IJMPR 2020, 4(6), 52-61

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Review Article
SJIF Impact Factor: 5.273

DETERMINATION OF HEPATOPROTECTIVE ACTIVITY USING PLANT EXTRACT IN ISONIAZID –RIFAMPICIN INDUCED HEPOTOTOXIC RAT MODELS

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Received on: 20/10/2020
Revised on: 10/11/2020
Accepted on: 30/11/2020

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ABSTRACT

The study was conducted which involves the investigation of protective action of liver by using tubers of juncus sabulatus and the leaves of cordia macleodii against isoniazid and rifamocin induced toxic rats models.the action was assayed using ethanolic extracts by using maceration technique. The extract were screened for standard phytochemical screening. The study was analysed by invitro and invivo methods by i.p administration and the mortality and toxic signs was observed for 24hrs.The study was carried out for 21days, and were administered with dose of 200mg/kg body weight and 400mg/kg bof dy weight both individually and in combination respectively against isoniazid and rifampicin toxicated rats. Silymarin was used as a refrence standard.The biochemical parameters ,ic50 value and histological resukts wre carried out. The reults shows the effects by decrement in the levels of GOT,ALP,MDA,alkaline phosphate and total bilirubin and increasing the levels of total protein,albumin,SOD and CAT. By this study it as found that the plants contain moderate effect of protective action which was supported by the histopathological reports.

KEYWORDS: J.sabulatus, C.macleodii, liver, hepatopertection, silymarin.

INTRODUCTION

The Liver is the solid multifunctional largest organ that serves a vital function in the human body.[1] It is the second largest organ and is placed on the right hand side of the abdomen, surrounding thoracic cage and diaphragm. The spatial position of the liver is seen above the stomach, gall bladder and pancreas.

Conventionally the liver is divided into four different lobes---right, left, caudate lobe, quadrate lobe.

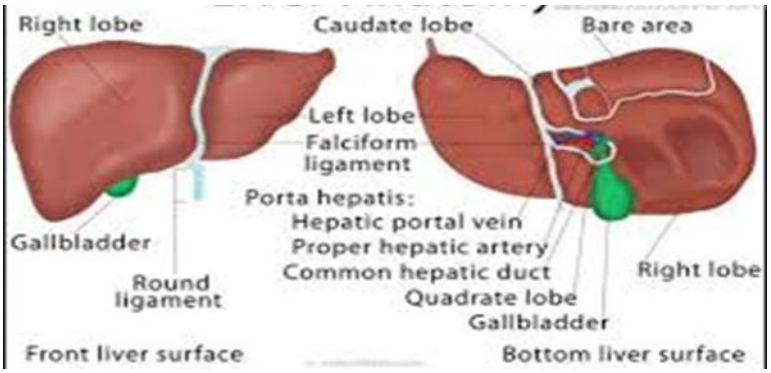


Image 1: Anatomy of Liver.

Histology of Liver:[2]

Lobule is the basic operating unit of the liver and is hexagonal in shape. The liver lobule consists of following basic structure:

- Plates of hepatocytes which occupies majority of the lobule
- Portal traids at each corner of hexagon
- Liver sinusoids
- Central vein
- Hepatic macrophages(kupffer cells)
- Bile canaliculi
- Space of disse present between sinusoids and hepatocytes.

The liver is made up of different types of cells but out of many the following type of cells are of outmost importance:

- Hepatocytes
- Endothelial cells
- Kupffer cells

- Stellate cells

Hepatoprotection
The term hepatoprotection is defined as the protective mechanism which involve in the attenuation of the damage liver which is caused due to toxifying agents.

Mechanism of Hepatoprotection:[3]
The herbal drugs elucidate their hepatoprotection action by using two or more mechanism on the hepatocytes either directly or indirectly to protect against harmful affects. The mdifferent type of mechanism are as follows:

- Decrease in the oxidants/increase in antioxidant level
- Hindrance of cytp450
- Increment and decrement in the levels of liver enzymes
- Reduced lipid peroxidation
- Rise in glutathione level or reducing equivalents

Hepatoprotective affirmation of silymarin
Silymarin is regarded as the protective drug with known mechanism of action employed for the eradication of effects of liver damage. It facilitates its action by multiple mechanisms,which includes the following

- Inhibition of toxin penetration into hepatic cells by binding to cell membranes
- Increment in the SOD activity,glutathione tissur level and hepatocyte protein synthesis, reduction of lipid peroxidation and elevation of synthesis of protein hepatocyte

Plant Profile
Juncus Sabulatus: It is indegenos genotype plant appeared as the pale green to brownish in colour which contains stem size of about 30-120cm with 3-4caulines leaves and basal sheaths. It belongs to the family juncacea.

