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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF SEBESTIANA CHAMELEA IN CHEMICALLY INDUCED HEPATOTOXIC RATS

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Abstract:
The primary point and goals are assessment of the phytochemical screening of different concentrates of leaves of Sebestiana chamelea. Sebestiana chamelea is has a place with the Euphorbiaceae family. The primary point and targets are assessment of the phytochemical screening of different concentrates of leaves of Sebestiana chamelea. Assessment of the hepatoprotective action of different concentrates of leaves of Sebestiana chamelea in Carbon tetrachloride prompted hepatotoxicity in rodents. The various concentrates of the leaves of Sebestiana chamelea had promising hepatoprotective action against CCl4 instigated hepatic harm. The hepatoprotective movement of Sebestiana chamelea is found on a mission to be more in ethanolic extract.The action could be because of the improvement in the cell reinforcement protein level and a reduction in free extreme levels. The presence of phytochemicals, for example, flavonoids has been demonstrated to be liable for hepatoprotective activity.Further studies can be done in the future to explain the system of activity of the ethanolic concentrate of leaves of Sebestiana chamelea, which may then be followed and clinical examinations to lay out its viability in people.

KEY WORDS: *Hepatoprotective activity, sebestiana chamelea leaves, albino rats*

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INTRODUCTION:

The liver is one of the fundamental organs of the body and assumes a vital part in the digestion and detoxification process; issues of this organ stay probably the most serious medical conditions [1]. Drug-initiated hepatic injury is considered as the essential driver of hepatotoxicity [2]. Acetaminophen (paracetamol, N-acetyl-p-aminophenol) by means of CYP450-interceded N-hydroxylation used to N-acetyl-pbenzoquinoneimine (NAPQI) [3]. N-acetyl-pbenzoquinoneimine (NAPQI), an exceptionally harmful, receptive metabolite of acetaminophen, which causes oxidative pressure and glutathione (GSH) exhaustion assumes a key part in portion subordinate hepatotoxicity [4]. N-acetylL-cysteine (NAC) is a sulfur-based amino corrosive and powerful cell reinforcement demonstrated compelling as a cure for hepatotoxicity because of acetaminophen glut [5]. NAC goes about as a forerunner for GSH combination and was demonstrated to be valuable against receptive oxygen species (ROS) age, mitochondrial dysfunctions and in mitochondrial reliant and free apoptotic cell passing in disease [6].

Sebestiana chamelea is has a place with the Euphorbiaceae family. Monoecious, erect to rambling yearly to lasting spice or bush up to 0.5(- 1) m tall with thin stems. Leaves substitute, straightforward, practically sessile; stipules praise, little; sharp edge direct lanceolate, 3-6 cm × c. 8 mm, base cuneate, summit heartless, edges finely toothed, short-bushy underneath. a little, terminal or leaf-went against spike, most blossoms male with 1-2 female blossoms at base; bracts with 2 huge organs at base. Blossoms unisexual, normal, sessile, sepals 3, praise, greenish yellow, petals missing, plate missing; male blossoms with 3 free, in no time exserted stamens, predominantly in South America; 4 species happen in tropical Africa. Microstachys was previously remembered for Sebastiania, which currently contains around 75 species in the New World jungles. Fundamental phytochemical screening of the concentrates uncovered the presence of Phenols, Flavonoids, Tannins, Steroids as primary constituents alongside Glycosides, Alkaloids, Lignins and Saponins. Presence of Phenolic intensifies upholds its antimicrobial movement and furthermore the home grown use against the runs. Bioassay directed fractionation of watery concentrate of these plants empowered the disengagement and ID of ellagic corrosive as the fundamental compound answerable for their antiplasmodial action. Along with ellagic corrosive, different subsidiaries having a place with various compound gatherings were separated however showed moderate antimalarial movement; gallic corrosive, brevifolin carboxylic corrosive, protocatechuic corrosive, corillogin, rutin and 3,4,8,9,10-pentahydroxy-dibenzo (b,d) pyran6-one. It showing Antibacterial action, Antifungal movement, Antioxidant action, Anthelmintic action, Antidiarrhoeal action, Antidiabetic action [7, 8].

The writing audit demonstrated that plant of Sebestiana chamelea for the most part rich wellsprings of cancer prevention agents and thus might be viewed as great potential for hepatoprotective action. From the writing survey plainly, no logical work has so far been done on the stem bark of Sebestiana chamelea for hepatoprotective movement. The primary point and targets are assessment of the phytochemical screening of different concentrates of leaves of Sebestiana chamelea. Assessment of the hepatoprotective action of different concentrates of leaves of Sebestiana chamelea in Carbon tetrachloride prompted hepatotoxicity in rodents.

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 the flask was added 0.30 ml of 5% $NaNO_2$, after 5min 0.3 ml of 10 % $AlCl_3$ 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. 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** Negative control	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** Positive control	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 4ºC, the serum samples were maintained at -80º 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 -80ºC 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	+	+	-

