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EVALUATION OF HEPATOPROTECTIVE AND ANTI OXIDANT POTENTIALS OF SEBESTIANA CHAMELEA LEAVES EXTRACT IN ALBINO RATS

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ABSTRACT
The liver is one of the vital organs of the body and plays a key role in the metabolism and detoxification process. Sebestiana chamelea is belongs to the Euphorbiaceae family. The main aim and objectives are evaluation of the phytochemical screening of various extracts of leaves of Sebestiana chamelea. Evaluation of the hepatoprotective activity of various extracts of leaves of Sebestiana chamelea in Carbon tetrachloride induced hepatotoxicity in rats. The different extracts of the leaves of *Sebestiana chamelea* possessed promising hepatoprotective activity against CCl_4 induced hepatic damage. The hepatoprotective activity of *Sebestiana chamelea is* found out to be more in ethanolic extract. The activity could be due to the improvement in the antioxidant enzyme level and a decrease in free radical levels. The presence of phytochemicals such as flavonoids has been shown to be responsible for hepatoprotective activity. Further studies can be carried out in the future to elucidate the mechanism of action of the ethanolic extract of leaves of *Sebestiana chamelea*, which may then be followed and clinical studies to establish its efficacy inhumans.

KEYWORDS: Hepatoprotective activity, Anti-oxidant activity, Sebestiana chamelea leaves, Albino rats.

INTRODUCTION
The liver is one of the vital organs of the body and plays a key role in the metabolism and detoxification process; disorders of this organ remain some of the most serious health problems.[1] Drug-induced hepatic injury is considered as the primary cause of hepatotoxicity.[2] Acetaminophen (paracetamol, N-acetyl-p-aminophenol) via CYP450-mediated N-hydroxylation metabolized to N-acetyl-pbenzoquinoneimine (NAPQI).[3] N-acetyl-pbenzoquinoneimine (NAPQI), a highly toxic, reactive metabolite of acetaminophen, which causes oxidative stress and glutathione (GSH) depletion plays a key role in dose-dependent hepatotoxicity.[4] N-acetylL-cysteine (NAC) is a sulfur-based amino acid and potent antioxidant proved effective as an antidote for hepatotoxicity due to acetaminophen overdose.[5] NAC acts as a precursor for GSH synthesis and was shown to be beneficial against reactive oxygen species (ROS) generation, mitochondrial dysfunctions and in mitochondrial dependent and independent apoptotic cell death in cancer.[6]

Sebestiana chamelea is belongs to the Euphorbiaceae family. Monoecious, erect to sprawling annual to perennial herb or shrub up to 0.5(–1) m tall with slender stems. Leaves alternate, simple, almost sessile; stipules ovate, small; blade linear-lanceolate, 3–6 cm × c. 8 mm, base cuneate, apex obtuse, margins finely toothed, short-hairy beneath. a small, terminal or leaf-opposed spike, most flowers male with 1–2 female flowers at base; bracts with 2 large glands at base. Flowers unisexual, regular, sessile, sepals 3, ovate, greenish yellow, petals absent, disk absent; male flowers with 3 free, shortly exserted stamens, mainly in South America; 4 species occur in tropical Africa. Microstachys was formerly included in Sebastiania, which now comprises about 75 species in the New World tropics. Preliminary phytochemical screening of the extracts revealed the presence of Phenols, Flavonoids, Tannins, Steroids as main constituents along with Glycosides, Alkaloids, Lignins and Saponins. Presence of Phenolic compounds supports its antimicrobial activity and also the herbal usage against diarrhoea. Bioassay guided fractionation of aqueous extract of these plants enabled the isolation and identification of ellagic acid as the main compound responsible for their antiplasmodial activity. Together with ellagic acid, other derivatives belonging to different chemical groups were isolated but showed moderate antimalarial activity; gallic acid, brevifolin carboxylic acid, protocatechuic acid, corillogin, rutin and 3,4,8,9,10-pentahydroxy-dibenzo (b,d) pyran6-one. It showing Antibacterial activity, Antifungal activity, Antioxidant activity, Anthelmintic activity, Antidiarrhoeal activity, Antidiabetic activity.[7,8]

The literature review indicated that plant of Sebestiana chamelea generally rich sources of antioxidants and hence may be considered to be good potential for hepatoprotective activity. From the literature review it is clear that no scientific work has so far been carried out on the stem bark of Sebestiana chamelea for hepatoprotective activity. The main aim and objectives are evaluation of the phytochemical screening of various extracts of leaves of Sebestiana chamelea. Evaluation of the hepatoprotective activity of various extracts of leaves of Sebestiana chamelea in Carbon tetrachloride induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh plant of *Sebestiana chamelea* was collected from the forest around August 2021 from chittur dist. The plant materials were identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference.

Preparation of plant extract

The freshly collected whole plant of this plant was shopped and dried. The dried material of leaves was powder. The powdered plant material (250 g) was extracted by hot continuous soxhlet extraction method and the plant material was extracted with Ethanol (99.9% v/v), Ethyl acetate and Petroleum ether for four days in a soxhlet apparatus.

Phytochemical qualitative analysis

The plant extracts were assessed for the existence of the phytochemical analysis.[9-12]

Flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To theflask was added 0.30 ml of 5% NaNO2, after 5min 0.3 ml of 10 % AlCl3 was added. After 5min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).[13,14]

In vivo studies

Experimental animals

The present study was conducted after obtained approval from the Institutional Animal Ethics Committee, the protocol met the requirements of national guidelines of CPCSEA (**PROPOSAL NO: CPCSEA/IAEC/JLS/17/03/22/29**) The Wistar rats (150-200g) used for this study were procured from, Central Animal House, Madras Medical College, Chennai-03.

In vivo hepatoprotective evaluation

The hepatoprotective activity of *Sebestiana chamelea*., was evaluated in Wistar rats. Liver toxicity was induced by intraperitoneal administration of carbon tetrachloride (CCl_4). The hepatoprotective effect of plant extract was compared with standard drug Silymarin.

Table 1: *In Vivo* hepatoprotective experimental design.