Vernacular name: English name-Smerset rush

Maltese name-Sima tal-ilma

Uses: They found to possess hepatoprotective and antioxidant property by the ethanolic,volatile oil and total alcoholic extraction. The juncus species were found to exhibit antitumot,anti-inflammatory,anti-angal and cytotoxic properties of action.

Image 2: Juncus Sabulatus.

Cordia Macleodii
It belongs to the family Boroginaceae which are distributed in the warmer regions of tropic and sub tropical areas around the world. The preliminary phytochemical screening was done by using different extraction which confirms the presence of triterpenoids.

Vernacular name: Telugu –palandekku
Oriya – baurlo, bhoto. sambarsinga, panki, hikari
Hindi – dahiman, dahiphalas, dhengan, gonni, kuhman
Kanada- billi challe, dodacalle, dadang, hirichalle
Telugu- botuku, peddabattava, peddabotuku
Marathi – daiwas, dhalm, bhoti, dhaim, dhaiwan, dhamam

Uses: It has many medicinal properties which are useful many research works in the medical field. Apart from the protective nature of liver it also yields for the usuage as the anti-inflammatory action,anti-fungal action,anti-bacterial, antioxidant property,radical scavenging action. It was also noticed it is usd as a analgesic.

Image 3: Cordia Macleodii.

METHODOLOGY

Plant Materials
The dried tubers of *Juncus sabulatus* and dried leaves of *Cordia macleodii* is obtained and authenticated from Department of Botany.

Ethanolic Extract Preparation
The extract will be prepared by Maceration Technique. The extracts are macerated with ethanol (99.9%) for 7 days and then it will be filtered. The filtrate will be evaporated to obtain dried extract.

Maceration

In this process, the uncut plant or the part of the plant is taken then it is made into britty form. Then this powder crude substance is placed in corked bottke in close contact with the particular dissolvable solution i.e.,99.9% ethanol inq:2 ratio at room temperature with persistent stirring for 7 days tillthe matter completely dissolves. Then at this point, the concoction is drained ith the help of muslin cloth, by pressing action the matc(soggy fabric cloth) the liquid exract is collected which was subjected for drying after completion of a week.[9]

Preliminary Phytochemical Screening

Standard screening tests of the plants extract will be carried out for various plants constituents. The crude extracts are screened for the presence of secondary metabolites such as alkaloids, quercetin, phenols, flavonoids, saponins, glycosides, terpenoids, rutin, tannins and anthraquinones etc., and it will be further analyzed by GC-MS analysis for confirmation of secondary metabolites.

Experimental animals

Wistar rats of either sex were taken which weighs about 180-200gms and the study was conducted out for 21days.

Before the experiment toxicity test are performed and the motality rate and the behavourial and the pharmacological changes are observed.Rats will be divided into respective groups and housed in standard conditions of temperature should be 25ºC and 12 hours light/dark cycles is set.Permission and approval of animal studies was obtained from IAEC, after submission of form B and protocol no. (IAEC-012/SES/2019/006).

Acute Toxicity Studies

- This toxicity studies will be performed for the extracts according to the OECD guidelines (no.425).
- Here up and down procedure is followed for acute oral toxicity studies.
- The first animal will be given a dose lesser than LD50 then,it is observed for 48hrs for signs of mortality or any toxicity.
- If it does not show any toxicity sign then the dose will be increased further or vice versa.
- The upper limit dose is taken as 2000mg/kg.
-

Experimental Design.