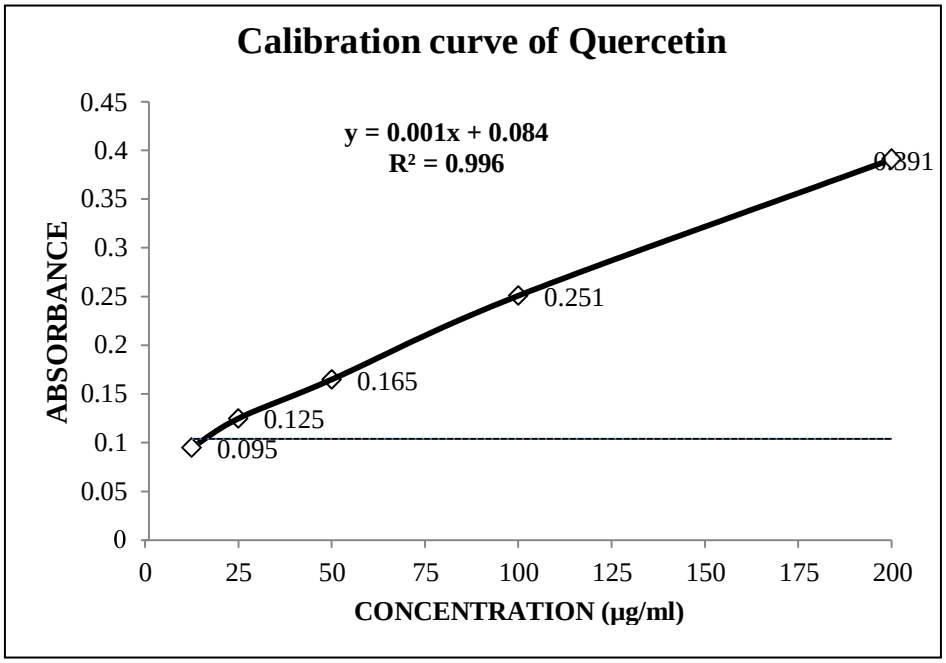
(+ Present) **(- absent)**

DETERMINATION OF TOTAL FLAVONOID CONTENT

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

S. No	Concentration ofQuercetin (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*.

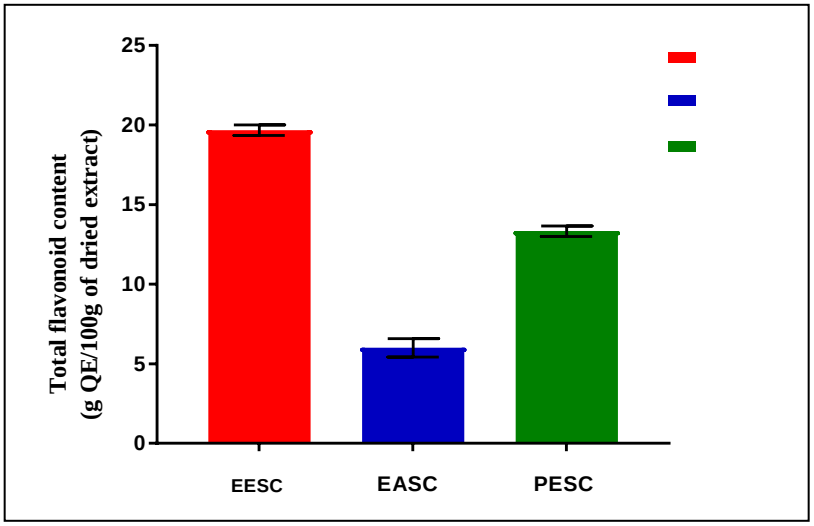


Table.4: Practical yield of *Sebestiana chamelea*

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

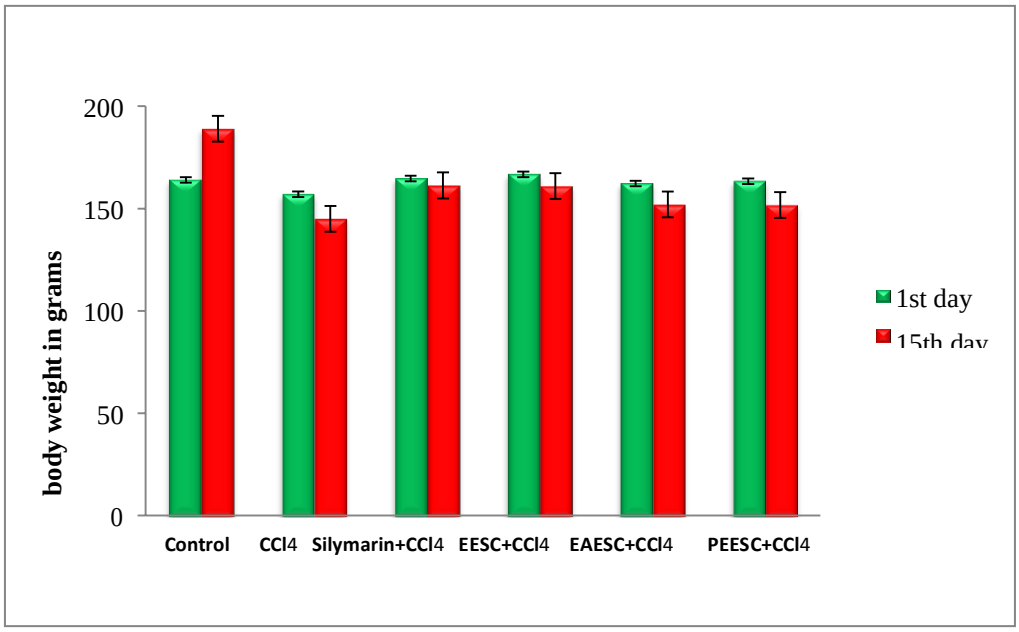
The body weight of the animals was determined on 1st and 15th day of the studyperiod and these are tabulated in **Table-5 and Fig.3**