S. no.	Groups	Treatment schedule	No. of animals
1	Group I Normal control	1 ml of 1% BCD p.o. daily for 14 days	6
2	Group II Negativecontrol	1 mg/kg CCl_4 in olive oil (1:1), i.p. once in 3 days for 14 days and received daily a singleoral dose of BCD (1ml of 1% w/v)	6
3	Group III Positivecontrol	25 mg/kg of Silymarin p.o. daily for 14 days + (CCl_4) 1mg/kg in olive oil (1:1), i.p. oncein 3days for 14 days	6
4	Group IV Test group 1	400mg/kg Ethanolic extract of *Sebestiana chamelea in* BCD p.o. daily for 14 days + (CCl_4) 1mg/kg in olive oil (1:1), i.p. once in 3days for 14 days	6
5	Group V Test group 2	400 mg/kg Ethyl acetate extract of *Sebestiana chamelea in* BCD p.o. daily for 14 days + (CCl_4)1mg/kg in olive oil (1:1), i.p. once in 3days for 14 days	6
6	Group VI Test group 3	400 mg/kg Petroleum ether extract of *Sebestiana chamelea in* BCD p.o. daily for 14 days + (CCl_4) 1mg/kg in olive oil (1:1), i.p. once in 3days for 14 days	6
Total animals			36

For all rats, body weight was measured before and after the induction of hepatotoxicity (1th and 15th days). On the 15th day, all the animals were mildly anesthetized and blood was collected by heart puncture and serum

was separated by centrifugation at 2000 rpm for 15-20 minutes at 4°C, the serum samples were maintained at -80° C, for estimation of biochemical parameters.

The animals were sacrificed by cervical dislocation method. The liver is removed and rinsed with ice cold saline and stored in 10% formalin solution. A part of liver was homogenate with phosphate buffer, PH 7.4 using a Teflon homogenizer in ice-cold condition. The homogenate of liver as centrifuged at 5000 rpm for 10 min, the supernatants solution are taken up for the evaluation of lipid peroxidation (LPO), superoxide dismutase (SOD) and glutathione peroxidase (GPx). The other part of liver was subjected to Histopathological study.

Biochemical parameters

The blood samples were collected and allowed to clot and centrifuged at 2000 rpm for 15-20 minutes using REMI (412 LAG) cooling centrifuge. The serum was kept at -80°C until analyzed. Levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Serum Alkaline Phosphate (ALP) Total protein, Albumin and Total Bilirubin were determined with an Automated Analyzer (Hitachi 911, Japan).

Histophathological studies

The liver from the animals was rinsed in ice cold 0.9% saline and was fixed in 10% formalin embedded in paraffin and cut into 5 µm thick section using a microtome. Sections were mounted on class slide using standard techniques. The sections were stained with Haematoxylin – Eosin and were examined under a microscope using 400 x magnifications and photographed under a light microscope equipped for photography (Olympus CK 40).

Statistical analysis

All the values were expressed as mean ± SEM. The data was statistically analyzed by one way ANOVA followed by Dunnet's test. One way analysis of variance (ANOVA) was used to correlate the statistical difference between the variables. $P<0.05$ was considered to be significant. Statistical analysis is done by using GraphPad prism.[15]

RESULTS

Preliminary phytochemical analysis

Table 2: Preliminary phytochemical analysis of various extract of *Sebestiana chamelea*.

Test	Ethanol extract	Ethyl acetate extract	Pet. Ether extract
Test for flavonoids a) Shinado's test b) Sodium hydroxide test	+	+	+
Test for tannins With lead acetate	+	+	-
Test for saponins Foam test	+	+	-
Test for terpenoids With tin and thiol chloride	+	+	-
Test for glycosides a) Libermann-burchard's test b) Legal's test c) Borntrager's test	+	+	-
Test for phytosterols Libermann test	+	+	+
Test for mucilage Swelling test	-	-	-
Test for protein a) Biuret test b) Million's test	+	+	+
Test for carbohydrate Molish's test	+	+	-
Test for alkaloids a) Dragendroff's test b) Mayer's test c) Hager's test d) Wagner's test	+	+	-

(**+ Present**) (**- absent**)

Determination of total flavonoid content

Table 3: Standard calibration curve of varying concentration of Quercetin.

S. No	Concentration of quercetin (µg/ml)	Mean absorbance
1	12.5	0.095
2	25	0.125
3	50	0.165
4	100	0.251
5	200	0.391

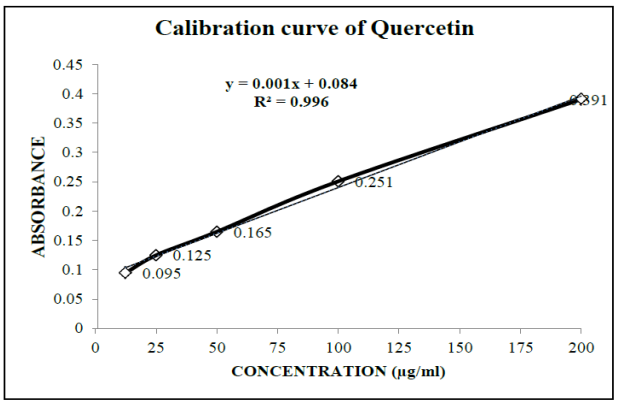


Fig. 1: Standard calibration curve of varying concentration of quercetin.

The total flavonoid content present in the extracts was determined using aluminium chloride colorimetric method from the calibration curve of standard quercetin. The total flavonoid content in the ethanolic extract of *Sebestiana chamelea*. was found to be 19.67±0.333g QE/100g of extract and ethyl acetate extract of *Erythrina indica* Lam. was 6±0.577g QE/100g of extract. Petroleum ether extract of *Sebestiana chamelea*. was 13.33±0.333g QE/100g of extract.

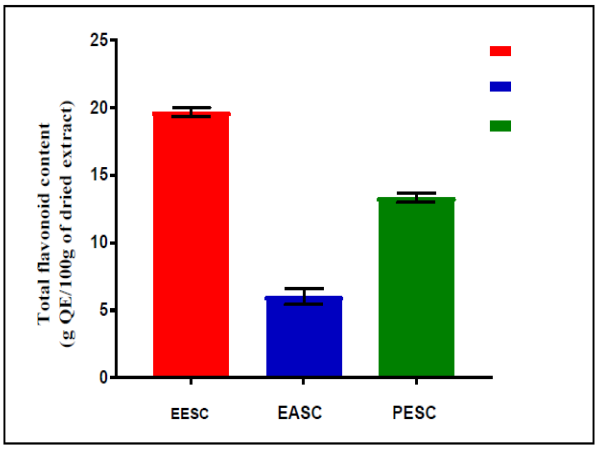


Fig. 2: Total flavonoid content of ethanol ethyl acetate and petroleum etherextracts of *Sebestiana chamelea*.