Groups	Drugs	Dosage and route	Number of animals
Group-1	Normal saline	10ml/kg b.w-p.o	6
Group-II	Toxic control	50+100mg/kg b.w i.p	6
Group-III	Toxic control + Standard drug	50mg/kg b.w - i.p	6
Group-IV	Toxic control+Test1+dose 1	200mg/kg b.w-p.o	6
Group-V	Toxic control+Test1+dose 2	400mg/kg b.w -p.o	6
Group-VI	Toxic control+Test2 +dose 1	200mg/kg b.w-p.o	6
Group-VII	Toxic control+Test2+dose 2	400mg/kg b.w-p.o	6

Hepatoprotective in Vivo Screening Activity

Wistar rats of either sex which weigh about 120-150g is taken and are subjected to acclimization for 7 days at 25º at 12 hours light-dark cycle and are fed with standard laboratory diet and water adlibitium before the experiment. The animals are divided into 7 groups of 6 rats each.

The experiment was carried for 21 days znd at the end of the experiment, the rats are fasted for 24 hours and blood samples was collected from retro orbital plexus by using capillary tubes for biochemical estmations.

For histopathological estimations, animals are exposed to ether anesthesia using dessicator chamber, then liver is dissected using standard technique then it is subjected to keep in a 10% formaldehyde solution and further used for histopathological examinations.[10]

In vitro method

- Hepg2 cells are taken and msntsined in 96 wel cultured plates,then IC50 value was deyermined by MTT assay.
- It was assessed by treating well cultered cells with respective standard dose,toxicant dose and different concentraton of test drug.
- Later cell viability was assayed by using standard merhods.

Biochemical Parameters Results

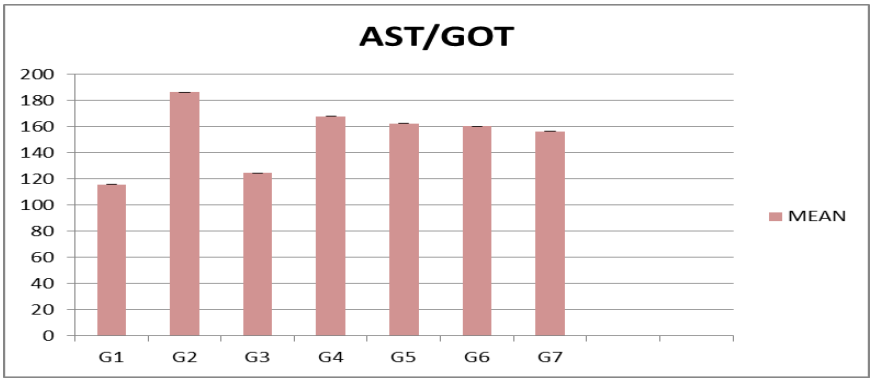
Table 2: Asessment of AST/SGOT Among treated groups, group 9th is most effective and 8th group accounts for the least effective among all groups.

Grouping	AST/SGOT VALUES
GROUP I	114.33±0.9545
GROUP II	177.17±0.8724
GROUP III	134.67±0.7149
GROUP IV	165.67±0.4944
GROUP V	163.17±0.7032
GROUP VI	161±0.5774
GROUP VII	157±0.3426

Data was represented as MEAN±SEM.

Toxicant rats+silymarin were compared toxic control rats.

P<0.05 compared to toxicant,*= p<0.05, **=p,0.01, ***=p<0.001

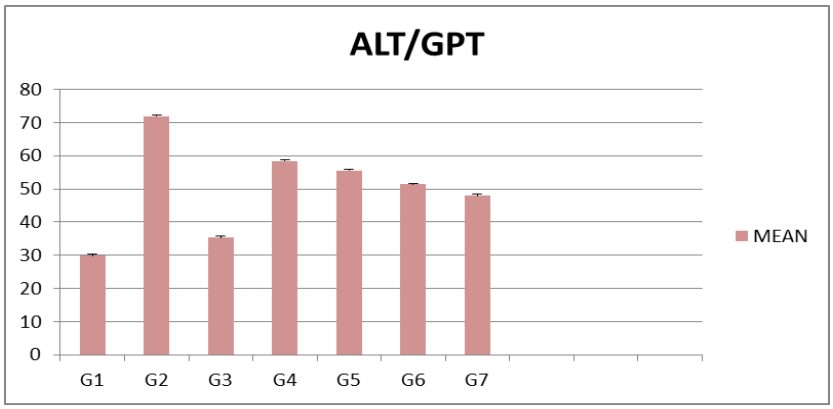


Graph 1: AST/SGOT.

