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RESEARCH ARTICLE

In Vivo Hepatoprotective Activity of Hydroalcohol Extract of *Gyrocarpus asiaticus* Willd and *Lactuca runcinata* DC

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Abstract

Objective: Investigation of hepatoprotective activity of *Gyrocarpus asiaticus* Willd (GA) and *Lactuca runcinata* DC (LR) prepared by hydroalcohol extraction. **Methodology:** Albino rats were used for the *in vivo* experiments for the determination of oral acute toxicity study. For these experiments, 6 groups were created of which 5 groups were for each plant extract and 1 group for control. A total of 11 groups were made for the toxicity study. Each group required 2 albino mice. Different doses of 0.025, 0.2, 0.5, 2, and 5 gm/kg body weight were administered orally for each plant extract. For the control group, these were administered with distilled water. For hepatoprotective activity, albino rats were randomly divided into 5 (control, toxic, standard, 2 samples) groups, and 4 animals were randomly divided into each group. For 2 plants, a total of 7 groups were made. 5% gum acacia was used as the vehicle. For induced hepatotoxicity CCl_4 and as a standard drug silymarin was used. **Result and Discussion:** Plant extracts did not show any toxicity, and no histopathological changes were seen in the liver, kidney, or lungs due to toxicity. In the study of GA Willd and LR DC extract prepared by hydroalcohol solution, there was evidence of protection against CCl_4-induced hepatotoxicity in the Histopathological study of the liver of albino rats. **Conclusion:** Hydroalcohol extract of LR DC and GA Willd shows no oral acute toxicity and LR DC shows no significant hepatoprotective activity but GA Willd shows significant hepatoprotective activity.

Keywords: Acute Toxicity, CCl_4, *Gyrocarpus asiaticus* Willd, Hepatoprotective, Hepatotoxicity, *Lactuca runcinata* DC

1. Introduction

The liver is compared to a well-equipped biochemical laboratory. Various metabolites are detoxified in the liver. The other roles of the liver include metabolism, storage of glycogen, production of hormones, and putrefaction of red blood cells. Liver diseases are one of the most common and serious causes of death. Hepatic injury caused by drugs is the main problem in public health care contributing to more than 50% of acute hepatic failure cases[1]. Drug-induced hepatotoxicity can be considered either predictable or unpredictable. Serious consequences may be associated with it. Liver diseases are one of the most common and serious causes of death all over the world. Despite tremendous advancements in modern therapy, mitigation, and treatment options still pose a challenge. Ingestion of poisonous chemicals, excessive alcohol consumption, infections, autoimmune disorders, and xenobiotics are the main causes of hepatotoxicity[2]. Hepatic injury caused by drugs is one of the mainly common problems in daily life and contributes to more than 50% of acute hepatic failure cases. Drug-induced hepatotoxicity can be considered either predictable or unpredictable. Serious consequences may be associated with it. Herbal drugs are widely used for the mitigation of liver disease or hepatic disorder. The plant extract from some selected medicinal plants is now in great

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demand for developing the treatment of liver disorders worldwide[3]. *Taniki* or *Nalla poliki* is the common name of GA Willd, which comes under the family Hernandiaceae. *Undirachakam* is the common name of LR DC and comes under the family Asteraceae. These two plants have traditionally claimed activity in chronic liver disease, diuretic, and prevention of mouth disorders[4]. Several plants and conventional polyherbal formulations are used for the treatment of the hepatic disorder. All over the world, about 600 trading polyherbal formulations are being sold with proven hepatoprotective activity[5]. The present investigation aims to perform an *in vivo* hepatoprotective activity study of hydroalcohol extracts of GA Willd and LR DC. To evaluate the hepatoprotective activity of these two plants Albino rats and albino mice were used for *in vivo* experiments according to OECD guideline 425. SGOT, SGPT, Serum alkaline phosphate, total bilirubin-like blood serum parameter and histo-pathological study of the liver before or after hepatotoxicity were performed[6].

2. Materials and Methods

2.1 Plant Materials and Hydroalcohol Extract Preparation

LR DC and GA Willd were collected from Palayamkottai, Tamil Nadu. The authentication certificate registration number of GA Willd is XCH-40373 and LR DC is XCH-40372. The extraction process was done by maceration. The first 500 ml hydroalcohol solvent (Alcohol: Water = 70: 30) was prepared. 200 gm dry whole plant parts of LR DC and GA Willd were placed on the alcohol solvent separately. After 5 days the crude extract of two plants was collected separately[7].

