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BIOMOLECULES IN GLYCOSMIS PENTAPHYLLA L. FRUIT-SEEDS MAY SERVE AS A HEPATOPROTECTIVE AGENT.

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Abstract:

*The primary purpose of this study was to determine the in vitro and Research Lab, GIET School of Pharmacy, NH-16, Chaitanya Knowledge City, Rajahmundry-533296. Research Lab, GIET School of Pharmacy, NH-16, Chaitanya Knowledge City, Rajahmundry-533296. Research Lab, GIET School of Pharmacy, NH-16, Chaitanya Knowledge City, Rajahmundry-533296.in vivo Hepatoprotective activity of biomolecules that could be extracted from Glycosmis pentaphylla L fruit seeds. The DPPH test was used to determine the Hepatoprotective activity in vitro. The EEFSGP was measured to have an IC50 value of 212.96 g/ml (50% inhibition). For the in-vivo Hepatoprotective activity, albino rats were used. Rats treated with an ethanolic extract of Glycosmis pentaphylla fruit seeds showed a substantial (*P0.05) decrease in their CCl4-induced increased levels of SGOT, SGPT, ALP, and serum bilirubin (EEFSGP). The levels of SGOT, SGPT, ALP, and serum bilirubin were all reduced by 6.23% ns(non significantly), 28.96% ns(non significantly), 8.81% ns(non significantly), and 11.11 % ns(non significantly), respectively, after treatment with EEFSGP at a dose of 250 mg/kg b. wt. A higher dose of 500 mg/kg b. wt. was A conventional dose of silymarin revealed inhibition by CCl4 of 55.09, 68.98, 57.46, and 35.04%, respectively. Based on these results, it can be concluded that the biochemical parameters of the ethanolic extract-treated group were considerably lower than those of the CCl4-treated group. The elevated levels of AST, ALT, ALP, and bilirubin in hepatotoxic rats were also dramatically decreased after treatment with the extract. Histopathological analysis demonstrated that the EEFSGP exhibited moderate to good hepatoprotective activity at both dosages (250 mg/kg b.w. and 500 mg/kg b.w.), but at 500 mg/kg b.w. performed exceptional hepatoprotective efficacy against CCl4-induced injured hepatocytes.*

Key words :*: Biomolecules; Intoxication; Hepatocytes; SGOT; SGPT; SALP; DPPH test;*

INTRODUCTION:

The Glycosmis pentaphylla, often called orangeberry and gin berry, is a flowering plant species in the Rutaceae (citrus) family. Its natural range includes India, northern Australia, and Southeast Asia. Its pink fruits are harvested for human consumption. Those who live in more temperate climates may grow it as an indoor houseplant [1].

Word shape [2]

Correa is a shrub or a small tree, and its scientific name is Glycosmis pentaphylla (Retz.) There are anywhere from three to five folioles on each leaf, and the leaflets may be anywhere from entirely obtuse to sharply crenate. There are also anything from two to twelve pairs of lateral nerves. 5-merous flowers that bloom in the axils and are very, very long tight racemes/cymes Globose to ellipsoid with a glandular pericarp characterizes the fruit.

Elements [2]:

In the leaves, you'll find the quinolone alkaloid glycolone. The alkaloids and amide (benzamide-2-methylamino) found in flowers include arborine, arborinine, skimmianine, glycorine, and glycosmicine. The carbazole alkaloid mupamine is also present. Dictamine, -fagarine, skimmianine, -sitosterol, coumarin, stigmasterol, myricylalcohol, base glyborine, triterpenes arborinolA, arborinolB, arborine, arborinine, carbazolealkaloid glycosinine, glycozolidol, and 3-formylcarbazole are all found in the roots. Glycophymine, glucosolone, glucocolone, and amide-glycomide are all alkaloids found in root bark, along with the acridine alkaloids noracronycine, de-methylacronycine, and e-N-methylnoracronycine.

This herb has been utilized for centuries in traditional medicine to treat a variety of conditions, including cough, rheumatism, anemia, and jaundice (see also [3]). The bitter leaf juice has medicinal uses for treating fever, easing liver issues, and as a vermifuge. Eczema and other skin problems are treated using a paste made from the leaves and ginger. Inflammations of the face are treated with a decoction made from the root.

Substances and Procedures: Drugs and Chemicals

All of the chemicals employed in the extraction process and the screening for phytochemicals were classified as LR or AR. Silymarin, the reference medicine, was acquired from a neighborhood drugstore, while the solvents and other chemicals used were all of a "AR grade" and sourced from a university or hospital chemistry warehouse.