Table 2: Assessment of ALT/GPT Among all toxicated groups 9,8,7 are having highest amount of Alt/GPT levels.

GROUPING	ALT/GPT
GROUP I	29.832±1.4017
GROUP II	72±0.8563
GROUP III	35.333±0.9888
GROUP IV	58.5±0.4282
GROUP V	55.5±0.5627
GROUP VI	51.333±0.5578
GROUP VII	48±0.4472

Data was represented as MEAN±SEM.
Toxicant rats+silymarin were compared toxic control rats.
P<0.05 compared to toxicant,*= p<0.05, **=p,0.01, ***=p<0.001



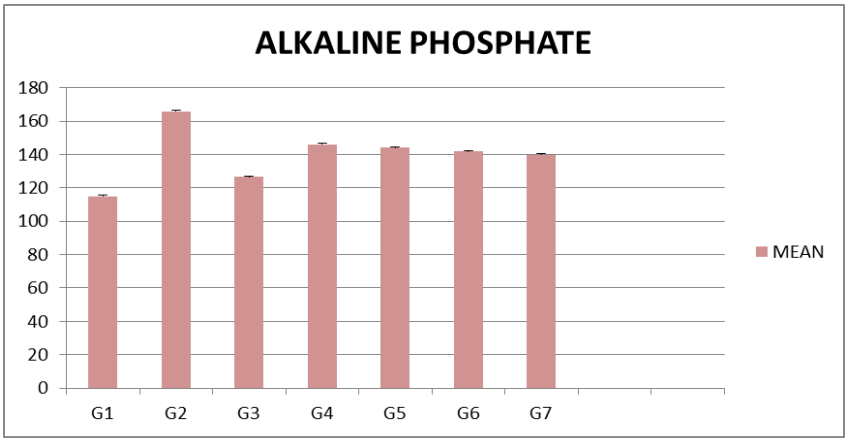
Graph 2: ALT/GPT.

Table 4: Assessment of alkaline phosphate Among treated groups, group 9th is the most effective and group 4th shows least effective results.

Grouping	Alkaline Phosphate
GROUP I	115±0.7330297
GROUP II	165.833±0.8333
GROUP III	126.5±0.619139
GROUP IV	146±0.818754
GROUP V	144±1.264911
GROUP VI	141.7933±1.313536
GROUP VII	139.833±1.301708

Data was represented as MEAN±SEM.
Toxicant rats+silymarin were compared toxic control rats.

P<0.05 compared to toxicant,*= p<0.05, **=p,0.01, ***=p<0.001



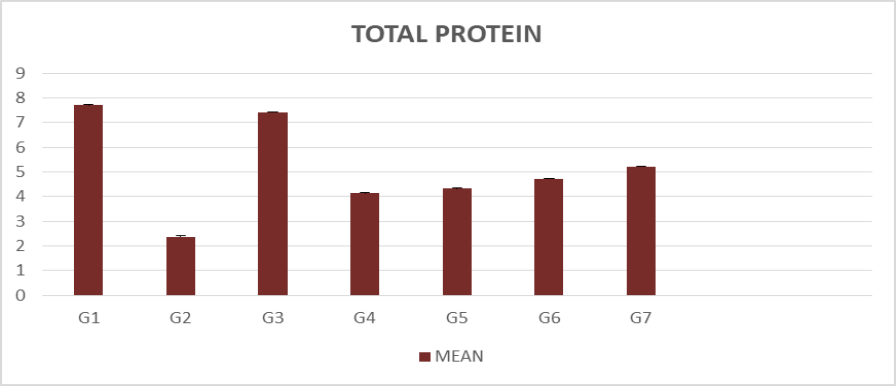
Graph 3: Alkaline Phosphate.

Table 5: Assessment of total protein Among all of the groups, group 9th shows the most effective results and the group 4th represents the least effective results.

Grouping	Total protein
Group I	7.71667±7.8
GROUP II	2.36667±2.4
GROUP III	7.41667±7.4
GROUP IV	4.1333±4.2
GROUP V	4.31667±4.4
GROUP VI	4.71667±4.7
GROUP VII	5.21667±5.4

Data was represented as MEAN±SEM.
Toxicant rats+silymarin were compared toxic control rats.