2.2 Experimental Animals

22 Swiss albino mice, non-pregnant, 8-10 weeks old, weighing 25-32gm. required for the acute toxicity experiment and hepatoprotective activity study and 28 Wister albino rats, male/female, and vice versa, 8-10 weeks old, weighing 150-200gm required for two plants. These animals were brought from the breeder SAHA Enterprises Kol-5, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Regd. no.- 1828/PO/Bt/S/15/CPCSEA. The animals were transferred to the polypropylene cages in the animal house, at the School of Pharmacy and Life Sciences, Centurion University of Technology and Management (CUTM), Bhubaneswar. The animals were transferred 7-8 days before to the experimental environment for adjustment. The animals were kept in environmental conditions of 12 hours light and 12 hours dark cycle, temperature (23 ± 2 ˚C), and RH 55-60 %. Each cage contained 10 animals. Standard food and water were given to the animals. *In vivo* experiments were approved by the guidelines of the CPCSEA, clearance was obtained from Institutional Animal Ethics Committee (IAEC) and the experiment was performed in the Pharmacology Lab, CUTM campus Bhubaneswar, Odisha[9-11].

2.3 Chemicals

Carbon tetrachloride (CCl_4), Silymarin, olive oil and gum acacia were supplied from the School of Pharmacy and Life Sciences, CUTM, Odisha. Kits were purchased from Span Diagnostics Ltd. Surat, for the determination of AST/SGOT, ALT/SGPT, ALP and TB. All other chemicals or reagents were purchased from standard commercial suppliers and those were of the highest analytical grade.

2.4 Acute Toxicity Study

According to OECD guideline 425, the acute toxicity of crude extract prepared by the hydroalcohol solvent of LR DC and GA Willd was performed by *in vivo* technique. The acute toxicity was carried out with the permission of IEAC Approval No-2024/PO/Re/S/18/CPCSEA. For these experiments 6 groups were created and out of 6 groups 5 groups for each plant extract and 1 group for control. A total of 11 groups were made for the toxicity study. Each group required 2 albino mice. Different doses of 0.025, 0.2, 0.5, 2, and 5gm/kg body weight were administered orally for each plant extract and with administration with distilled water for the group named as control (Table 1). After administration of the oral dose, the result was observed continuously for 2 hours and in the first four hours daily for 14 days. The LD_{50} and ED_{50} values of both plant extracts were determined. For acute toxicity determination, one animal from each group was sacrificed for histopathological study of the liver, lungs and kidney.

2.5 Hepatoprotective Activity (CCl_4-induced Hepatotoxicity)

For 1 plant, 5 groups of animals, each having 4 animals were created. For 2 plants, a total of 7 groups were made.

5% gum acacia was used as the vehicle. Group I (Control): This group was treated with aqueous 5% gum acacia with 1ml/200gm body weight from the 1st to 7th day and on the 7th day it was treated with olive oil 0.12ml/100gm body weight. Group II (Toxic): This group was treated with aqueous 5% gum acacia with 1ml/200gm body weight from the 1st to 7th day and on the 7th day treated with CCl_4 with vehicle olive oil (1:1) 0.12ml/100gm body weight. Group III (Standard): This group was treated with 0.5% standard drug silymarin with 50mg/kg body weight from the 1st to 7th day and on the 7th day treated with CCl_4 with vehicle olive oil (1:1) 0.12ml/100gm body weight. Group IV (LR extract low dose): This group was treated with LR DC plant extract with 50mg/kg body weight dose with 5% gum acacia vehicle from the 1st to 7th day and on the 7th day treated with CCl_4 with vehicle olive oil (1:1) 0.12ml/100gm body weight. Group V (LR extract high dose): This group was treated with LR DC plant extract with 100mg/kg body weight dose with 5% gum acacia vehicle from the 1st to 7th day and on the 7th day treated with CCl_4 with vehicle Olive oil (1:1) 0.12ml/100gm body weight. Group VI (GA extract low dose): this group was treated with GA Willd plant extract with 50mg/kg body weight dose with 5% gum acacia vehicle from 1st to 7th day and on the 7th day treated with CCl_4 with vehicle olive oil (1:1) 0.12ml/100gm body weight. Group VI (GA extract high dose): This group was treated with GA Willd plant extract with 100mg/kg body weight dose with 5% gum acacia vehicle from the 1st to 7th day and on the 7th day treated with CCl_4 with vehicle olive oil (1:1) 0.12ml/100gm body weight (Table 2). 24 hours later (on the 8th day), the blood collection was done by retro-orbital plexus, and one animal was sacrificed for the histopathological study of the liver. Various blood parameters like SGOT/AST, SGPT/ALT, ALKP and TB were determined to understand the liver function[12-14].