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

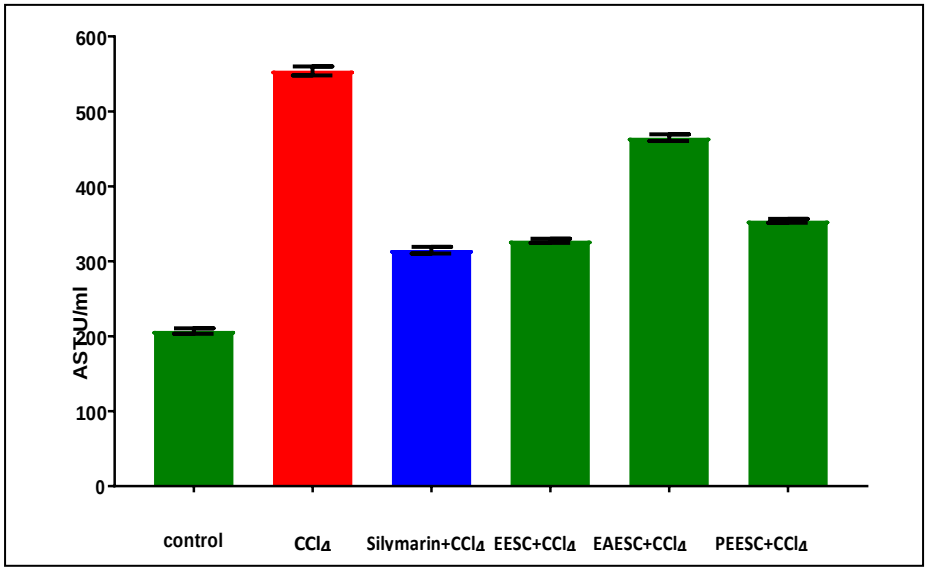
GROUP	TREATMENT	AST (U/ml)
I	Control	207±3.57***
II	Disease control	554±5.96***
III	Silymarin (25 mg/kg)	314.7±4.43***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	327.3±4.43***
V	Ethyl acetate extract of *Sebestiana chamelea* (400 mg/kg)	464.7±4.55***
VI	Pet. ether extract of *Sebestiana 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

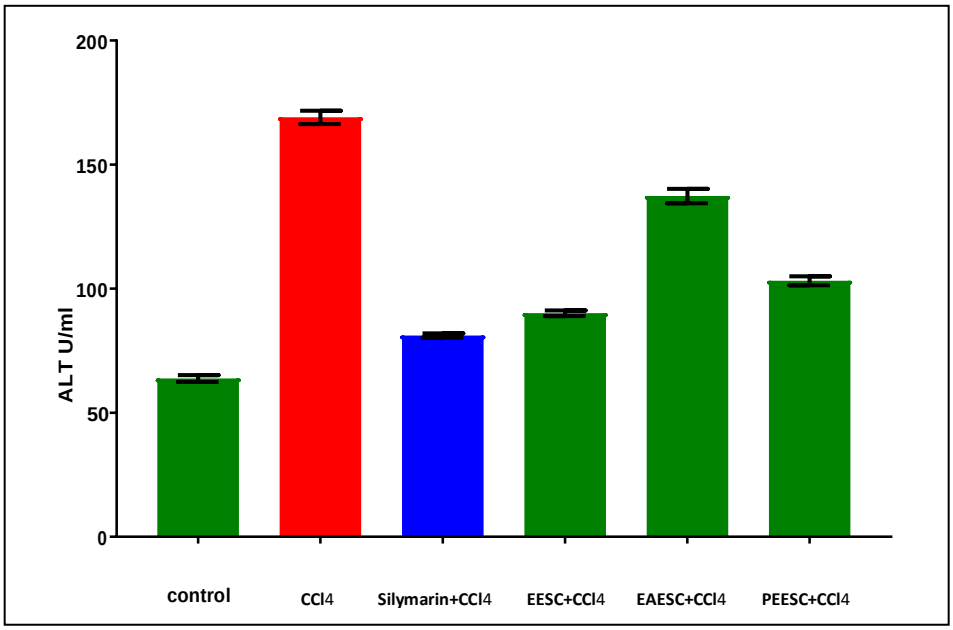
GROUP	TREATMENT	ALT (U/ml)
I	Control	63.83±1.38***
II	Disease control	169.1±2.69***
III	Silymarin (25 mg/kg)	81.13±0.85***
IV	Ethanolic extract of Sebestiana chamelea Lam (400 mg/kg)	90.16±1.14***
V	Ethyl acetate extract of *Sebestiana chamelea*(400 mg/kg)	137.4±2.89***
VI	Pet. ether extract of *Sebestiana 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

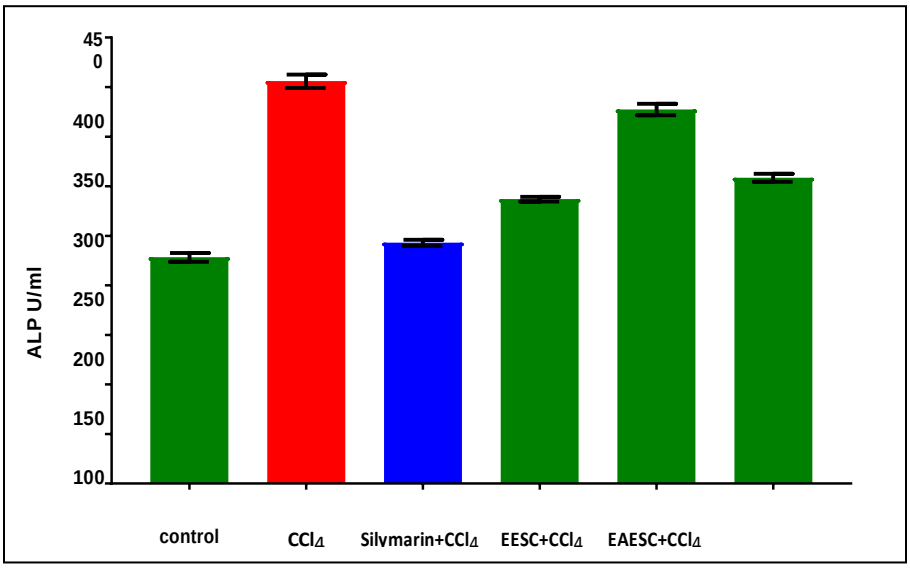
GROUP	TREATMENT	ALP (U/ml)
I	Control	228.2±4.32***
II	Disease control	405.9±6.76***
III	Silymarin (25 mg/kg)	242.8±3.18***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	286.9±2.41***
V	Ethyl acetate extract of*Sebestiana chamelea* (400 mg/kg)	377.4±5.75#
VI	Pet. ether extract of *Sebestiana 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