P<0.05 compared to toxicant,*= p<0.05, **=p,0.01, ***=p<0.001.



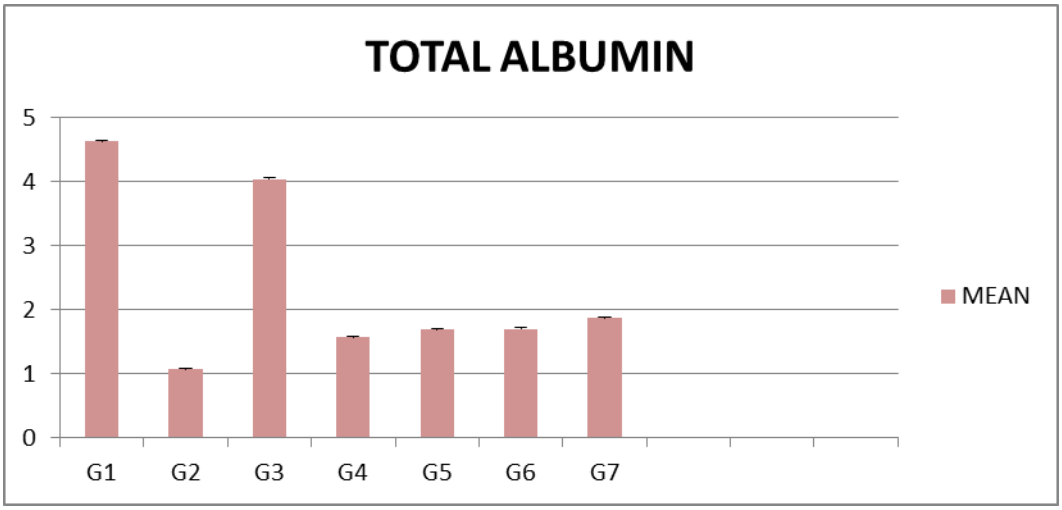
Graph 4: total protein.

Table 6: Assessment of total ALBUMIN Among all extracts, group 9th shows effective results whereas 4th group shows least total protein.

GROUPING	TOTAL ALBUMIN
GROUP I	4.6333±0.0333
GROUP II	1.066667±0.061464
GROUP III	4.035±0.053526
GROUP IV	1.57±0.021134
GROUP V	1.69±0.026833
GROUP VI	1.705±0.029297
GROUP VII	1.86833±0.027978

Data was represented as MEAN±SEM.
Toxicant rats+silymarin were compared toxic control rats.

P<0.05 compared to toxicant,*= p<0.05, **=p,0.01, ***=p<0.001

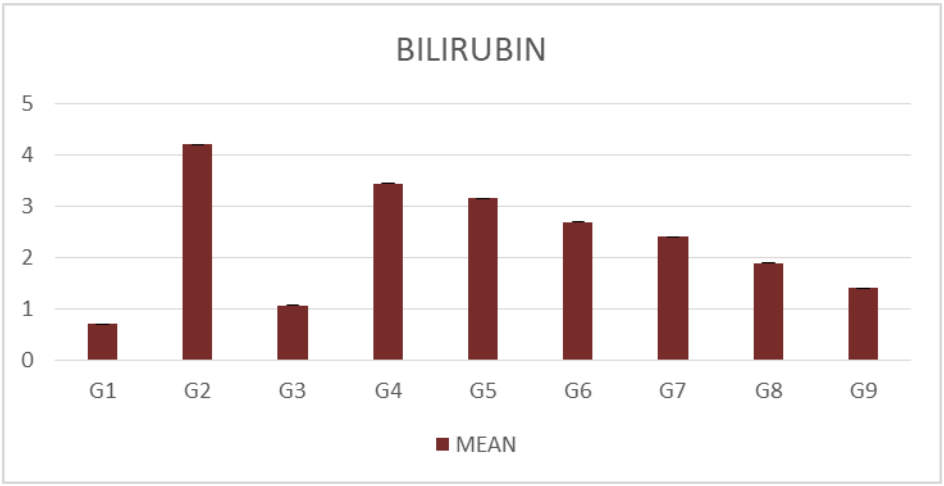


Graph 5: Total Albumin.

