

## EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF GLYCOSMIS *PENTAPHYLLA* ROOTS AGAINST CCl<sub>4</sub> – INDUCED ACUTE LIVER INJURY IN RATS

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### ABSTRACT

Glycosmis *pentaphylla* (Rutaceae) are well known in India as Ashvashakota, Vananimbuka, Pathalagarudi (Sanskrit), Ban-nimbu (Hindi), Kirmira (Marathi) Golugu and Gongipadu (Telugu) of ethyl acetate and methanolic extracts (100, 200 and 400 mg/kg) was prepared and tested for its hepatoprotective effect against carbon tetrachloride induced in rats. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, bilirubin were tested in both treated and untreated groups. Carbon tetrachloride has enhanced the SGPT, SGOT, ALP and bilirubin levels. Treatment with ethyl acetate extract of Glycosmis *pentaphylla* (Rutaceae) roots has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner. This was evident from significant reduction in serum enzyme, SGOT, SGPT, ALP and Total bilirubin (TB). Though both the extracts were recorded with significant hepatoprotective activity with same "p" value (p<0.01). Various pathological changes like centribular necrosis and vacuolization were observed in CCl<sub>4</sub> treated rats, which were significant protective activity in groups treated with GP and silymarin. It was concluded from the study that ethyl acetate and methanolic extracts of GP possess hepatoprotective activity against CCl<sub>4</sub> induced hepatotoxicity in rats.

**Keywords:** Hepatoprotective, Glycosmis *pentaphylla*, Carbontetrachloride, Silymarin.

### INTRODUCTION

Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated<sup>1,2</sup>. However there are several herbs/herbal formulations claimed to possess beneficial activity in treating hepatic disorders.

Glycosmis *pentaphylla* (Rutaceae) is commonly called as orange berry, Juice of leaves is used in fever and liver complaints and as a vermifuge, Leaves are considered good antidote for eczema and other skin troubles and applied in the form of paste<sup>3</sup>.

The plant is used in indigenous medicine for cough, rheumatism, anaemia and jaundice. A decoction of roots is given for facial inflammations<sup>4</sup>. Roots pounded and mixed with sugar are given in low fever and wood is used in snake bite<sup>5</sup>.

Common names:

Glycosmis *pentaphylla* is called as Jamaica mandarin orange, Orange berry (English), Ashvashakota, Vananimbuka, Pathalagarudi (Sanskrit), Ban-nimbu (Hindi), Kirmira (Marathi) Golugu and Gongipadu (Telugu)<sup>3</sup>.

Traditional uses:

In Hindu medicines, it has been used traditionally in bilious complaints, cough, worms, jaundice and fever. This drug has been proved by CCRH, monograph of which

has also been published. This medicine has been found clinically useful in amoebiasis, dyspepsia, migraine and irritable bowel syndrome. This drug is proved to be effective in bilious complaints like nausea, vomiting, bitter taste in the mouth, heart burn with desire for lime juice, which ameliorates and gastric symptoms are aggravated by eating, Migraine-pain in temples, throbbing type with bilious vomiting followed by weakness<sup>6</sup>. Juice of leaves is used in fever, liver complaints and as a vermifuge, while leaves are considered good antidote for eczema and other skin troubles<sup>3</sup>.

It is reported to contain arborinine<sup>7</sup>, glycozolicine, 3-formyl carbazole, glycosinine<sup>8</sup>, mupamine<sup>9</sup>, varbazole, 3-methyl carbazole<sup>10</sup>, glycolone<sup>11</sup>, glycozolidol<sup>12</sup>, glycozolinine<sup>13</sup>, glycophymoline<sup>14</sup>, glycophymine, glycomide<sup>15</sup>, glycozoline<sup>16</sup>, noracronycine, des-N-methyl-acronycine and des-N-methylnoracronycine<sup>17</sup>. In the present investigation, the anti-inflammatory activities of ethyl acetate and methanol extracts of the plant roots have been studied.