Hepatotoxin

A rat model of hepatotoxicity caused by CCl4 was used to assess hepatoprotective efficacy in this investigation.

Animals used in experiments

Two hundred and fifty to two hundred and fifty gram male albino rats were utilized. They came from the animal facility of the C.L. Baid Metha College of Pharmacy in Chennai (Reference Number IAEC-CPCSEA).

IAEC/XXIX/10/2010. The participants were closely monitored for around 7 days before to the start of the trial to rule out the possibility of a concomitant infection, and they were given unrestricted access to their regular meals and drinking water during this time. The animals were kept in clean, well-ventilated plastic cages at room temperature (255 °C) with a standard 12-hour light/dark cycle.

Method of Extraction (Soxhlet Extraction)Introduction

To isolate an organic compound from a solid, we use an organic solvent that does not dissolve the unwanted byproducts. Extraction from solids is a time-consuming process that often requires prolonged contact and heating with the solvent. To do this, the Soxhlet Extractor is used. It's a glass cylinder with a siphon and a tube on the side. Water condenser is located at the top of the cylinder, and the whole thing is placed into the neck of a boiling round bottom flask [4].

Methodology

Before the equipment can be assembled, the dried fruits and seeds must be ground into a fine powder in a food processor. Suitable solvent, such as ethanol, is heated in a flask placed in a water bath or on a heating mental. When the solvent is heated to a boil, the vapors escape through the side tube and condense on the top of the water tank. The powdered material in the thimble is exposed to the condensed liquid, which dissolves any organic compounds present and then filters out into the gap between the thimble and the glass cylinder. The solution drains back into the boiling flask via the siphon as the liquid level in this container increases. Once again, the solvent is evaporated, and the extracted material is left behind in the flask. A steady stream of pure solvent is dripped over the solid, where it dissolves the substance of interest and then flows back into the original flask. The organic material is separated from the solvent by distillation at the conclusion of the procedure [4]. After concentrating the ethanolic extract in a water bath, you may chill it and store it in the freezer in a clean, dry beaker. The first phytochemical analysis must be performed on the ethanolic extract of Glycosmis pentaphylla fruit seeds (EEFSGP).

Preliminary Phytochemical Screening [5, 6, 7, 8] The preliminary phytochemical screening of EEFSGP revealed the presence of a wide variety of biologically active molecules, including carbohydrates, amino acids and peptides, phytosterols,

carotenoids, alkaloidsds (higher concentration), terpenoids especially diterpenoids, tri and tetra terpinoids di, and aromatic acids and alcohols, etc.

Evaluation of Acute Toxicity [9]

The current investigation assessed the EEFSGP for acute oral toxicity using the acute toxic class technique. Three Wister rats were to be used at each stage of this technique to determine the extract's toxicity. Prior to treatment, the rats were fasted for three to four hours (food but not drink should be withheld). After the fasting period was over, the animals were weighed again, and an oral dosage of 2000 mg/Kg b.w. of the extract was given to them. After administration of the dose, each animal was watched at least once within the first 30 minutes, then at regular intervals over the next 24 hours (with extra care provided during the first 4 hours), and then every day for 14 days.

Evaluation of In vitro Antioxidant Activity by DPPH Assay (Free Radical Scavenging Activity) [10, 11]

Principle

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) test measures the reduction in color intensity that happens when a free radical scavenger contributes a hydrogen atom to quench a DPPH radical.

Method

The DPPH free radical scavenging activity of the ethanolic plant extract was determined using a modified version of the technique described by Pan et al. [10]. We mixed 8 milliliters of a 0.004% (w/v) stock solution of DPPH in 95% ethanol with 0.2 milliliters of extract solutions at varying concentrations. Absorbance at 517 nm was measured using a UV-Visible spectrophotometer until the reaction reached a steady state, at which point the DPPH radical scavenging activity could be calculated. Gallic acid, a synthetic antioxidant, was utilized as a positive control. All counts were done three times for accuracy. This equation was used to determine the percent scavenging activity against DPPH radicals:

S% = [(Acontrol-Asample) /Acontrol].

×100 Asample = absorbance of test sample; Acontrol = absorbance of blank control (containing all reagents except the extract solution).

Study Design for Evaluating In Vivo Hepatoprotetive Activity [12]

Thirty rats in total were used, with each group consisting of six animals. Group I received just vehicle (1 mL/kg/day of 1% CMC; p.o.)