Assessment of Bilirubin

GROUPING	TOTAL ALBUMIN
GROUP I	0.7±0.037
GROUP 2	4.2±0.096
GROUP 3	1.06±0.042
GROUP 4	3.4±0.042
GROUP 5	3.15±0.024
GROUP 6	2.63±0.03607
GROUP 7	2.4±0.0365

Data was represented as MEAN±SEM.
Toxicant rats+silymarin were compared toxic control rats.
P<0.05 compared to toxicant,*= p<0.05, **=p,0.01, ***=p<0.001

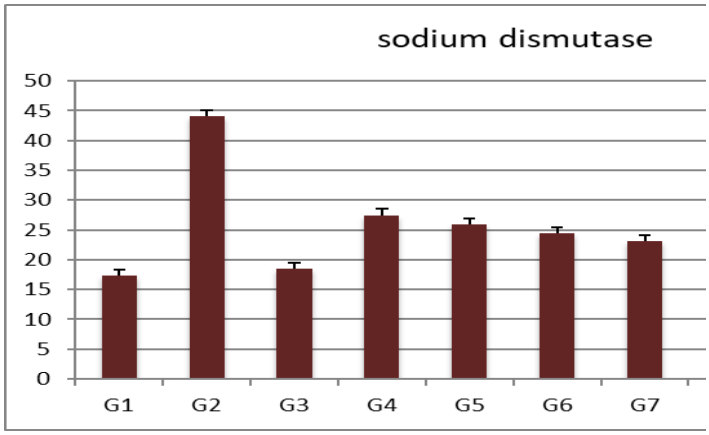


Graph 6: Bilirubin.

ASSESSMENT OF SOD

GROUPING	SOD
GROUP I	37.84±0.606
GROUP II	10.68±1.56
GROUP III	36.36±0.659
GROUP IV	26.74±1.114
GROUP V	28.40±1.35
GROUP VI	30.29±0.851
GROUP VII	32.154±1.04

Data was represented as MEAN±SEM.
Toxicant rats+silymarin were compared toxic control rats.
P<0.05 compared to toxicant,*= p<0.05, **=p,0.01, ***=p<0.001



Graph 7: Sodium Dismutase.

% Protection Assay of Juncus Sabulatus.

CONCµG/ML	TRAIL 1	TRAIL 2	TRAIL 3	SEM	MEAN
100	72	73	75	0.88192	73.3333
80	66	69	67	0.88192	67.3333
60	45	46	48	0.88192	46.3333
40	33	36	38	1.45297	35.6667
20	22	25	26	1.20185	24.3333
10	15	14	18	1.20185	15.6667

% Protection of Cordia Meclodia.

CONCµG/ML	TRAIL 1	TRAIL 2	TRAIL 3	SEM	MEAN
100	77	76	75	0.57735	76
80	55	57	56	0.57735	56
60	44	45	43	0.57735	44
40	33	36	34	0.88192	34.3333
20	23	26	25	0.88192	24.6667
10	12	15	14	0.88192	13.6667

% Protection of Silymarin.

CONCµG/ML	TRAIL 1	TRAIL 2	TRAIL 3	SEM	MEAN
100	95	92	93	0.88192	93.3333
80	82	80	79	0.88192	80.3333
60	77	78	75	0.88192	76.6667
40	44	46	48	1.1547	46
20	23	24	21	0.88192	22.6667
10	12	10	11	0.57735	11

Histopathological Results

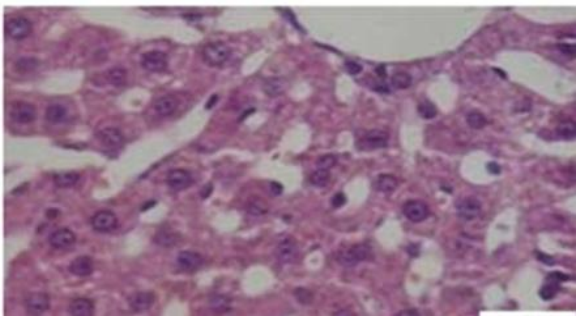


Fig. I: (GROUP I) misrcopic view of noral control (group I) group rat H&E×400 hepatocytes of the normal control group shows normal lobular architecture of the liver with distinct hepatocytes and sinusoidal spaces.