Table 4: Practical yield of *sebestiana chamelea.*

Solvent	Practical yield in percentage
ethanol	10.04% w/w
Ethyl acetate	4.2% w/w
Petroleum ether	2.6% w/w

***In vivo* hepatoprotective activity**

Body weight

The body weight of the animals was determined on 1st and 15th day of the studyperiod and these are tabulated in **Table-5 and Fig.3.**

Table 5: Body weight of the animals in the various groups.

Groups	Treatment	Animal body weight in gms	
		1st day	15th day
I	Control	164±3.43	189±2.96
Ii	Disease control	157±2.31	145±2.73
Iii	Silymarin (25 mg/kg)	164.7±2.94	161.3±3.18
Iv	Ethanolic extract of sebestiana chamelea (400 mg/kg)	166.7±2.46	161±2.21
V	Ethyl acetate extract of *Sebestiana chamelea* (400 mg/kg)	162.3±2.36	152±1.95
Vi	Pet. Ether extract of *Sebestiana chamelea* (400 mg/kg)	163.3±3.82	151.8±2.75

Values are expressed by Mean ± SEM

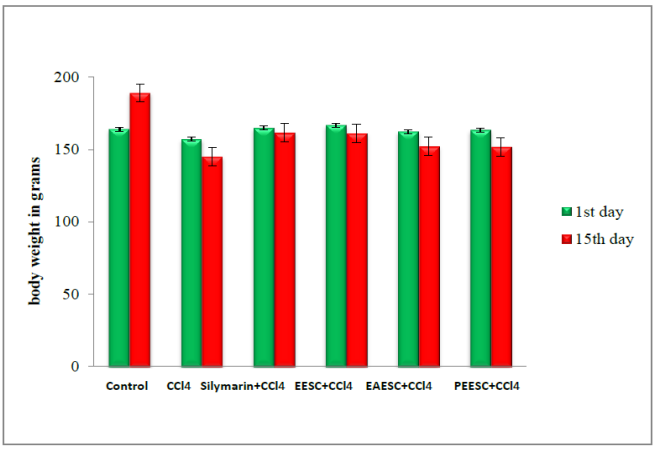


Fig. 3: Body weight of animals in the various groups.

It is seen from the data that in the CCl_4 treated group there was a slight decrease in body weight on the 15th day. In the Silymarin and extracts of *Sebestiana chamelea* treated group shows the reduction in the body weight was lesser than that of CCl_4 treated group.

Biochemical estimation

Aspartate Aminotransferase (AST) evaluations

The AST level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ Extracts of *Sebestiana chamelea* were estimated on Day 15. They are tabulated in **Table-6 and Fig.4.**

Table 6: Aspartate aminotransferase levels.

Group	Treatment	Ast (u/ml)
I	Control	207±3.57***
Ii	Disease control	554±5.96***
Iii	Silymarin (25 mg/kg)	314.7±4.43***
Iv	Ethanolic extract of sebestiana chamelea (400 mg/kg)	327.3±4.43***
V	Ethyl acetate extract of *sebestiana chamelea* (400 mg/kg)	464.7±4.55***
Vi	Pet. Ether extract of *sebestiana chamelea* (400 mg/kg)	354±2.75***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group

###P<0.001 compared to disease control group

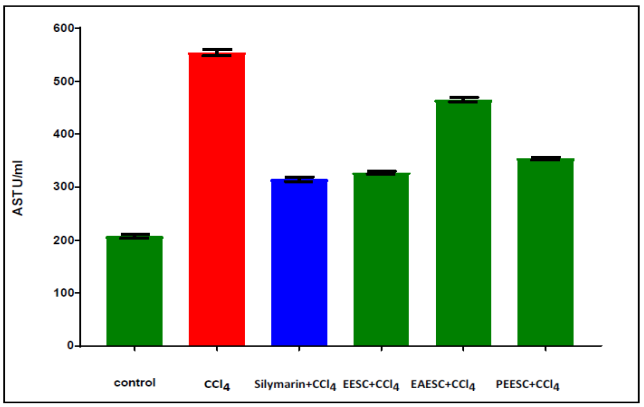


Fig. 4: Aspartate aminotransferase levels.

In the **Table-6 and Fig.4:** It was seen that AST level 554±5.96 had increased significantly in animals which were given CCl_4 as compared to normal group 207±3.57. Treatment with Silymarin showed a significant decrease in the level of AST 314.7±4.43 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant decrease in the level of AST 327.3±4.43, 464.7±4.55 and 354±2.75 respectively. The reduction was more in the group treated with the ethanolic extracts of *Sebestiana chamelea 327*.3±4.43, when compare to other extracts treated groups.

Alanine aminotransferase (ALT) evaluation

The ALT level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated on day 15. They are tabulated in **Table-7** and **Fig. 5**

Table 7: Alanine aminotransferase levels.

Group	Treatment	Alt (u/ml)
I	Control	63.83±1.38***
Ii	Disease control	169.1±2.69***
Iii	Silymarin (25 mg/kg)	81.13±0.85***
Iv	Ethanolic extract of sebestiana chamelea Lam (400 mg/kg)	90.16±1.14***
V	Ethyl acetate extract of *tiana chamelea*(400 mg/kg)	137.4±2.89***
Vi	Pet. Ether extract of *sebestiana chamelea* (400 mg/kg)	103.2±1.82***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group

###P<0.001 compared to disease control group

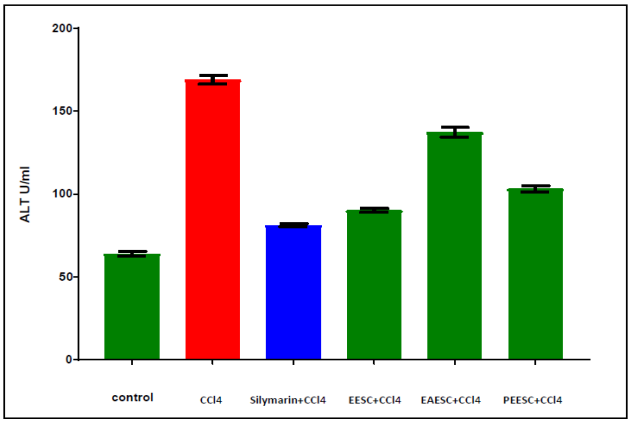


Fig. 5: Alanine aminotransferase levels.