However, there is no significant basis or reports in the modern literature regarding its usefulness as hepatoprotective agent. Thus the present study was conducted to evaluate the hepatoprotective activity of the ethyl acetate and methanol extract of the Glycosmis *pentaphylla* roots by using CCl<sub>4</sub> induced hepatic injury in rats.



## MATERIALS AND METHODS

### Plant Material Collection:

*Glycosmis pentaphylla* (Rutaceae) roots were collected from the Ananthagiri region of Visakhapatnam district, Andhra Pradesh in the month of November 2007 and Authenticated by the taxonomist, Dept of Botany, Andhra University and the specimen Voucher No AUCP/BGR/2007/A55 has been preserved in the Department.

### Animals:

Wistar albino rats of either sex weighing between 200-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of  $22 \pm 1^\circ\text{C}$  with an alternating 12 h light-dark cycle and relative humidity of  $60 \pm 5\%$ ), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/a/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Food and water was allowed *ad libitum* during the experiment.

### Acute toxicity studies:

Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality even at a dose of 1000mg/kg and therefore considered safe.

Toxicological studies were conducted in mice (N=6) for all the extracts as per the Irvin's method<sup>18</sup> at the doses of 100, 300 and 1000 mg/kg, no mortality was observed.

## MATERIALS

All the materials used for this experiment are of Pharmacopoeial grade. Carbon tetrachloride (E. Merck), silymarin (Sigma Chemical Co.) and olive oil were purchased from the local supplier. Diagnostic kits for the estimation of SGOT, SGPT, SALKP and serum bilirubin were purchased from local supplier (Sai chemicals) manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Water represents the double distilled water; standard orogastric cannula was used for oral drug administration.

### Carbontetrachloride-Induced Hepatotoxicity:

The animals were divided into nine groups of six animals each. Group-I served as normal control received 5% acacia mucilage (1 ml/kg.p.o) daily once for 7 days. Group-II served as toxic control and received  $\text{CCl}_4$  (1 ml/kg i.p) daily once for 7 days<sup>19</sup>. Group-III was treated with the standard drug Silymarin (50 mg/kg .p.o) and followed by  $\text{CCl}_4$  (1 ml/kg i.p) daily once for 7 days<sup>20</sup>. Groups IV-VI were treated with ethyl acetate extract of *Glycosmis*

*pentaphylla* (Rutaceae) roots at doses of 100, 200 & 400mg/kg p.o. in acacia mucilage respectively followed by  $\text{CCl}_4$  (1 ml/kg i.p) daily once for 7 days. Groups VII-IX were treated with methanol root extract of *Glycosmis pentaphylla* (Rutaceae) at doses of 100, 200 & 400mg/kg p.o. in acacia mucilage respectively followed by  $\text{CCl}_4$  (1 ml/kg i.p) daily once for 7 days. After completion of treatment blood was collected, serum was separated and used for determination of biochemical parameters.

### Collection of Blood Samples:

All the animals were sacrificed on 7th day under light ether anesthesia. The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at  $37^\circ\text{C}$ . The clear serum was separated at 2500rpm (Microcentrifuge) for 10min and subjected to biochemical investigation viz, serum glutamic oxaloacetate transe aminase (SGOT), serum glutamic Pyruvate transe aminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB).

### Assessment of Liver Function:

The Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by UV kinetic method in which both SGOT and SGPT were assayed based on enzyme coupled system; where keto acid formed by the aminotransaminase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm for SGOT malate dehydrogenase (MDH) reduces to maleate with simultaneous oxidation of NADH to NAD. The rate of oxidation of NADH is measured, where as SGPT<sup>21</sup> the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase. Estimation of Alkaline phosphate (ALKP)<sup>22</sup> involves hydrolysis of P-nitrophenyl phosphate by alkaline phosphatase to give P-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALKP) activity. Estimation of total bilirubin (TB)<sup>23</sup> involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546 nm. All the estimations were carried out using standard kits in semi auto analyzer Screen Master 3000.