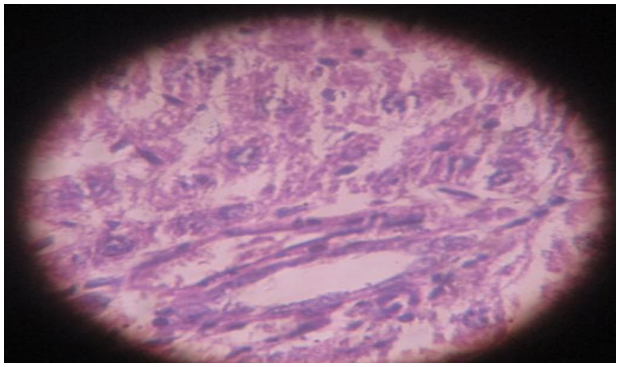


Fig. 2: (group II) microscopic view of standard control group (silymarin) rat H&E×400 Hepatocytes of the standard control group shows normal architecture with normal range of hepatocytes.

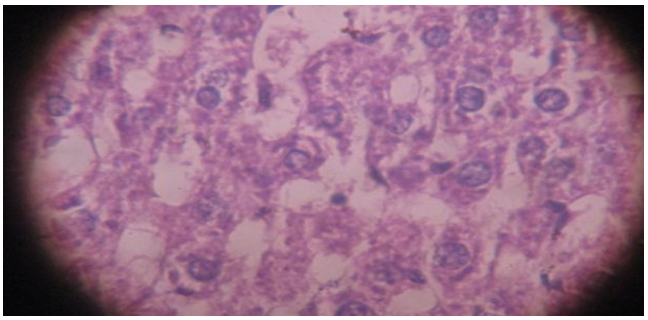


Fig. 3: (group III) microscopic view of toxic control group(INH+RIF) RAT H&E×400 Hepatocytes of toxic control group shows disarrangement of normal hepatocytes with necrosis inflammation.

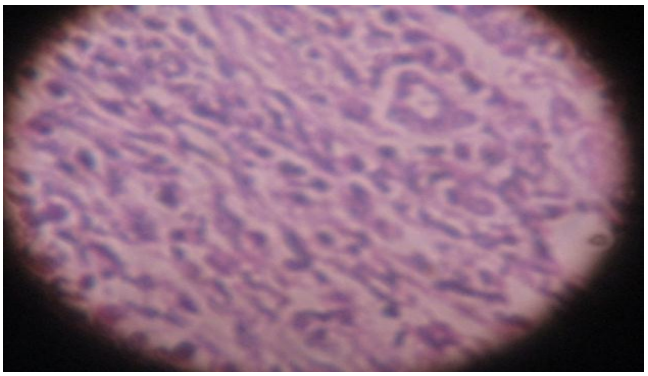


Fig. 4: (GROUP IV) microscopic view of pretreated test 1(juncus sabulatus) dose 1(100mg/kg) control group rats H&E×400.

Hepatocytes of this group shows minimal inflammation with moderate lobular structure

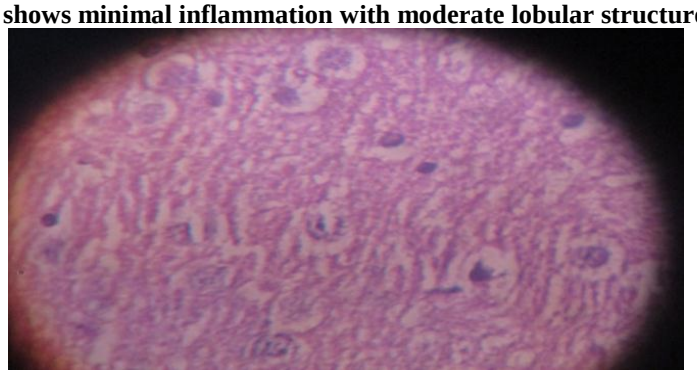


Fig 5: (GROUP V) microscopic view of pretreated test 1 dose 2(200mg/kg) control group rats H&E×400 HEepatocytes of this group shows minimal inflammation with normal cellular architecture.

