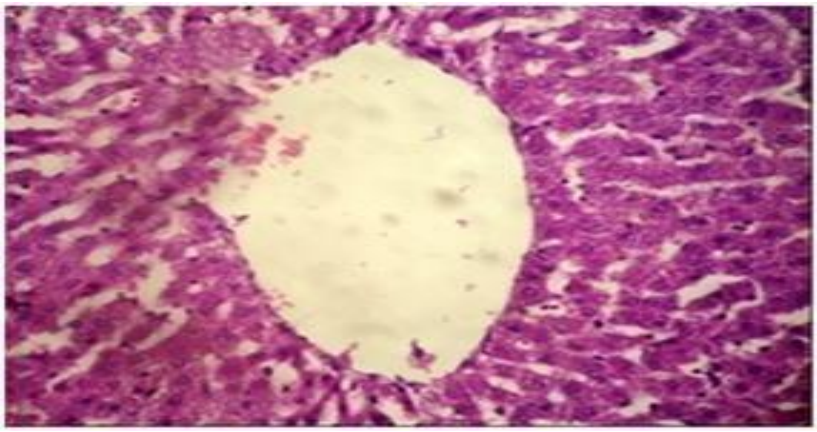
Liver of normal rat.



Liver of Ethanol Induced rat.



Liver of *Hibiscus sabdariffa* petals extract 200mg/kg treated rat



Liver of *Hibiscus sabdariffa* petals extract 400 mg/kg treated rat.

Fig. 7.5: Effect of Silymarin and *Hibiscus sabdariffa* petals extract on liver histology in Ethanol inducedliver toxicity.

CONCLUSION

In conclusion, the present study evaluated the hepatoprotective activity of Hibiscus sabdariffa petals extract in rats with ethanol-induced liver necrosis. The results showed that the extract significantly decreased serum levels of AST, ALT, ALP, and bilirubin in a dose-dependent manner. The histopathological evaluation also indicated a protective effect of the extract against hepatic damage induced by ethanol. Therefore, it can be suggested that Hibiscus sabdariffa petals extract has potential as a natural hepatoprotective agent.

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