### Statistical Analysis:

Results of biochemical estimation were reported as mean  $\pm$ SEM for determination of significant inter group difference was analyzed separately and one-way analysis of variance (ANOVA) was carried out<sup>24</sup>. Dunnet's test was used for individual comparisons<sup>25</sup>.

## RESULTS

Serum levels of SGOT, SGPT, SALKP and total bilirubin were significantly increased ( $p < 0.001$ ) in carbon tetrachloride (1ml/kg.i.p) treated Group-2 rats. Group-3 rats treated with Silymarin (50mg/kg.p.o) produced



significant reduction ( $p < 0.001$ ) in SGOT, SGPT, SALKP and total bilirubin

In Groups: 4-6 treated with ethyl acetate extract of *Glycosmis pentaphylla* at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease

in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The activity of the extracts is found to be dose dependant. The results were given in Table-(1 & 1.1) and Fig-1 & 1A

**Table 1:** Effect of ethyl acetate and methanol extracts of *G. pentaphylla* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCl<sub>4</sub> induced toxicity in rats.

Groups	SGOT (IU/ml)	SGPT (IU/ml)	SALKP (IU/ml)	Total Bilirubin (mg/dl)
Control 1ml/kg	160.5 ± 0.62	96.95 ± 1.34	179.5 ± 0.99	0.82 ± 0.06
CCl <sub>4</sub> 1ml/kg	295.5 ± 0.39 <sup>+</sup>	269.5 ± 1.8 <sup>+</sup>	296.5 ± 1.45 <sup>+</sup>	2.02 ± 0.03 <sup>+</sup>
Silymarin 50mg/kg	174.8 ± 1.88 <sup>***</sup>	107.5 ± 1.45 <sup>***</sup>	187.7 ± 2025 <sup>***</sup>	0.89 ± 0.04 <sup>***</sup>
GPEE 100 mg/kg	234.6 ± 3.52 <sup>*</sup>	198.1 ± 4.52 <sup>*</sup>	210.2 ± 5.15 <sup>*</sup>	1.26 ± 1.32 <sup>*</sup>
GPEE 200 mg/kg	201.5 ± 2.47 <sup>**</sup>	171.3 ± 2.67 <sup>**</sup>	197.3 ± 1.11 <sup>***</sup>	1.01 ± 2.23 <sup>***</sup>
GPEE 400 mg/kg	175.7 ± 2.35 <sup>***</sup>	110.2 ± 2.38 <sup>***</sup>	190.2 ± 1.50 <sup>***</sup>	0.99 ± 0.18 <sup>***</sup>
GPME 100 mg/kg	252.1 ± 2.02 <sup>*</sup>	231.2 ± 3.16 <sup>*</sup>	248.1 ± 2.46 <sup>*</sup>	1.42 ± 1.24 <sup>*</sup>
GPME 200 mg/kg	228.0 ± 3.34 <sup>**</sup>	200.5 ± 3.51 <sup>**</sup>	239.4 ± 2.30 <sup>**</sup>	1.35 ± 2.45 <sup>**</sup>
GPME 400 mg/kg	207.3 ± 1.16 <sup>***</sup>	181.8 ± 1.23 <sup>***</sup>	220.3 ± 2.10 <sup>***</sup>	1.19 ± 1.37 <sup>***</sup>

Values are mean ± SEM for six observations; P: <sup>+</sup> <0.001 Compared to respective control group-1

P: \* <0.05, \*\* <0.01, \*\*\* <0.001 Compared to respective control CCl<sub>4</sub> group-2