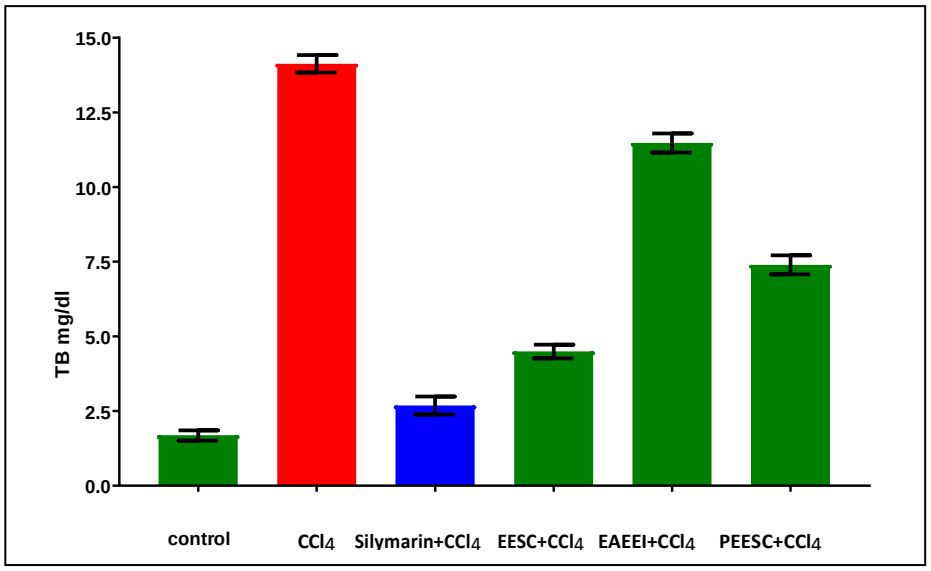
GROUP	TREATMENT	TB (mg/dl)
I	Control	1.68±0.17***
II	Disease control	14.12±0.29***
III	Silymarin (25 mg/kg)	2.68±0.30***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	4.49±0.23***
V	Ethyl acetate extract of *Sebestiana chamelea*(400 mg/kg)	11.47±0.32##
VI	Pet. ether extract of *Sebestiana chamelea*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

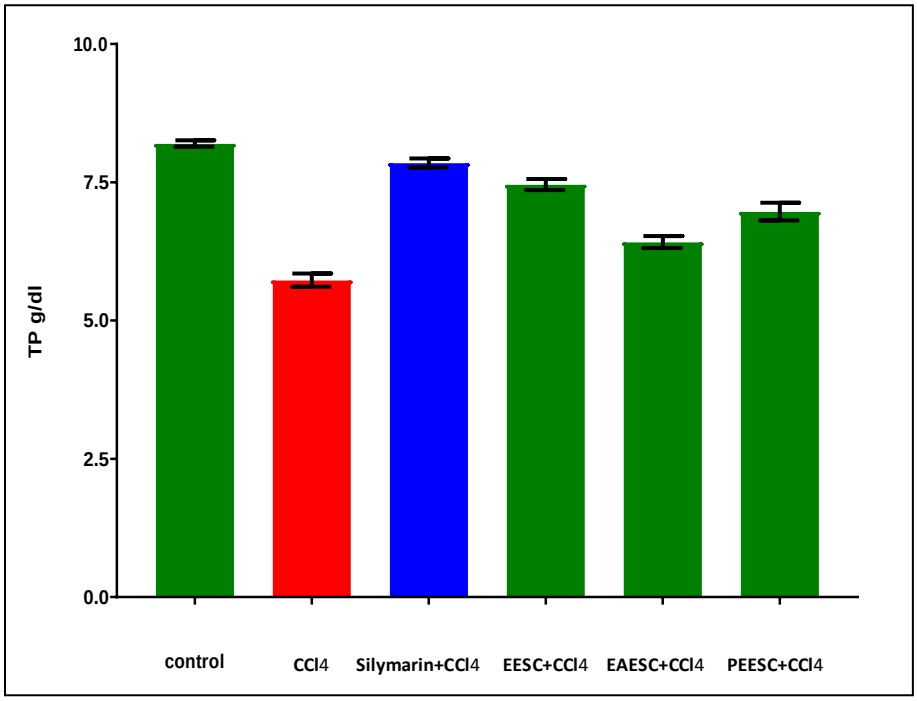
GROUP	TREATMENT	TP (g/dl)
I	Control	8.20±0.06***
II	Disease control	5.73±0.12***
III	Silymarin (25 mg/kg)	7.85±0.08***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	7.46±0.1***
V	Ethyl acetate extract of *Sebestiana chamelea*(400 mg/kg)	6.42±0.11#
VI	Pet. ether extract of *Sebestiana chamelea* (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