In **Table 7** and **Fig.5:** It was seen that ALT level 169.1±2.69 had increased significantly in animals which were given CCl_4 as compared to normal group 63.83±1.38. Treatment with Silymarin showed a significant decrease in the level of ALT 81.13±0.85 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant decrease in the level of ALT

90.16±1.14, 137.4±2.89 and 103.2±1.82 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Sebestiana chamelea* 90.16±1.14, when compare to other extracts treated groups.

Alkaline phosphatase (ALP) evaluation

The ALP level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated on day 15. They are tabulated in **Table-8** and **Fig.6**

Table 8: Alkaline phosphatase levels.

Group	Treatment	Alp (u/ml)
I	Control	228.2±4.32***
Ii	Disease control	405.9±6.76***
Iii	Silymarin (25 mg/kg)	242.8±3.18***
Iv	Ethanolic extract of sebestiana chamelea (400 mg/kg)	286.9±2.41***
V	Ethyl acetate extract of*sebestiana chamelea* (400 mg/kg)	377.4±5.75#
Vi	Pet. Ether extract of *sebestiana chamelea* (400 mg/kg)	308.4±4.002***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group

#P<0.001 compared to disease control group

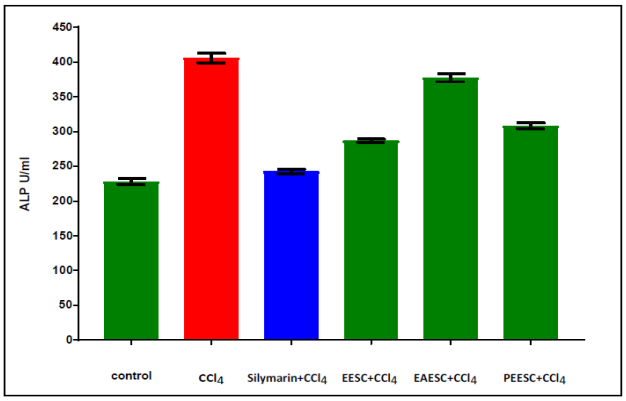


Fig. 6: Alkaline phosphatase levels.

In the **Table-8** and **Fig.6:** It was seen that ALP level 405.9±6.76 had increased significantly in animals which were given CCl_4 as compared to normal group 228.2±4.32. Treatment with Silymarin showed a significant decrease in the level of ALP 242.8±3.18 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant decrease in the level of ALP 286.9±2.41, 377.4±5.75 and 308.4±4.002 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Sebestiana chamelea 286*.9±2.41, when compare to other extracts treated groups.

Total bilirubin (TB) evaluation

The CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated on day 15. They are tabulated in **Table-9** and **Figure. 7**

Table 9: Total bilirubin levels.

Group	Treatment	TB (mg/dl)
I	Control	1.68±0.17***
II	Disease control	14.12±0.29***
III	Silymarin (25 mg/kg)	2.68±0.30***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	4.49±0.23***
V	Ethyl acetate extract of *tiana chamelea*(400 mg/kg)	11.47±0.32##
VI	Pet. ether extract of *Sebestiana chamelea* (400 mg/kg)	7.39±0.32***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group

##P<0.001 compared to disease control group

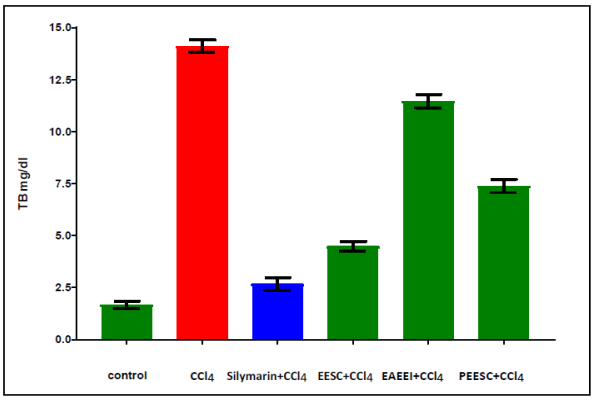


Fig. 7: Total bilirubin levels.

In the **Table-9** and **Fig.7:** It was seen that Total Bilirubin level 14.12±0.29 had increased significantly in animals which were given CCl_4 as compared to normal group 1.68±0.17. Treatment with Silymarin showed a significant decrease in the level of Total Bilirubin 2.68±0.30 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant decrease in the level of Total Bilirubin 4.49±0.23, 11.47±0.32 and 7.39±0.32 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Sebestiana chamelea*, when compare to other extracts treated groups 4.49±0.23.

Total Protein (TP) evaluation

The Total Protein level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated on day 15. They are tabulated in **Table-10** and **Figure. 8**

Table 10: Total Protein levels.

Group	Treatment	Tp (g/dl)
I	Control	8.20±0.06***
Ii	Disease control	5.73±0.12***
Iii	Silymarin (25 mg/kg)	7.85±0.08***
Iv	Ethanolic extract of sebestiana chamelea (400 mg/kg)	7.46±0.1***
V	Ethyl acetate extract of *tiana chamelea*(400 mg/kg)	6.42±0.11#
Vi	Pet. Ether extract of *sebestiana chamelea* (400 mg/kg)	6.97±0.16#

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group**

#P<0.001 compared to disease control group

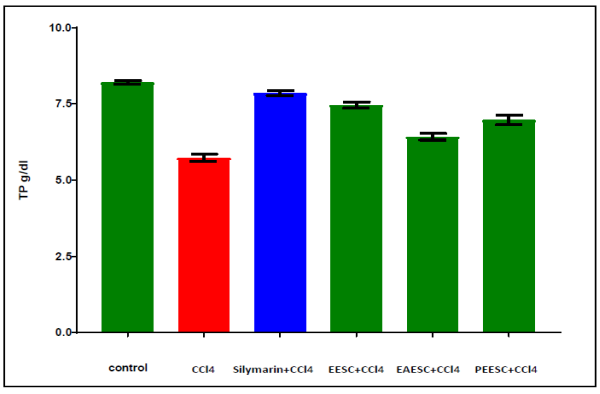


Fig. 8: Total protein levels.