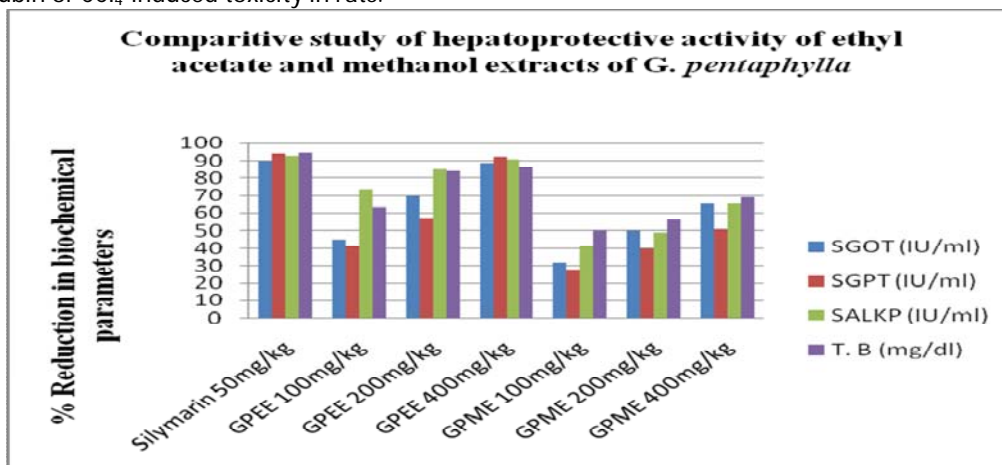
GPEE-Glycosmis *pentaphylla* ethyl acetate extract; GPME-Glycosmis *pentaphylla* methanol extract

**Table 1.1:** Percentage reduction of various biochemical parameters due to treatment with ethyl acetate & methanol extracts of *g. pentaphylla* against ccl4 induced hepatotoxicity in rats.

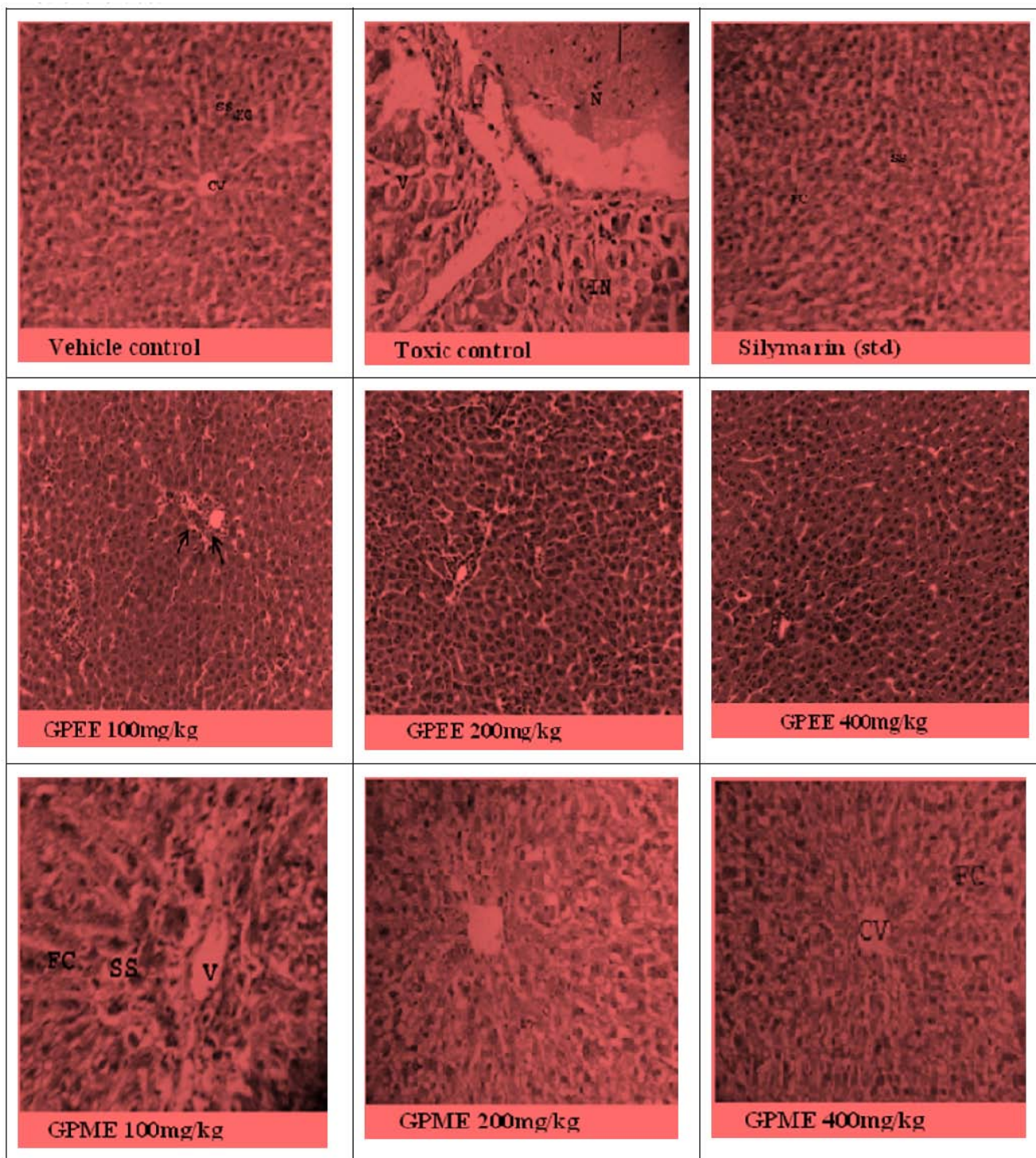
S.NO	TREATMENT	SGOT (IU/ml)	SGPT (IU/ml)	SALKP (IU/ml)	T. B (mg/dl)
1	Silymarin 50mg/kg	89.63	93.8	92.99	94.16
2	GPEE 100mg/kg	45.11	41.36	73.76	63.33
3	GPEE 200mg/kg	69.6	56.89	84.78	84.16
4	GPEE 400mg/kg	88.74	92.29	90.85	85.83
5	GPME 100mg/kg	32.14	27.98	41.36	49.99
6	GPME 200mg/kg	49.99	39.9	48.8	55.83
7	GPME 400mg/kg	65.33	50.81	65.12	69.16