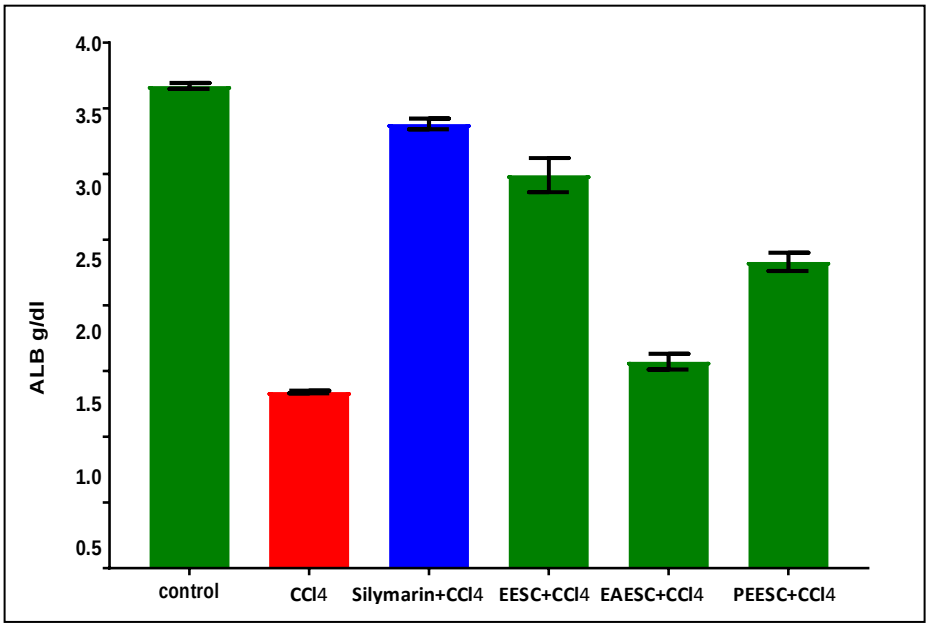
GROUP	TREATMENT	Albumin (g/dl)
I	Control	3.67±0.02***
II	Disease control	1.34±0.01***
III	Silymarin (25 mg/kg)	3.38±0.04***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	2.99±0.13***
V	Ethyl acetate extract of *Sebestiana chamelea* (400 mg/kg)	1.57±0.06#
VI	Pet. ether extract of *Sebestiana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

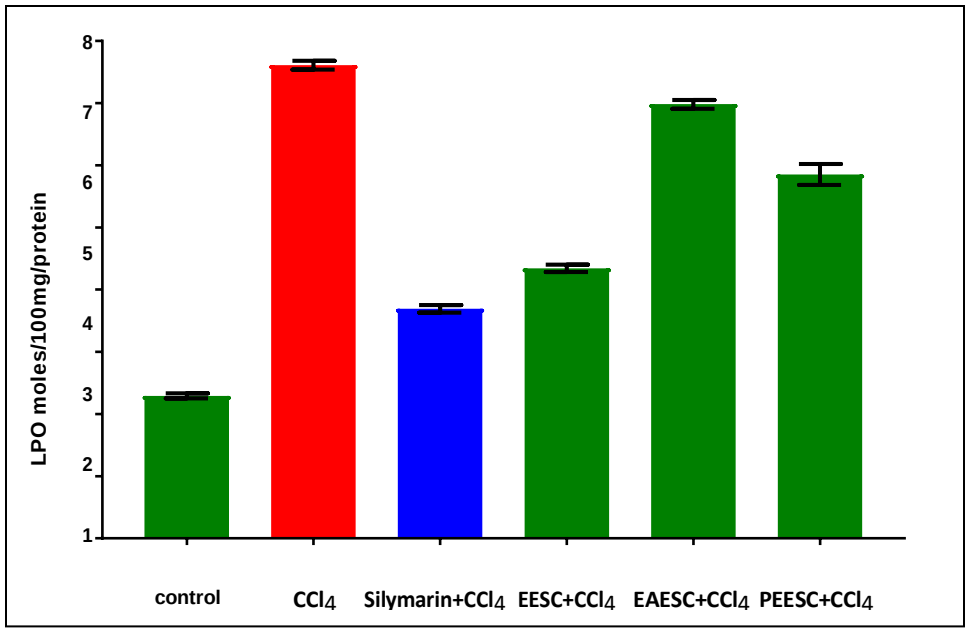
GROUP	TREATMENT	LPO (moles/100mg/protein)
I	Control	2.29±0.04
II	Disease control	7.61±0.07***
III	Silymarin (25 mg/kg)	3.69±0.06***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	4.34±0.06***
V	Ethyl acetate extract of *Sebestiana chamelea* (400 mg/kg)	6.98±0.07##
VI	Pet. ether extract of *Sebestiana 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 increasedsignificantly 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 ascompare 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

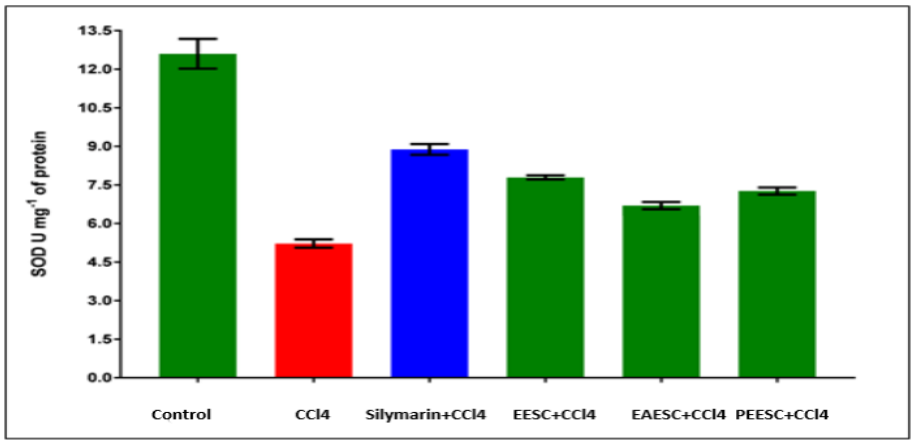
GROUP	TREATMENT	SOD (U mg^{-1} of protein)
I	Control	$12.6\pm0.58^{***}$
II	Disease control	$5.22\pm0.16^{***}$
III	Silymarin (25 mg/kg)	$8.88\pm0.21^{***}$
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	$7.79\pm0.08^{***}$
V	Ethyl acetate extract of *Sebestiana chamelea*(400 mg/kg)	$6.40\pm0.1^{\#\#}$
VI	Pet. ether extract of *Sebestiana chamelea* (400 mg/kg)	$7.26\pm0.14^{***}$