In the **Table-10** and **Fig.8:** It was seen that Total Protein level 5.73±0.12 had decreased significantly in animals which were given CCl_4 as compared to normal group 8.20±0.06. Treatment with Silymarin showed a

significant increase in the level of Total Protein 7.85±0.08 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant increase in the level of Total Protein 7.46±0.1, 6.42±0.11 and 6.97±0.16 respectively. The elevation was more in the group treated with the Ethanolic extracts of *Sebestiana chamelea* 7.46±0.1, when compare to other extracts treated groups.

Albumin (ALB) evaluation

The Albumin level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated on day 15. They are tabulated in **Table-11** and **Figure. 9**

Table 11: Albumin levels.

Group	Treatment	Albumin(g/dl)
I	Control	3.67±0.02***
II	Disease control	1.34±0.01***
III	Silymarin (25 mg/kg)	3.38±0.04***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	2.99±0.13***
V	Ethyl acetate extract of *Sebestiana chamelea* (400 mg/kg)	1.57±0.06#
VI	Pet. ether extract of *Sebestiana chamelea* (400 mg/kg)	2.33±0.07***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group

#P<0.001 compared to disease control group

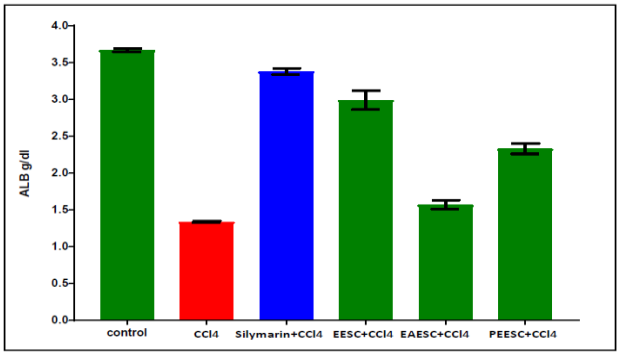


Fig. 9: Albumin levels.

In the **Table-11** and **Fig.9:** It was seen that albumin level 1.34±0.01 had decreased significantly in animals which were given CCl_4 as compared to normal group 3.67±0.02. Treatment with Silymarin showed a significant increase in the level of albumin 3.38±0.04 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant increase in the level of albumin 2.99±0.13, 1.57±0.06 and 2.33±0.07 respectively. The elevation was more in the group treated with the ethanolic extract of *Sebestiana chamelea* 2.99±0.13, when compare to other extracts treated groups.

Estimation of antioxidant enzymes levels

Lipid peroxidation (LPO)

Lipid peroxidase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-12** and **Fig. 10**

Table 12: Lipid peroxidation levels.

Group	Treatment	Lpo (moles/ 100mg/ protein)
I	Control	2.29±0.04
Ii	Disease control	7.61±0.07***
Iii	Silymarin (25 mg/kg)	3.69±0.06***
Iv	Ethanolic extract of sebestiana chamelea (400 mg/kg)	4.34±0.06***
V	Ethyl acetate extract of *sebestiana chamelea* (400 mg/kg)	6.98±0.07##
Vi	Pet. Ether extract of *sebestiana chamelea* (400 mg/kg)	5.85±0.17##

The value are expressed as Mean ± SEM (n=6)
***P<0.001 compared to control group
##P<0.001 compared to disease control group

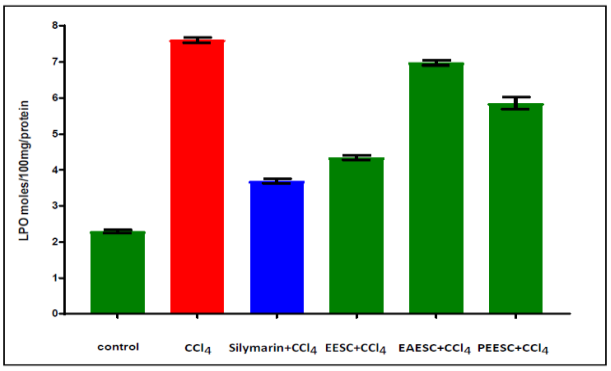


Fig. 10: Lipid peroxidation levels.

In the **Table-12** and **Fig.15:** It was seen that the LPO level 7.61±0.07 had increased significantly in animals which were given CCl_4 as compared to normal group 2.29±0.04. Treatment with Silymarin showed a significant decrease in the level of LPO 3.69±0.06 as compare to the disease control. The extracts *Sebestiana chamelea* treated groups of the animals also showed a significant decrease in the level of LPO 4.34±0.06, 6.98±0.07 and 5.85±0.17 respectively. The reduction was more in the group treated with the Ethanolic extracts of *Sebestiana chamelea* 4.34±0.06, when compare to other extracts treated groups.

Superoxide dismutase (SOD)

Superoxide dismutase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-13** and **Fig. 11**

Table 13: Superoxide dismutase levels.

Group	Treatment	SOD (U mg^{-1} of protein)
I	Control	12.6±0.58***
II	Disease control	5.22±0.16***
III	Silymarin (25 mg/kg)	8.88±0.21***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	7.79±0.08***
V	Ethyl acetate extract of *tiana chamelea*(400 mg/kg)	6.40±0.1##
VI	Pet. ether extract of *Sebestiana chamelea* (400 mg/kg)	7.26±0.14***

The value are expressed as Mean ± SEM (n=6)
***P<0.001 compared to control group
##P<0.001 compared to disease control group

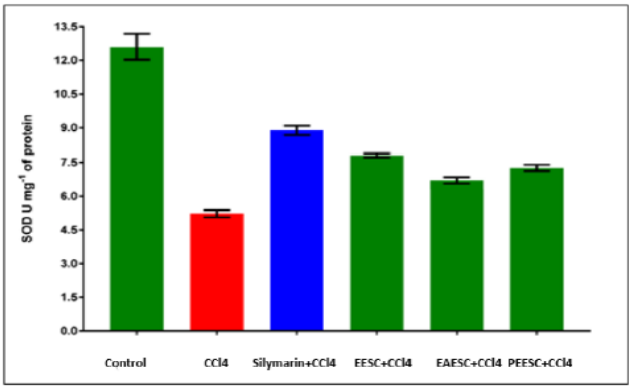


Fig. 11: Superoxide dismutase levels.