Class II: The Negative Control 1 milliliter of CCl4 per kilogram intramuscularly or intraperitoneally (1:1 CCl4 in olive oil).

Positive Control/Standard Group (Group III) Standard Silymarin 100 mg/kg p.o. for 7 days Plus CCl4 1 mL/kg i.p. (1:1 of CCl4 in olive oil)

Organizations that Provide Different Forms of Therapy

High Dose Group [CCl4 1 mL/kg (1:1 of CCl4 in olive oil) i.p + EEFSGP (500 mg/ kg b. w., p.o.]

Low Dose Group [CCl4 1 mL/kg (1:1 of CCl4 in olive oil) i.p + EEFSGP (250 mg/ kg b. w., p.o.] Over the course of seven days, the patients received an oral medication.

Blood Donation: After an overnight fast of 8 hours, blood was drawn from the retro orbital abyss under light ether anesthesia on day 8. In order to separate the blood components, the samples were centrifuged at 3000 rpm for 20 minutes. Before undergoing biochemical assessments, serum was isolated and frozen at -200 C for later use.

The Use of Biochemical Methods

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin were all tested on the serum samples taken.

Histopathological Analysis

Hepatic tissue was removed by dissection and then preserved in 10% formalin solution. Ethanol (between 50% and 100%) was used to remove moisture, then xylene was used to clean it, and finally paraffin wax was used to embed it. Following this, thick slices (5-6 mm) were cut and stained with hematoxylin and eosin dye for photo microscopic examination. V.H.S. Hospital in Chennai was responsible for all of the biochemical and histological testing.

RESULT AND DISCUSSION:

Table 1: Results of DPPH Scavenging Activity

Sl. No.	Concentration (ug/mL)	Absorbance (A)	S% = [(A_0-A)÷A_0] X 100	IC_{50} (µg/ml)
Control (DPPH Sol.)				
1.	0.1mM in ethanol	1.174 (A_0)		-
Std (Ascorbic Acid)				
1	4	0.983	16.26	
2	6	0.954	18.73	
3	8	0.917	21.89	
4	10	0.870	25.89	39.87

5		25	0.565	51.87	
6		50	0.037	96.84	
EEFSGP					
1		10	1.044	11.07	
2		25	0.936	20.01	
3		50	0.850	27.59	212.96
4		75	0.786	33.04	
5		100	0.702	40.20	
6		250	0.568	51.61	