3. Statistical Analysis

The data was calculated with ± S.E.M. by one-way ANOVA using Graph Pad Prism software, and the difference between the mean values was analysed. The data is considered significant if $p<0.05$[15].

4. Result and Discussion

4.1 Oral Acute Toxicity

The study of oral acute toxicity of hydroalcohol extract of GA Willd and LR DC was done according to OECD guidelines 425. Extract of GA Willd and LR DC was administrated orally to separate groups of albino mice with the doses 0.05g/kg, 0.2g/kg, 0.5g/kg, 2g/kg, and 5mg/kg (Table 1). The highest dose (5000mg/kg) of both plants does not show any mortality. Results

Table 1. Protocol of animal study for acute toxicity. GA Willd, LR DC

S. No.	Group	Sample	Numbers of animals	Dose Concentration
1	I (Control)	Distilled Water	2	Adequate
2	II	(LR extract)	2	25 mg/ kg
3	III	(LR extract)	2	200 mg/kg
4	IV	(LR extract)	2	500 mg/kg
5	V	(LR extract)	2	2000 mg/kg
6	VI	(LR extract)	2	5000 mg/kg
7	VII	(GA extract)	2	25 mg/ kg
8	VIII	(GA extract)	2	200 mg/kg
9	IX	(GA extract)	2	500 mg/kg
10	X	(GA extract)	2	2000 mg/kg
11	XI	(GA extract)	2	5000 mg/kg

Table 2. Protocol of animal study for hepatoprotective activity. GA Willd, LR DC

S. No.	Group	Treatment (1-7 Days)	Toxicant on 7th day	Animals Required
1	I (Control)	Aq. 5% gum acacia (1ml/200g)	Olive oil (0.12ml/100 gm)	6
2	II (Toxic)	Aq. 5% gum acacia (1ml/200g)	CCl_4 + Olive oil (1:1) 0.25ml/100 gm	6
3	III (Standard)	0.5% silymarin 25 mg/kg (1ml/200g)	CCl_4 + Olive oil (1:1) 0.25ml/100 gm	6
4	IV (LR extract)	LR extract 50 mg/kg in Aq. 5% gum acacia	CCl_4 + Olive oil (1:1) 0.25ml/100 gm	6
5	V (LR extract)	LR extract 100 mg/kg in Aq. 5% gum acacia	CCl_4 + Olive oil (1:1) 0.25ml/100 gm	6
6	VI (GA extract)	GA extract 50 mg/kg in Aq. 5% gum acacia	CCl_4 + Olive oil (1:1) 0.25ml/100 gm	6
7	VII (GA extract)	GA extract 100 mg/kg in Aq. 5% gum acacia	CCl_4 + Olive oil (1:1) 0.25ml/100 gm	6

show no changes in the behaviour. No salivation, blood pressure fluctuation, breathing problems, or any type of lethargy, diarrhoea, or coma was observed in any of the groups of albino mice. A histopathological investigation was done to find if any morphological changes in the liver, kidney, and lung occurred due to the acute toxicity produced by the hydroalcohol extract of GA Willd and LR DC. As there was no mortality in any group, only one albino mouse from each plant (Two for two plants) was sacrificed from the group which was administered with 5000 mg/kg dose. In the histopathological findings, no major morphological changes occurred in the liver, kidney, or brain of the albino mice (Figure 1).