In the **Table-13** and **Fig.11:** It was seen that superoxide dismutase enzyme level 5.22±0.16 had decreased significantly in animals which were given CCl_4 as compared to normal group 12.6±0.58. Treatment with Silymarin showed a significant increase in the level of superoxide dismutase 8.88±0.21 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant increase in the level of superoxide dismutase 7.79±0.08, 6.40±0.1 and 7.26±0.14 respectively. The elevation was more in the group treated with the ethanolic extracts of *Sebestiana chamelea* 7.79±0.08, when compare to other extracts treated groups.

Glutathione peroxidase (GPx)

Glutathione peroxidase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-14**and **Fig.12**

Table 14: Glutathione peroxidase levels.

Group	Treatment	Gpx (nmol nadph min^{-1} mg^{-1} of protein)
I	Control	167.5±0.83***
Ii	Disease control	79.1±0.81***
Iii	Silymarin (25 mg/kg)	145.6±0.91***
Iv	Ethanolic extract of sebestiana chamelea (400 mg/kg)	135.2±0.69***
V	Ethyl acetate extract of *sebestiana chamelea* (400 mg/kg)	93.85±0.50***
Vi	Pet. Ether extract of *sebestiana chamelea* (400 mg/kg)	104.6±1.64***

The value is expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group

###P<0.001 compared to disease control group

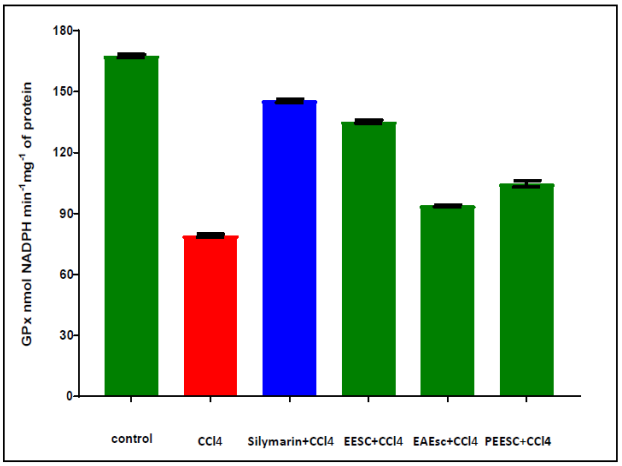


Fig. 12: Glutathione peroxidase levels.

In the **Table-14** and **Figure.12:** It was seen that Glutathione peroxidase enzyme level 79.1±0.91 had decreased significantly in animals which were given CCl_4 as compared to normal group 167.5±0.83. Treatment with Silymarin showed a significant increase in the level of Glutathione peroxidase 145.6±0.91 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant increase in the level of Glutathione peroxidase 135.2±0.69, 93.85±0.50 and 104.6±1.64 respectively. The elevation was more in the group treated with the Ethanolic extracts of *Sebestiana chamelea*, when compare to other extracts treated groups 135.2±0.69.

Catalase (CAT)

Catalase enzyme level of the animals treated with CCl_4 alone and those that were given CCl_4 and Silymarin/ extracts of *Sebestiana chamelea* were estimated in liver homogenized solution on Day 15. They are tabulated in **Table-15** and **Fig.13**

Table 15: Catalase levels.

Group	Treatment	Catalase (nmol min^{-1} mg^{-1} ofprotein)
I	Control	489.2±2.75***
Ii	Disease control	213.6±0.99***
Iii	Silymarin (25 mg/kg)	363.1±0.54***
Iv	Ethanolic extract of sebestiana chamelea (400 mg/kg)	352.8±0.76***
V	Ethyl acetate extract of *tiana chamelea*(400 mg/kg)	253.6±2.38***
Vi	Pet. Ether extract of *sebestiana chamelea* (400 mg/kg)	286±1.58***

The value are expressed as Mean ± SEM (n=6)

***P<0.001 compared to control group
###P<0.001 compared to disease control group

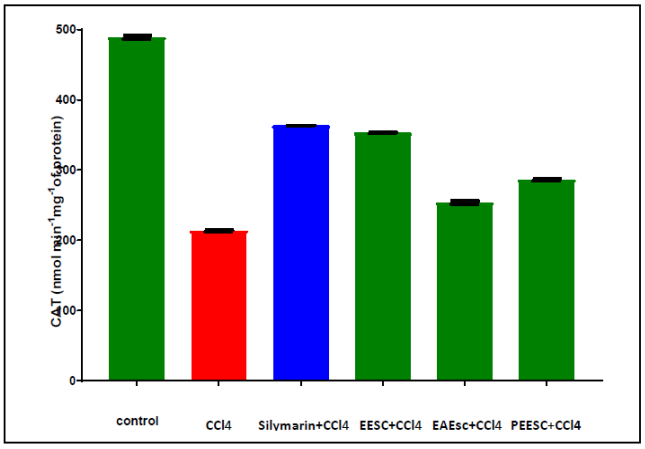
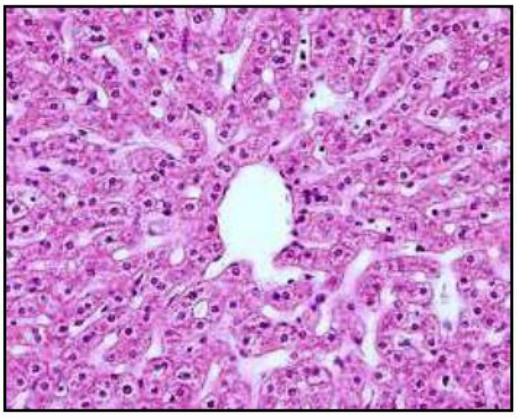


Fig. 13: Catalase levels.