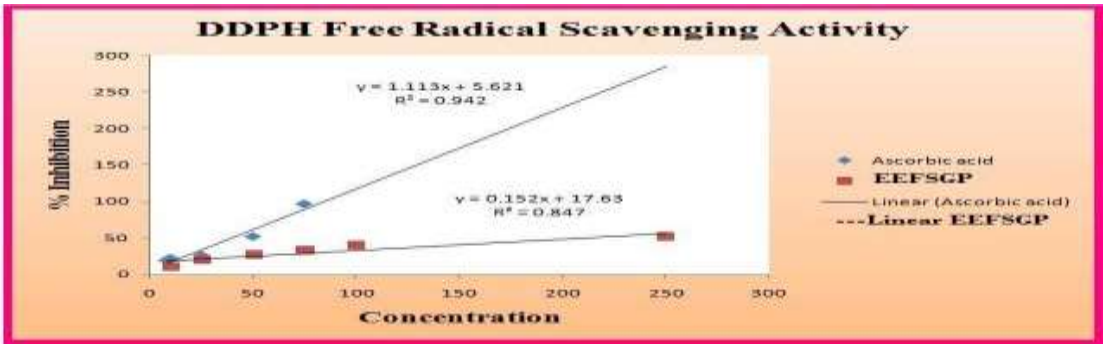

Fig 1: DDPH scavenging Activity of Ascorbic Acid and EEFSGP

Table 2: Results of Hepatoprotective Activity

Group	Treatment	AST(SGOT)	ALT(SGPT)	ALP(SALP)	Serum bilirubin
		IU/L	IU/L	IU/L	mg/dL
1	Normal Control Group (only the vehicle, 1% CMC; p.o.)	53.00±8.672***	46.60±11.95***	139.2±6.914***	0.58±0.08***
2	Negative Control (1:1 of CCl_4 in olive oil; i.p.)	202.2±30.45	204.4±47.74	399.2±16.18	1.17±0.16
3	Low dose [(1:1 of CCl_4 in olive oil) i.p + EEFSGP (250 mg/ kg b. w., p.o.)]	189.6±14.48ns	145.2±39.75*	364.0±16.52*	1.04± 0.15ns
4	High dose [(1:1 of CCl_4 in olive oil) i.p + EEFSGP (500 mg/ kg b. w., p.o.)]	151.6±13.52***	107.0±19.47***	303.0±38.78***	0.85±0.20**
5	Positive Control/Standard				
	Group[(1:1 of CCl_4 in olive oil)i.p.+ Silymarin 100 mg/kg orally (p.o.)]	90.80±17.61***	63.40±15.73***	169.8±8.55***	0.76±0.14***

Data are expressed as mean±SD (n = 6). One-way ANOVA followed by Dunnett's Multiple Comparison Test (P< 0.05) compared with group 2 ;(ns=non significant.*

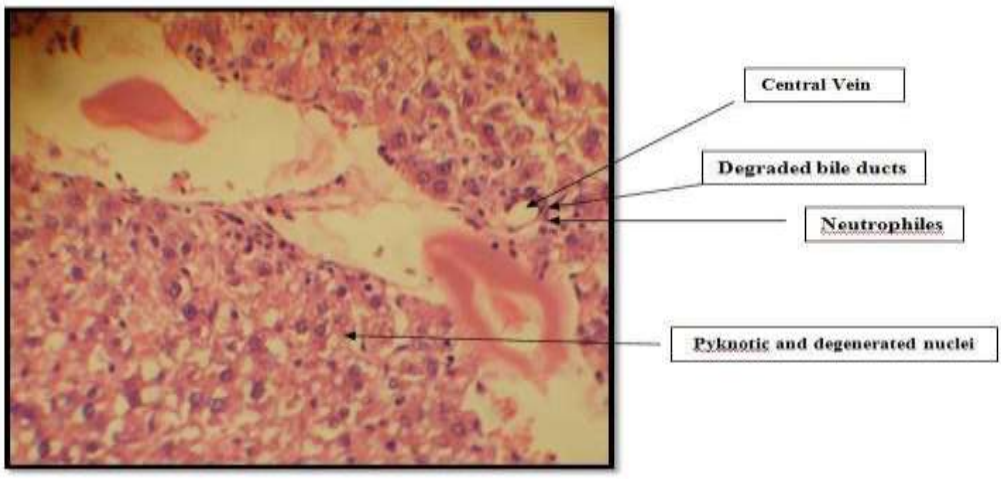

Fig 2: Liver Section of CCl4 Treated Rats.

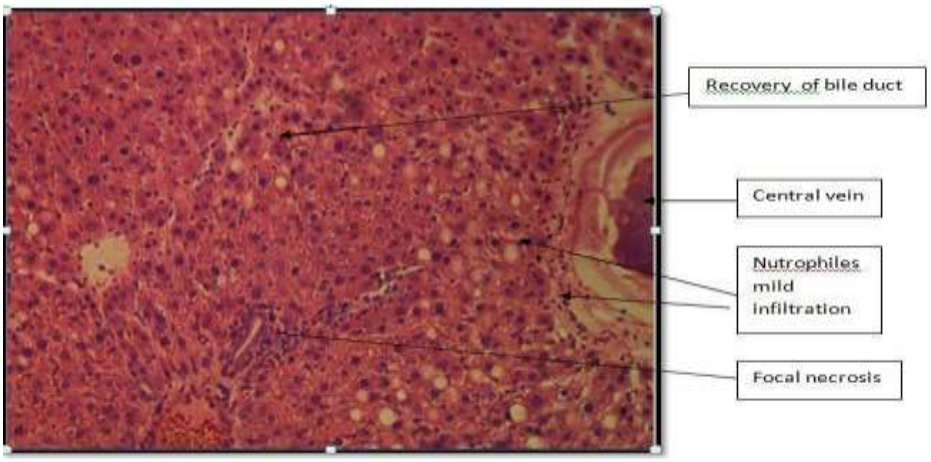

Fig 3: Liver Section of Rats Treated CCl_4 and 100 mg/kg of Silymarin.