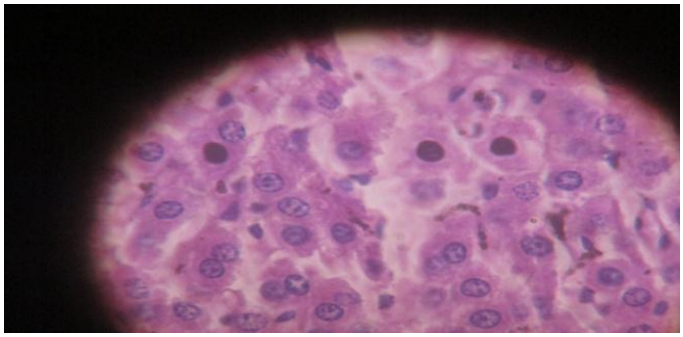


Fig 6: (GROUP VI) microscopic view of pretreated test 2 dose 1 control rats H&E×400 Hepatocytes of this group shows slight inflammation with normal lobular structure with hepatic cells.

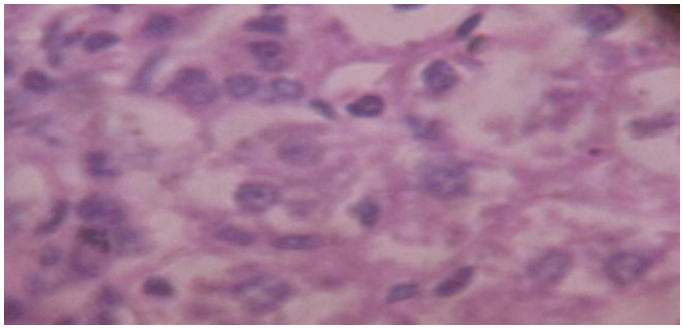


Fig 7: (GROUPVII) microscopic view of pretreated test 2 dose2 control rta H&E×400 Hepatocytes of this group shows minimal inflation with normal lobular architecture with distich hepatic cells.

DISCUSSION

It is evident that derangement of liver causes alter in the permeability ,which leads to leakage of enzymes from cells. Isoniazid and rifampicin are the commonest antitubercular drugs. Ingestion of combination of both results in functional loss of liver which leads to fatal effects . free radicals are formed due to the metabolic action of reactive molecules of isoniazid and rifampicinSilymarin shows its effective action by enhancing oxidative stability by modulating antioxidant related genes..Silymarin has a potential of stabilizing biological membrane and increases protein synthesis. Higher total phenolic content have shown to contribute to the antioxidant activity.

In this study, to halt the dangerous effect of toxicity caused due to the antitubercular drugs herbal plants such as juncus sabulatus and cordia macleodii are used. Apart from protective action it also shows anti-inflammatory, antioxidant,antifungal and scavenging properties.it contains the chemical compounds such as flavonoids, quercetin,alkaloids,phenols and luetinols. Among all of these flavonoids is the active constitute which are to contain antioxidant property.

In this study,for the management of oxidative stress antioxidant parameters are studied,the such as superoxide dismutase(SOD),CAT,MDA. It was found that levels of SOD shows the increasing response as the effectiveness
In the present study,the degree of protective action was appraised along with biochemical parameters. The toxocated group i.e.,animal group induced with INH+RIF shows drastic effects such as lossof cellular boundary, disaray of hepatic cells,sinusoidal inflammation, centriloblar necrosis,congestion of cental veins,fatty degeneration where as in contrast to normal group it shows opposite signs such as normal lobular and cellular architecture,sinusoidal spaces and central veins. The standard group shows signs of recovery with absec=nce of necrosis,no generative changes. The treated groups exhibit different effects,thelow doses of both the plant(100mg/kg) dose shows less effect without ny significant recovery signs where as dose 2(200mg/kg) plants shows slight protective action with regeneration of hepatocytes.,but in relation with both plants plant 2 exhibits much more positive effects and rate of recovery is aloso high.

CONCLUSION

The present study shows significant hepatoprotective effect by juncus sabulatus and cordia macleodii with their potent effect. The resilt showcasese efficient effect and futher study can be conducted for more effect by certain changes and combination effect of both the plants can be studied for more potent action.

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