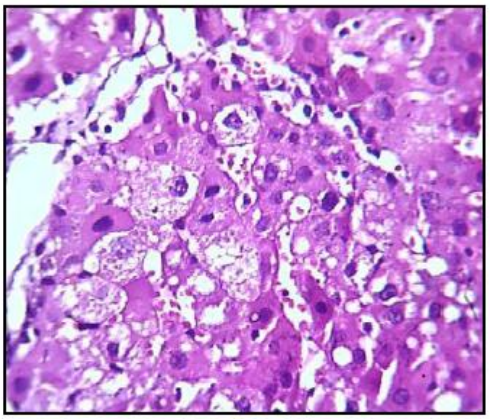
In the **Table-15** and **Fig.13:** It was seen that CAT enzyme level 213.6±0.09 had decreased significantly in animals which were given CCl_4 as compared to normal group 489.2±2.75. Treatment with Silymarin showed a significant increase in the level of CAT 363.1±0.54 as compare to the disease control. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant increase in the level of CAT 352.8±0.76, 253.6±2.38 and 286±1.58 respectively. The elevation was more in the group treated with the ethanolic extracts of *Sebestiana chamelea* , when compare to other extracts treated groups 352.8±0.76.

Histopathological study

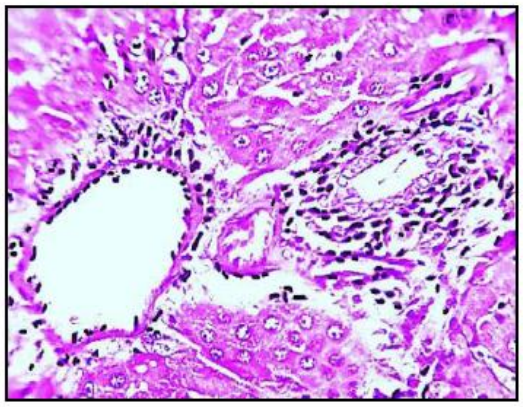
A part liver tissue subjected to histopathological evaluation.



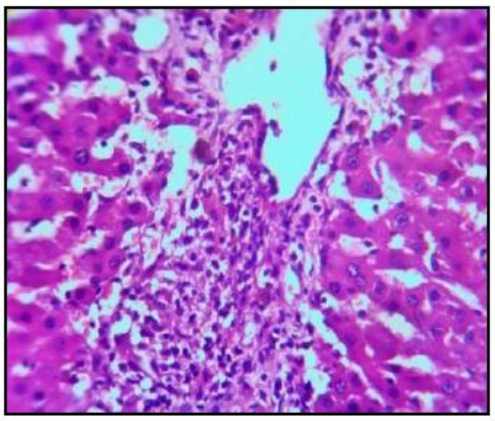
1) Normal control



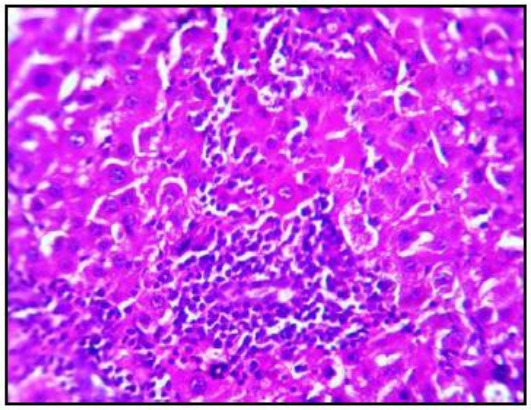
2) CCl_4 treated



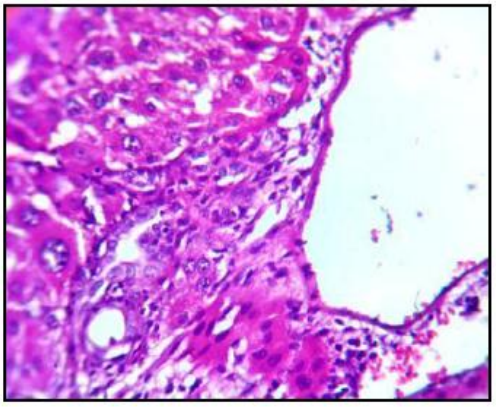
3) CCl_4 + Silymarin 25 mg/kg



4) CCl_4 + EESC 400 mg/kg



5) CCl_4 + EAESC 400 mg/kg



6) CCl_4 + PEESC 400 mg/kg

Fig. 14: Histopathological studies of liver.

1. In control group, Liver section showing normal histological appearance.
2. CCl_4 induced group of Liver section showed diffuse areas of vacuolar degeneration, lobular inflammation, portal to portal fibrosis and centrilobular necrosis with mononuclear cell infiltration.
3. Liver section, standard drug silymarin treated group was showing mild hepatocyte vacuolation.
4. Liver section of CCl_4 along with ethanolic extract of *Sebestiana chamelea 400* mg/kg: treated group was showing mild vacuolar degeneration and mild hepatocyte swelling.
5. Section of CCl_4 along with ethyl acetate extract of *Sebestiana chamelea 400* mg/kg treated group has shown vacuolar degeneration, lymphocyte present in portal tract and mononuclear cell infiltration in parenchyma and portal areas.
6. Liver section of CCl_4 along with pet ether extract of *Sebestiana chamelea 400* mg/kg treated group has shown mild vacuolar degeneration and mild hepatocyte swelling.

DISCUSSION

Liver is an important organ involved in metabolism of many xenobiotics. It removes toxins from the body. It is also exposed to several drugs and xenobiotics which cause hepatic damage. In the present study hepatoprotective activity on the various extracts of stem bark of *Sebestiana chamelea* was evaluated.

The powdered plant material was extracted by hot continuous soxhlet extraction method and the plant material was extracted with Ethanol (99.9% v/v), Ethyl acetate and Petroleum ether for four days in a soxhlet apparatus. The practical yield of ethanolic extract showed 10.04% w/w, ethyl acetate extract showed 4.2% w/w and petroleum etherextract showed 2.6% w/w.
In phytochemical evaluation of ethanolic extract of stem bark of *Sebestiana chamelea* showed the presence of flavonoids, tannins, saponins, terpenoids, phytosterol, protein, carbohydrate and alkaloids. Ethyl acetate extract of stem bark of *Sebestiana chamelea* showed the presence of flavonoids, tannins, saponins, terpenoids, phytosterol, protein, carbohydrate and alkaloids. Petroleum ether extract of stem bark of *Sebestiana chamelea* showed the presence of flavonoids, phytosterol and protein.