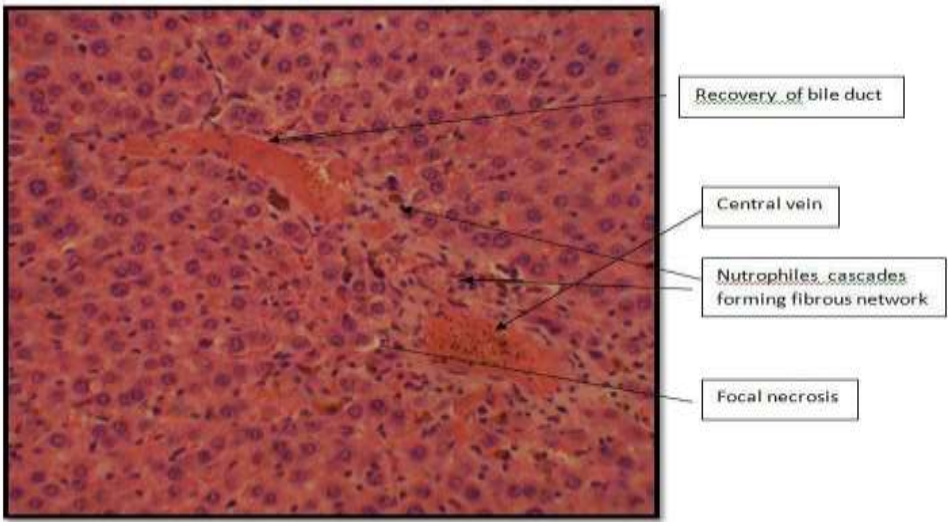

Fig 4: Liver Section of Rats Treated CCl_4 and 500 mg/kg of EEFSGP.

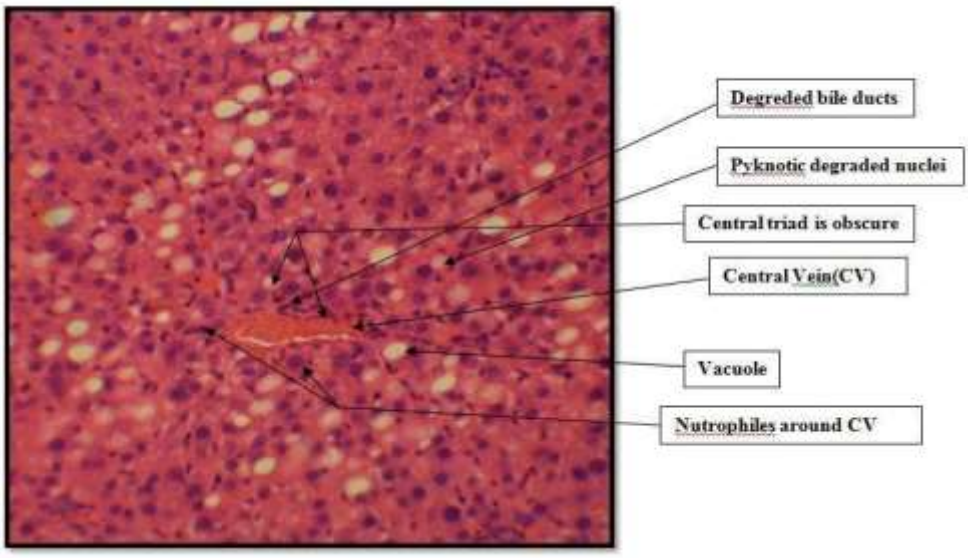

Fig 5: Liver Section of Rats Treated CCl_4 and 250 mg/kg of EEFSGP.

1. Phytochemical Screening

Carbohydrates, amino acids, peptides, phytosterols, carotenes, alkaloidsds (in greater concentration), terpenoids (particularly diterpenoids, triterpenoids, and tetraterpenoids di), aromatic acids, and alcohols were all found in EEFSGP during preliminary phytochemical screening..

2. Acute Oral Toxicity Studies

"Acute toxic class techniques (OECD guideline- 423)" were used to determine the acute oral toxicity in this research. The extract was given orally at a dosage of 2,000 milligrams per kilogram of body weight. Neither major toxicity nor any other significant changes in behavior were seen over the observation period. Significant dosages were determined to be 250 mg/kg body weight per oral (LD) and 500 mg/kg body weight per oral (ED) (HD) considerably.

3. Hepatoprotective Activity

(i) Statistical analysis

Methods from the "acute toxic class" (OECD guideline 423), which measure oral toxicity quickly, were employed in this study. The oral administration of the extract was at a rate of 2 milligrams per kilogram of body weight. During the course of the study, no substantial toxicity or behavioral alterations were detected. An oral LD (lowest effective dose) of 250 mg/kg and an ED (highest effective dose) of 500 mg/kg were shown to be significantly effective..