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

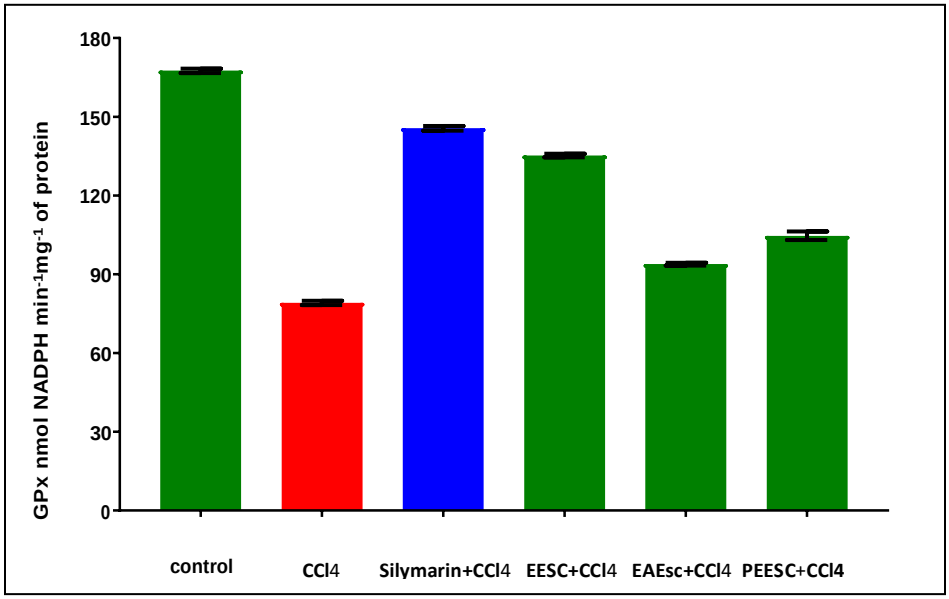
GROUP	TREATMENT	GPx (nmol NADPH $min^{-1}mg^{-1}$ of protein)
I	Control	167.5±0.83***
II	Disease control	79.1±0.81***
III	Silymarin (25 mg/kg)	145.6±0.91***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	135.2±0.69***
V	Ethyl acetate extract of *Sebestiana chamelea* (400 mg/kg)	93.85±0.50***
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

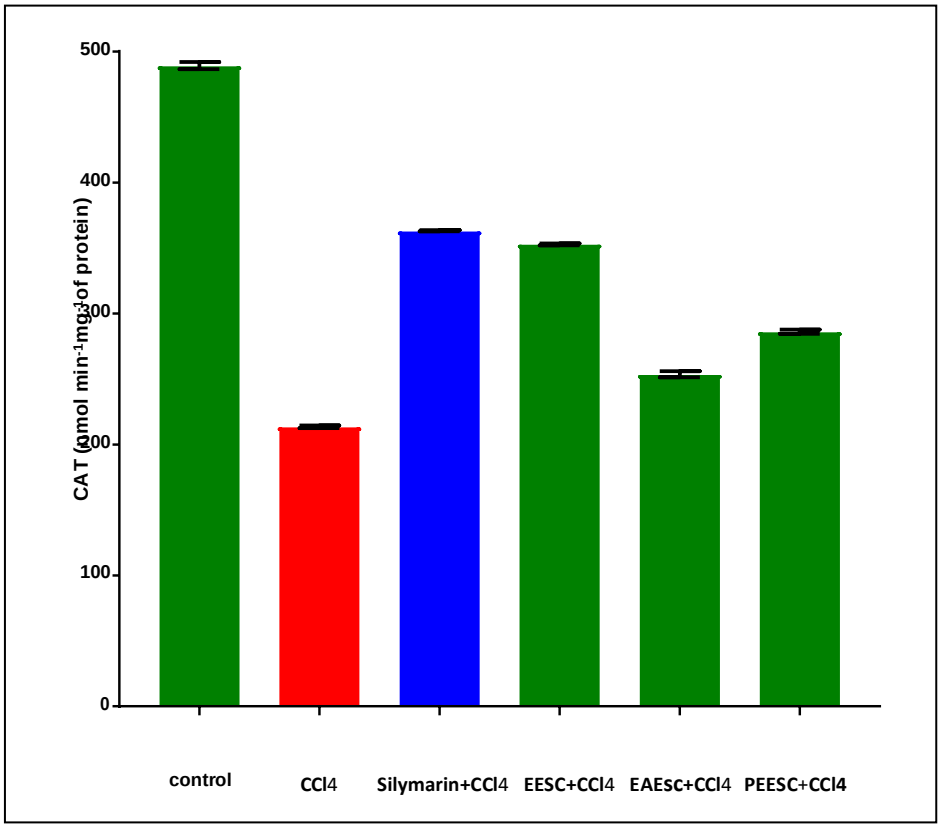
GROUP	TREATMENT	CATALASE (nmol min^{-1} mg^{-1} ofprotein)
I	Control	489.2±2.75***
II	Disease control	213.6±0.99***
III	Silymarin (25 mg/kg)	363.1±0.54***
IV	Ethanolic extract of Sebestiana chamelea (400 mg/kg)	352.8±0.76***
V	Ethyl acetate extract of *Sebestiana chamelea*(400 mg/kg)	253.6±2.38***
VI	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