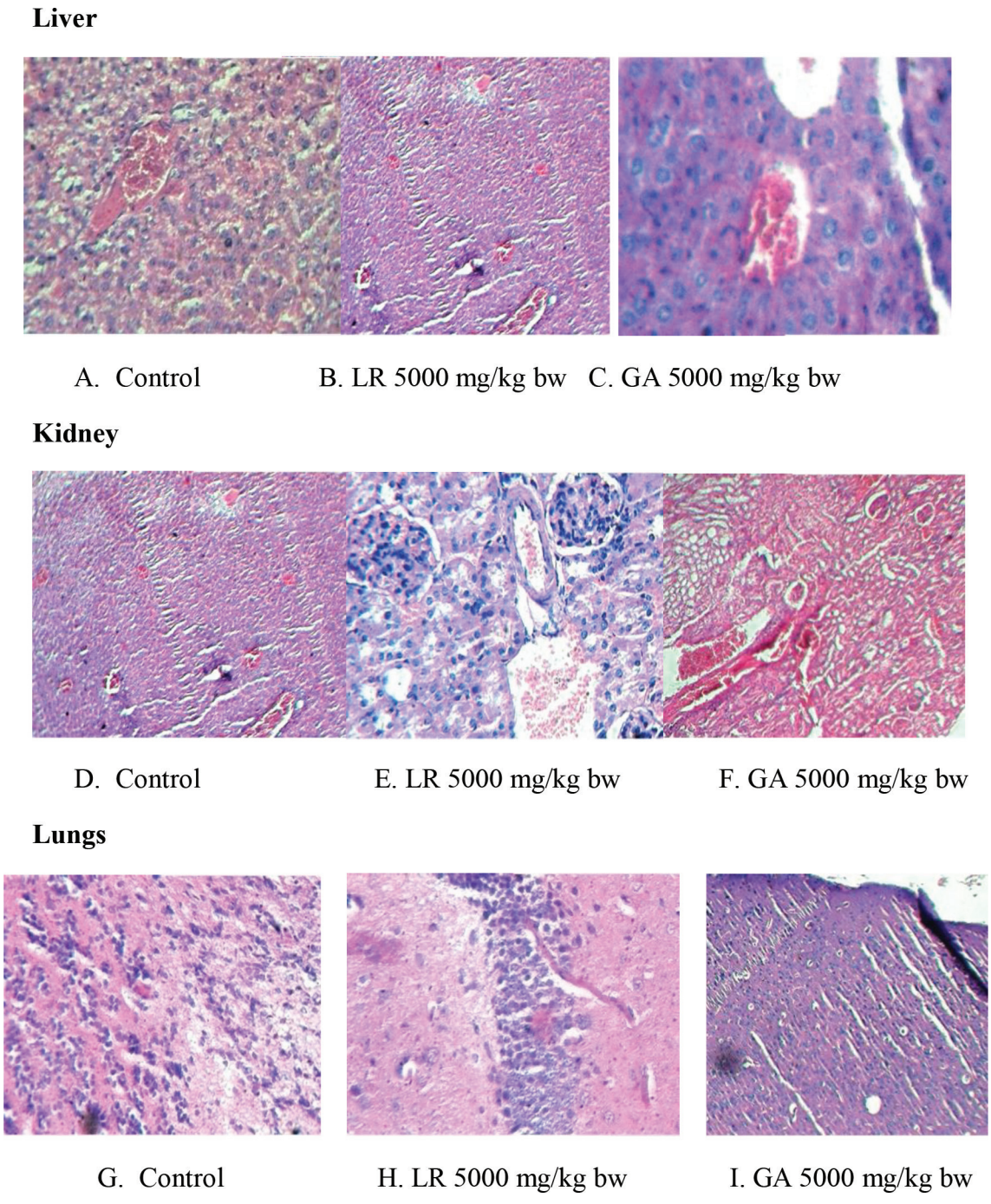

Figure 1. Oral acute toxicity study: Histopathological study of morphological changes of liver, kidney and lungs of albino mice after 5000 mg/kg bw administrated dose group and control group. GA Willd, LR DC.