GPEE-Glycosmis *pentaphylla* ethyl acetate extract; GPME-Glycosmis *pentaphylla* methanol extract

**Figure 1:** Effect of ethyl acetate and methanol extracts of *G. pentaphylla* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCl<sub>4</sub> induced toxicity in rats.



**Figure 1A:** Representative photographs of histopathological changes showing effect of the test material on the rats intoxicated with carbon tetrachloride. GPPE-Glycosmis *pentaphylla* ethyl acetate extract, GPME-Glycosmis *pentaphylla* methanol extract



Carbontetrachloride (1ml/kg.i.p) intoxication in normal rats produced elevated levels of serum biochemical parameters significantly SGOT( $160.5 \pm 0.62$ ,  $295.5 \pm 0.39$ ), SGPT( $96.95 \pm 1.34$ ,  $269.5 \pm 1.8$ ), ALKP( $179.5 \pm 0.99$ ,  $296.5 \pm 1.45$ ), T.B( $0.82 \pm 0.06$ ,  $2.02 \pm 0.03$ ) indicating acute hepatocellular damage and biliary obstruction. The percentage reduction of various serum biochemical parameters in case of standard drug Silymarin (50mg/kg.p.o) in CCl<sub>4</sub> intoxicated rats revealed a significant reduction ( $p < 0.01$ ) in the levels of

SGOT(89.63%), SGPT(93.8%), ALKP(92.99%) and T.B(94.16%).

When compared to the CCl<sub>4</sub> toxic control group, the group treated with the ethyl acetate extracts of Glycosmis *pentaphylla*, at doses of 100, 200 and 400mg/kg; p.o in CCl<sub>4</sub> intoxicated rats exhibited a significant reduction ( $p < 0.01$ ) of SGOT(45.11%, 69.60%, 88.74%), SGPT(41.36%, 56.89%, 92.29%), ALKP(73.76%, 84.78%, 90.85%) and T.B(63.33%, 84.16%, 85.83%) levels respectively.

In Groups: 7-9 treated with methanol extract of *Glycosmis pentaphylla* at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The activity of the extracts is found to be dose dependant.