It was seen that ethanolic extract of stem bark of *Sebestiana chamelea* showed increased total flavonoid content when compared to other extracts. The observed *in vivo* antioxidant and hepatoprotective activity for this extract therefore may be due to the presence of

flavonoids.[46,47] The acute toxicity test suggested that the crude extracts of the plant was non-toxic to rat upto the dose 4000 mg/ kg.

CCl_4- induced hepatic injury is an experimental model widely used for the screening of hepatoprotective drugs. CCl_4 undergoes a biotransformation by hepatic microsomal cytochrome P-450 to produce trichloromethyl free radicals. This hepatotoxic metabolite can react with protein and lipid in the membrane of cells or organelles leading to necrosis of hepatocytes. As a result of hepatic injury, permeability of the cell membrane is altered causing the cytosolic transaminase (ALT, AST), in the circulation. Hence evaluation of AST and ALT are definite indicators of hepatoprotective activity. The rise in the serum levels of ALP, AST, ALT and bilirubin as observed in the present study could be attributed to the damaged structural integrity of the liver. Liver damage is also associated with elevated levels of ALT, and Bilirubin. It is also associated with decrease in levels of Total Protein and Albumin.[48]

It was seen that AST, ALT, ALP and bilirubin levels had increased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant decrease in the level of AST, ALT, ALP and bilirubin. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant decrease in the level of AST, ALT, ALP and bilirubin. The reduction was more in the group treated with the Ethanolic extracts of *Sebestiana chamelea* , when compare to other extracts treated groups.

Total Protein, Albumin levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the level of Total Protein, Albumin. The extracts of *Sebestiana chamelea* treated groups of animals also showed a significant increase in the level of Total Protein, Albumin. The elevation was more in the group treated with the ethanolic extracts of *Sebestiana chamelea* , when compare to other extracts treated groups.

The administration of various extracts of stem bark of *Sebestiana chamelea* showed improvement in the biochemical parameters profile of the animals. The effect was seen with ethanolic extract of stem bark of *E. indica* is almost equal to that of the standard drug Silymarin. These evaluation studies confirm the hepatoprotective potential of various extracts of stem bark of *Sebestiana chamelea*.

It has been hypothesized that one of the principle causes of CCl_4- induced liver injury is formation of lipid peroxidases by free radical derivatives of CCl_4 (CCl_3^-). Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4- induced hepatotoxicity.

Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by compounds like CCl_4 and is responsible for the cell membrane alteration. In the present study, elevated level of LPO observed in CCl_4 administered rats indicated excessive formation of free radicals and activation of LPO system resulting in hepatic damage.[81] It was seen that the LPO levels had increased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant decrease in the level of LPO. The various extracts of stem bark of *Sebestiana chamelea* treated groups of the animals also showed a significant decrease in the levels of LPO. The reduction was more in the group treated with the ethanolic extracts of *Sebestiana chamelea*, when compare to other extracts treated groups. Hence, it is possible that the mechanism of hepatoprotection of *Sebestiana chamelea* might be due to its antioxidant action.

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, Glutathione peroxidase and Catalase. SOD has been reported as one of the most important enzyme in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Decrease in enzymatic activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury.[83] It was seen that Superoxide dismutase enzyme levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the level of Superoxide dismutase. The various extracts of stem bark of *Sebestiana chamelea* causes a significant increase in hepatic SOD level indicating a reduction of reactive free radical induced oxidative damage to liver. The elevation was more in the group treated with the ethanolic extracts of *Sebestiana chamelea* , when compare to other extracts treated groups.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.[84,85] It was seen that Glutathione peroxidase enzyme levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the levels of Glutathione peroxidase. The various extracts of stem bark of *Sebestiana chamelea* causes a significant increase in hepatic GPx level indicating a reduction of reactive free radical induced oxidative damage to liver. The elevation was more in the group treated with the ethanolic extracts of *Sebestiana chamelea*, when compare to other extracts treated groups.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) and protects the tissues from highly reactive hydroxyl radicals.[49] Therefore reduction in the level of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide.[50,51] It was seen that catalase enzyme levels had decreased significantly in animals which were given CCl_4 as compared to normal group. Treatment with Silymarin showed a significant increase in the levels of catalase. The various extracts of stem bark of *Sebestiana chamelea* causes a significant increase in hepatic Catalase level indicating a reduction of reactive free radical induced oxidative damage to liver. The elevation was more in the group treated with the ethanolic extracts of *Sebestiana chamelea*, when compare to other extracts treated groups.

From the control group, Liver section showing normal histological appearance. CCl_4 induced group of Liver section showed diffuse areas of vacuolar degeneration, lobular inflammation, portal to portal fibrosis and centrilobular necrosis with mononuclear cell infiltration. The liver section of standard drug silymarin treated group was showing mild hepatocyte vacuolation. The liver section of CCl_4 along with ethanolic extract of *Sebestiana chamelea* 400 mg/kg: treated group was showing mild vacuolar degeneration and mild hepatocyte swelling. The liver section of CCl_4 along with ethyl acetate extract of *Sebestiana chamelea* 400 mg/kg treated group has shown vacuolar degeneration, lymphocyte present in portal tract and mononuclear cell infiltration in parenchyma and portal areas. The liver section of CCl_4 along with pet ether extract of *Sebestiana chamelea* 400 mg/kg treated group has shown mild vacuolar degeneration and mild hepatocyte swelling.

Histopathological liver sections also revealed that the hepatic architecture was altered by hepatotoxin in Carbon tetrachloride group, whereas in the liver sections of the rat treated with the various stem bark extracts of *Sebestiana chamelea* and intoxicated with CCl_4, the hepatic architecture was not altered and was comparable with the standard drug Silymarin. The histopathological study confirms the significant hepatoprotective effect of ethanolic extract of stem bark of *Sebestiana chamelea*.

CONCLUSION

The different extracts of the leaves of *Sebestiana chamelea* possessed promising hepatoprotective activity against CCl_4 induced hepatic damage. The hepatoprotective activity of *Sebestiana chamelea is* found out to be more in ethanolic extract.The activity could be due to the improvement in the antioxidant enzyme level and a decrease in free radical levels. The presence of phytochemicals such as flavonoids has been shown to be responsible for hepatoprotective activity.Further studies can be carried out in the future to elucidate the mechanismof action of the ethanolic extract of leaves of *Sebestiana chamelea*, which may then be followed and clinical studies to establish its efficacy in humans.

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