4.2 Hepatoprotective Activity

4.2.1 Serum Biochemical Parameters Study

In the hepatoprotective activity study Carbon tetrachloride (CCl_4) treatment significantly increased the serum liver enzyme levels, SGOT/AST, SGPT/ALT, ALKP, and TB. The activity of SGOT (174.8 ± 2.819), SGPT (137.9 ± 1.625), ALP (179.0 ± 2.121), TB (1.578 ± 0.1226) was higher (P<0.05) compared to normal control SGOT (60.49±2.325), SGPT (68.48 ± 1.769), ALP (87.58 ± 2.269), TB (0.5767± 0.02290) indicating marked hepatocellular necrosis. Hepatocellular necrosis leads to a significant increase in the levels of certain enzymes in the bloodstream. These enzymes are originally contained within the liver cells and play essential roles in various metabolic processes. Within liver tissues, two specific enzymes, SGOT and SGPT, are predominantly located in the cytoplasm, with SGOT also being present in the mitochondria. When hepatocytes in the liver are damaged, their ability to regulate enzyme transport becomes disrupted, causing these enzymes to escape through the plasma membrane and consequently elevating the i r concentrations in the blood. The values were higher (*P<0.05) compared to Control + CCl_4 treated group. Pretreatment with Silymarin (25 mg/kg, b.w.) + CCl_4 shows results in SGOT (58.43 ± 2.127), SGPT (46.09 ± 1.459), ALP (68.63±1.579), TB (0.4800 ± 0.0139). The values of LR DC extract 50 mg/kg in aq. The 5% gum acacia + CCl_4 group shows results SGOT (96.76 ± 2.439), SGPT (94.43 ± 2.292), ALP (102.4 ± 1.631), TB (0.8567 ± 0.02860) and the values of LR extract 100 mg/kg in Aq. 5% gum acacia + CCl_4 group shows result SGOT (96.67 ± 2.176), SGPT (98.39 ± 1.649), ALP (101.3 ± 1.421), TB (0.8583 ± 0.03280). In the case of GA Willd**,** the values of GA extract 50 mg/kg in aq. The 5% gum acacia + CCl_4 group shows results SGOT (67.79± 2.44), SGPT (58.29 ± 1.680), ALP (77.52 ± 1.807), TB (0.5283 ± 0.02522) and the values of GA extract 100 mg/kg in Aq. The 5% gum acacia + CCl_4 group shows results SGOT (69.18± 1.429), SGPT (56.53 ± 1.744), ALP (77.52 ± 1.807TB (0.5283 ± 0.02522) (Table 3). Treatment with extract of LR DC (50 and 100 mg/kg, b.w) and extract of GA Willd (50 and 100mg/ kg, b.w) for continuous 7 days offered significant protection against the CCl_4-induced hepatic damage.

4.2.2 Histopathological Findings

The histopathological examination of the livers from the control group of albino rats reveals a typical, healthy tissue structure. This includes a Central Vein (CV), Layers of Hepatocytes (SOP), and Sinusoids (S), as observed under a 100X magnification with H and E staining (Figure 2A). In the toxic group, CCl_4-induced hepatotoxicity shows a Congested Central Vein (CCV), Necrosis with Inflammatory Cells (NWIC), and H and E stain 100X (Figure 2B). Pretreatment with the standard drug silymarin shows complete protection of hepatocytes and H and E stain 100 X (Figure 2C). Prior administration of a 50mg/kg dose of the hydroalcohol extract from LR DC resulted in the observation of specific regions of necrosis surrounding the CV in a CCl_4-induced liver injury model. This was visualized

Table 3. Serum biological parameters in CCl_4-induced liver toxicity rat models. Values are shown as mean ± SEM, n=6. *P<0.05 and P=<0.0001, when control vs toxic, toxic vs standard, standard vs LR 50 and LR 100; **P<0.05 when standard vs GA 50, GA 100

S. No.	Groups	SGOT IU/L (Mean±S.E.M)	SGPT IU/L (Mean±S.E.M)	Alkaline Phosphatase (ALKP) IU/L (Mean±S.E.M)	Total Bilirubin (TB) (mg/dl) (Mean±S.E.M)
1	Control Group-I	60.49±2.325*5.47	68.48 ± 1.769*	87.58 ± 2.269*	0.5767± 0.02290*
2	Toxic Control Group-II	174.8 ± 2.819* ± 3.61	137.9 ± 1.625*	179.0 ± 2.121*	1.578 ± 0.1226*
3	Standard Group-III (25mg/kg)	2458.43 ± 2.127* ± 1.28*	46.09 ± 1.459*	68.63±1.579*	0.4800 ± 0.01390*
4	GA Group-VII (50 mg/kg)	69.67.79± 2.44** ± 1.36*	58.29 ± 1.680**	78.89 ± 1.825**	0.6083 ± 0.02386**
5	GA Group-VI (100mg/kg)	6669.18± 1.429** 23 ± 1.03*	56.53 ± 1.744**	77.52 ± 1.807**	0.5283 ± 0.02522**
6	LR Group-IV (50mg/kg)	8 96.76 ± 2.439* ± 2.05*,#	94.43 ± 2.292*	102.4 ± 1.631*	0.8567 ± 0.02860*
7	LR Group-V (10 mg/kg)	8396.67 ± 2.176* 1.23*	98.39 ± 1.649*	101.3 ± 1.421*	0.8583 ± 0.03280*