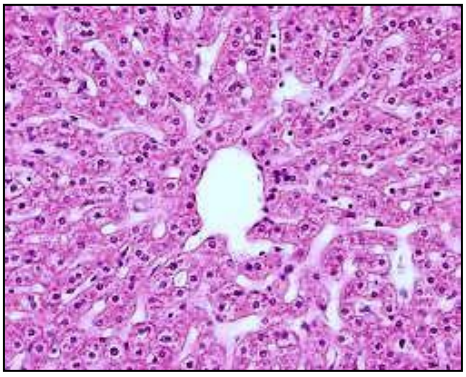


the **Table-15** and **Fig.13:** It was seen that CAT enzyme level 213.6±0.09 had decreased significantly in animals which were given CCl_4 as compared to normal group 489.2±2.75. Treatment with Silymarin showed a significant increase in the level of CAT 363.1±0.54 as compare to the disease control. 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.

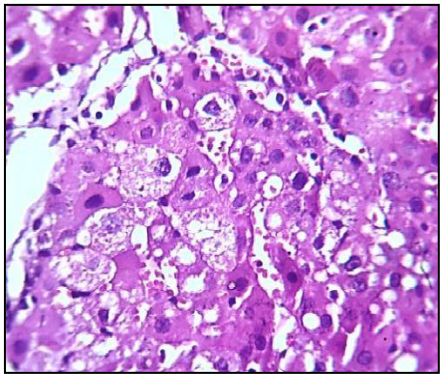
HISTOPATHOLOGICAL STUDY

A part liver tissue subjected to histopathological evaluation.

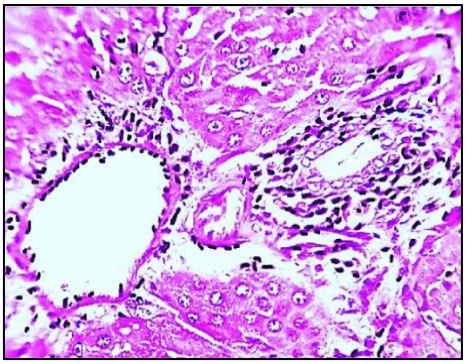
Fig. 14: Histopathological studies of liver



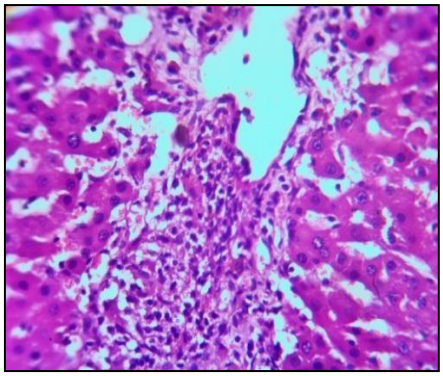
1) Normal control



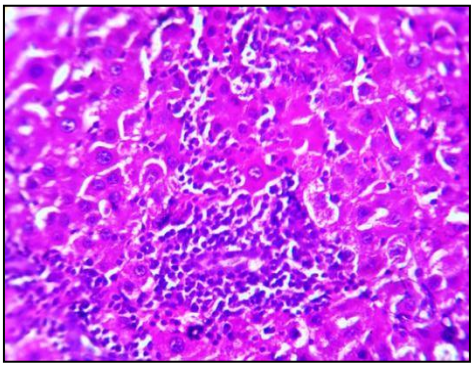
2) CCl_4 treated



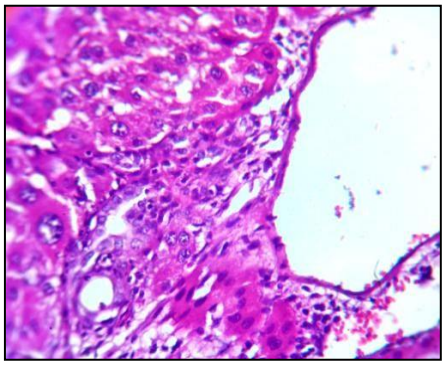
3) CCl_4 + Silymarin 25 mg/kg



4) CCl_4 + EESC 400 mg/kg



5) CCl_4 + EAESC 400 mg/kg



6) CCl_4 + PEESC 400 mg/kg

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 a significant organ associated with digestion of numerous xenobiotics. It eliminates poisons from the body. It is likewise presented to a few medications and xenobiotics which cause hepatic harm. In the current review hepatoprotective movement on the different concentrates of stem bark of Sebestiana chamelea was assessed.

The powdered plant material was separated by hot ceaseless soxhlet extraction technique and the plant material was extricated with Ethanol (99.9% v/v), Ethyl acetic acid derivation and Petroleum ether for four days in a soxhlet device. The pragmatic yield of ethanolic separate showed 10.04% w/w, ethyl acetic acid derivation remove showed 4.2% w/w and petrol ether extricate showed 2.6% w/w.

In phytochemical assessment of ethanolic concentrate of stem bark of Sebestiana chamelea showed the presence of flavonoids, tannins, saponins, terpenoids, phytosterol, protein, carb and alkaloids. Ethyl acetic acid derivation concentrate of stem bark of Sebestiana chamelea showed the presence of flavonoids, tannins, saponins, terpenoids, phytosterol, protein, carb and alkaloids. Petrol ether concentrate of stem bark of Sebestiana chamelea showed the presence of flavonoids, phytosterol and protein.