(ii) Analysis of DPPH free Radical Scavenging Activity

Antioxidant activity in the extract was supposed to be measured in vitro using DPPH free radical scavenging. Ascorbic acid, itself a natural antioxidant, was used as the reference antioxidant throughout the research. Antioxidant activity was measured using DPPH free radical scavenging, and the findings were reported as a percentage of suppression of produced free radicals at different doses. One may see a concentration-dependent impact, as greater concentrations were shown to show a greater percentage of inhibition. The X-axis of the graph represents the percentage of inhibition achieved, while the Y-axis represents the different doses used in the experiment (Fig 1). In every study, both the EEFSGP and the gold standard ascorbic acid were tested to find their respective IC50 values (50% inhibition).

Table 1 displays the findings of an experiment where an ethanolic extract of the fruit-seeds of Glycosmis pentaphylla (EEFSGP) was tested for its ability to scavenge DPPH radicals, in comparison to ascorbic acid. Figure 6.3 shows a Microsoft Office Excel 2007 plot of the percentage inhibition at several concentrations of ethanolic extract of fruit-seeds of Glycosmis pentaphylla (4-50 g/ml) and standard Ascorbic acid (10-250 g/ml). Graph analysis revealed that the IC50 values for Ascorbic acid and EEFSGP, respectively, are 39.87 g/ml and 212.96 g/ml.**)**.

(iii) Biochemical Analysis

Table 2 shows how EEFSGP affects liver marker enzymes and bilirubin levels in the blood. The results indicated that whereas the Normal Control Group had levels of AST, ALT, and bilirubin within the normal range, the CCl4-treated group showed raised levels of these markers, demonstrating that CCl4 caused hepatocellular degeneration at higher dosages. An sign of liver cell disruption and the subsequent release of enzymes is an increase in cytoplasmic AST and ALT. Chemically induced hepatic damage has been measured by measuring bilirubin levels.

Table 2 shows that in rats treated with EEFSGP, the raised levels of SGOT, SGPT, ALP, and Serum bilirubin caused by CCl4 intoxication were considerably (*P 0.05) decreased. The levels of SGOT, SGPT, ALP, and serum bilirubin were reduced by 6.23 percent (nonsignificant), 28.96 percent (nonsignificant), 8.81 percent (nonsignificant), and 11.1 percent (nonsignificant), respectively, after treatment with ethanolic extract at a dose of 250 milligrams per kilogram of body weight. A higher dose of 500 milligrams per kilogram of body weight was even more effective. A conventional dose of silymarin revealed inhibition by CCl4 of 55.09, 68.98, 57.46, and 35.04%, respectively. Data in Table 2 revealed that the EEFSGP-treated group had considerably lower biochemical parameters than the CCl4-treated group. Treatment with the EEFSGP also dramatically decreased the elevated levels of AST, ALT, ALP, and bilirubin seen in hepatotoxic rats.

Histopathological Analysis

Section of a CCl4-treated liver is shown in Fig. The loss of cellular boundaries, pyknotic, and degraded nuclei, as well as extensive infiltration of the lymphocytes surrounding the central vein, were all seen in the affected rats' tissues.

Liver sections from CCl4 and 100 mg/kg Silymarin-treated rats showed minor fatty change, necrosis, and localized necrosis in the central vein, as well as in other areas of the liver (Fig. B) (dilatation).

C. Figure 4: Liver sections from CCl4 and 500 mg/kg EEFSGP-treated rats showed normal liver architecture, with minimal inflammatory cellular infiltration around the central vein, no necrosis, and a fibrous network formed by neutrophil cascades providing substantial protection and large septa of connective tissue flowing together and penetrating the parenchyma.

D. Fig. 5: Liver sections from CCl4 and 250 mg/kg EEFSGP-treated rats showed very little recovery, with an indistinct central triad and infiltration of neutrophils surrounding the central vein, a deteriorated fatty change, necrosis, and localised necrosis (dilatation), and a loss of

cellular boundaries.

CONCLUSION:

Finally, we demonstrate that ethanolic extract of Glycosmis pentaphylla fruit-seeds (EEFSGP) showed the capacity to regenerate hepatocytes in vivo and had potential antiinflmmatory action, as validated by liver biopsy, with an IC50 value of 212.96 g/ml. When compared to the gold standard medicine silymarin, the hepatoprotective activity of EEFSGP may be regarded outstanding.

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