The comparative efficacy of the extracts tested for their hepatoprotective activity, the relationship between dose and percentage reduction in each case were depicted in the form of a bar diagram as shown Fig-1.

Carbontetrachloride (1ml/kg.i.p) intoxication in normal rats produced elevated levels of serum biochemical parameters significantly SGOT ( $160.5 \pm 0.62$ ,  $295.5 \pm 0.39$ ), SGPT ( $96.95 \pm 1.34$ ,  $269.5 \pm 1.8$ ), ALKP ( $179.5 \pm 0.99$ ,  $296.5 \pm 1.45$ ), T.B ( $0.82 \pm 0.06$ ,  $2.02 \pm 0.03$ ) indicating acute hepatocellular damage and biliary obstruction. The percentage reduction of various serum biochemical parameters in case of standard drug Silymarin(50mg/kg.p.o) in CCl<sub>4</sub> intoxicated rats revealed a significant reduction ( $p < 0.01$ ) in the levels of SGOT(89.63%), SGPT(93.8%), ALKP(92.99%) and T.B(94.16%).

When compared to the CCl<sub>4</sub> toxic control group, the group treated with the methanol extracts of *Glycosmis pentaphylla*, at doses of 100, 200 and 400mg/kg; p.o in CCl<sub>4</sub> intoxicated rats exhibited a significant reduction ( $p < 0.01$ ) of SGOT(32.14%, 49.99%, 65.33%), SGPT(27.98%, 39.90%, 50.81%), ALKP(41.36%, 48.80%, 65.12%) and T.B(49.99%, 55.83%, 69.16%) levels respectively.

Though both the extracts were recorded with significant hepatoprotective activity with same "p" value ( $p < 0.01$ ). The ethyl acetate extract was found to be more potent than methanol extract because of effect on percentage reduction in elevated levels of biochemical parameters and effect was dose dependant.

The effect of ethyl acetate and methanol extracts of stem on CCl<sub>4</sub> induced liver damage in rats with reference to biochemical changes in serum was shown. Percentage decrease or increase was calculated by Histopathology of liver tissues. Group I (vehicle control)—section shows central vein surrounded by hepatic cord of cells (normal architecture). Group II (toxic control)—section shows patches of liver cell necrosis with inflammatory collections, around central vein. Group III (standard silymarin)—almost near normal. Group IV (GPEE 100mg/kg)—inflammatory collections around central vein and focal necrosis. Group V (GPEE 200mg/kg)—inflammation decreasing around central vein. Group VI (GPEE 400mg/kg)—less inflammatory cells around central vein, absence of necrosis. Group VII (GPME 100mg/kg)—less inflammation around central vein. Group VIII (GPME 200mg/kg)—less inflammatory cellular infiltration. Group IX (GPME 400mg/kg)—minimal inflammatory cells (Fig-1A).

## CONCLUSION

The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin.

Orally administered doses of 100, 200 and 400mg/kg of methanol of stem heart wood of *Glycosmis pentaphylla* produced significant decrease in SGOT, SGPT, SALKP and total bilirubin levels. The activity of the extracts is found to be dose dependant. In CCl<sub>4</sub> induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures<sup>26</sup>. Administration of methanolic extracts of *Glycosmis pentaphylla* showed recovery against the toxic effects of CCl<sub>4</sub>. The hepatoprotective effect of the drugs was further concluded by the histopathological examinations of the liver sections which reveal that the normal liver shape was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with ethyl acetate extract and methanolic extract and intoxicated with CCl<sub>4</sub> the normal cellular shape was retained as compared to silymarin, thereby confirming the protective effect of the extracts of *Glycosmis pentaphylla*

The hepatoprotective activity of *Glycosmis pentaphylla* could be due to the presence of bioflavonoids which have hepatoprotective properties<sup>27-29</sup>. The result of this investigation indicated that the methanolic extract of stem heart wood of *Glycosmis pentaphylla* possess hepatoprotective activity against CCl<sub>4</sub> induced liver damage in rats. Attempts are being made to isolate and characterize the active principle to which the hepatoprotective activity can attribute.

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