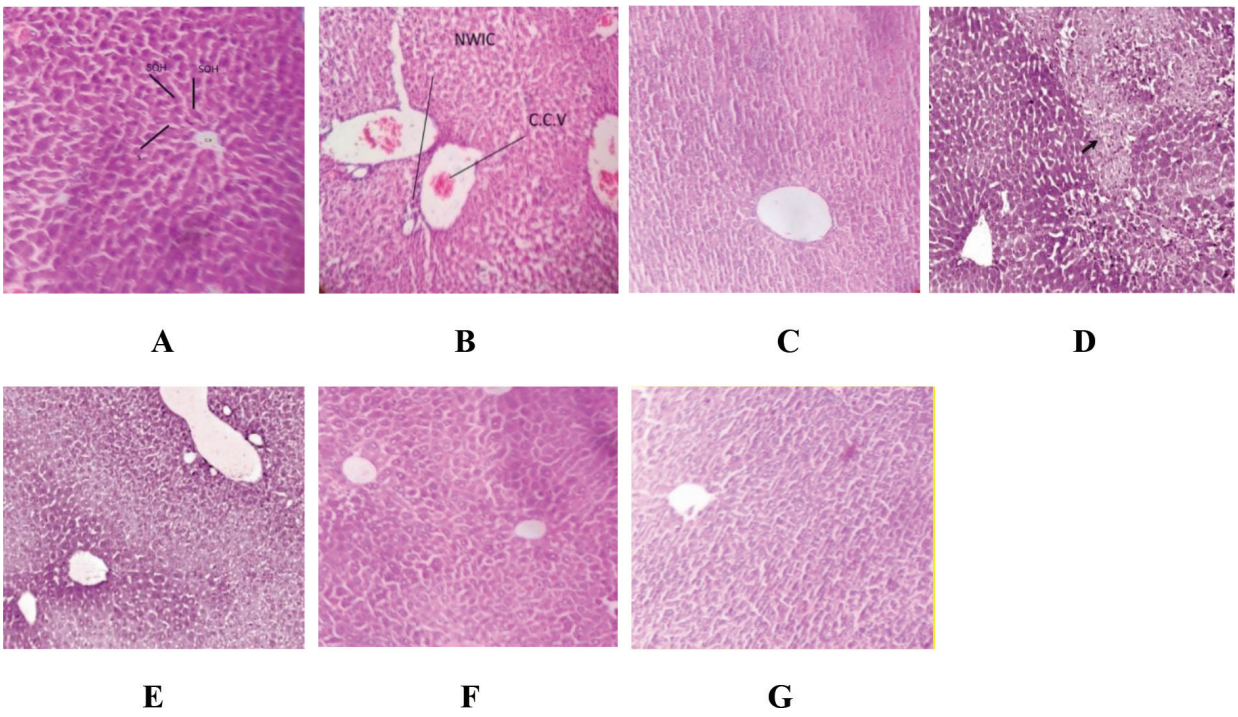

Figure 2. Hepatoprotective activity: Histopathological study of liver effects by hydroalcohol extract of *L. runcinata* DC and *G. asiaticus* WILLD. **A.** Control, **B.** Toxic, **C.** Standard, **D.** LR Extract (50mg/kg,bw), **E.** LR Extract (100mg/kg,bw), **F.** GA Extract (50mg/kg,bw), GA Extract (100mg/kg,bw).

under 100X magnification using H and E staining. (Figure 2D). Pretreatment with hydroalcohol extract of LR DC with 100mg/kg,b.w dose shows complete protection of hepatocytes, H and E stain 100X. (Figure 2E) Beforehand administration of a 50 mg/kg dosage of the hydroalcohol extract derived from GA Willd resulted in the observation of clusters of lymphoid cells infiltrating the liver tissue (indicated by an arrow). This was visualised at a 100X magnification using H and E staining. (Figure 2F). Pretreatment with hydroalcohol extract of GA Willd with 100 mg/kg showed foci of lymphoid cell infiltration in the liver parenchyma (arrow), H and E stain 100X (Figure 2G).

5. Conclusion

In this study, the hydroalcohol extracts from *L. runcinata* DC and *G. asiaticus* Willd were found to have a diminishing effect on certain parameters when administered to albino rats at the specified dosage, especially over a certain duration. This can be attributed to the fact that the blood biochemical values showed a shift away from those of the groups that received carbon tetrachloride separately, moving closer to the values observed in the control group. Hydroalcohol extract of *L. runcinata* DC shows no oral acute toxicity but also does not show significant hepatoprotective activity. Hydroalcohol extract of *G. asiaticus* Willd shows no oral acute toxicity and has good and significant hepatoprotective activity.

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