It was seen that ethanolic concentrate of stem bark of Sebestiana chamelea showed expanded complete flavonoid content when contrasted with different concentrates. The saw in vivo cell reinforcement and hepatoprotective movement for this concentrate accordingly might be because of the presence of flavonoids46,47. The intense poisonousness test recommended that the rough concentrates of the plant was non-harmful to rodent upto the portion 4000 mg/kg.

CCl4-actuated hepatic injury is a trial model generally utilized for the screening of hepatoprotective medications. CCl4 goes through a biotransformation by hepatic microsomal cytochrome P-450 to create trichloromethyl free revolutionaries. This hepatotoxic metabolite can respond with protein and lipid in the film of cells or organelles prompting corruption of hepatocytes. Because of hepatic injury, porousness of the cell film is adjusted causing the cytosolic transaminase (ALT, AST), in the dissemination. Subsequently assessment of AST and ALT are unequivocal marks of hepatoprotective movement. The ascent in the serum levels of ALP, AST, ALT and bilirubin as seen in the current review could be credited to the harmed primary uprightness of the liver. Liver harm is likewise connected with raised degrees of ALT, and Bilirubin. It is additionally connected with decline in degrees of Total Protein and Albumin.48

It was seen that AST, ALT, ALP and bilirubin levels had expanded fundamentally in creatures which were given CCl4 when contrasted with ordinary gathering. Treatment with Silymarin showed a huge decline in the degree of AST, ALT, ALP and bilirubin. The concentrates of Sebestiana chamelea treated gatherings of creatures likewise showed a critical lessening in the degree of AST, ALT, ALP and bilirubin. The decrease was more in the gathering treated with the Ethanolic concentrates of Sebestiana chamelea , when contrast with different concentrates treated gatherings.

Absolute Protein, Albumin levels had diminished essentially in creatures which were given CCl4 when contrasted with typical gathering. Treatment with Silymarin showed a critical expansion in the degree of Total Protein, Albumin. The concentrates of Sebestiana chamelea treated gatherings of creatures likewise showed a critical expansion in the degree of Total Protein, Albumin. The rise was more in the gathering treated with the ethanolic concentrates of Sebestiana chamelea , when contrast with different concentrates treated gatherings.

The organization of different concentrates of stem bark of Sebestiana chamelea showed improvement in the biochemical boundaries profile of the creatures.

The impact was seen with ethanolic concentrate of stem bark of E. indica is practically equivalent to that of the standard medication Silymarin. These assessment studies affirm the hepatoprotective capability of different concentrates of stem bark of Sebestiana chamelea.

It has been estimated that one of the standard reasons for CCl4-actuated liver injury is development of lipid peroxidases by free extreme subsidiaries of CCl4 (CCl3-). In this manner, the cancer prevention agent action or the restraint of the age of free extremists is significant in the assurance against CCl4-actuated hepatotoxicity.

Lipid peroxidation has been embroiled in the pathogenesis of hepatic injury by intensifies like CCl4 and is answerable for the cell film modification. In the current review, raised degree of LPO saw in CCl4 regulated rodents showed unnecessary development of free revolutionaries and actuation of LPO framework bringing about hepatic damage81. It was seen that the LPO levels had expanded fundamentally in creatures which were given CCl4 when contrasted with ordinary gathering. Treatment with Silymarin showed a critical reduction in the degree of LPO. The different concentrates of stem bark of Sebestiana chamelea treated gatherings of the creatures likewise showed a huge reduction in the degrees of LPO. The decrease was more in the gathering treated with the ethanolic concentrates of Sebestiana chamelea , when contrast with different concentrates treated gatherings. Consequently, it is conceivable that the system of hepatoprotection of Sebestiana chamelea may be because of its cell reinforcement activity.

The body has a powerful safeguard instrument to forestall and kill the free extremist actuated harm. This is achieved by a bunch of endogenous cell reinforcement chemicals like SOD, Glutathione peroxidase and Catalase. Grass has been accounted for as one of the main catalyst in the enzymatic cancer prevention agent guard framework. It rummages the superoxide anion to frame hydrogen peroxide and hence reducing the harmful impact brought about by this revolutionary. Decline in enzymatic action of superoxide dismutase (SOD) is a touchy record in hepatocellular harm and is the most delicate enzymatic file in liver injury83. It was seen that Superoxide dismutase catalyst levels had diminished fundamentally in creatures which were given CCl4 when contrasted with typical gathering. Treatment with Silymarin showed a huge expansion in the degree of Superoxide dismutase. The different concentrates of stem bark of Sebestiana chamelea causes a huge expansion in hepatic SOD level demonstrating a decrease of responsive free revolutionary prompted oxidative harm to liver. The height was more in the gathering treated with the ethanolic concentrates of Sebestiana chamelea , when contrast with different concentrates treated gatherings.

Glutathione is one of the most plentiful tripeptide, non-enzymatic organic cell reinforcement present in the liver. The biochemical capability of GPx is to lessen lipid hydroperoxides to their comparing alcohols and to diminish free hydrogen peroxide to water84,85. It was seen that Glutathione peroxidase chemical levels had diminished altogether in creatures which were given CCl4 when contrasted with ordinary gathering. Treatment with Silymarin showed a huge expansion in the degrees of Glutathione peroxidase. The different concentrates of stem bark of Sebestiana chamelea causes a critical expansion in hepatic GPx level demonstrating a decrease of receptive free extremist prompted oxidative harm to liver. The rise was more in the gathering treated with the ethanolic concentrates of Sebestiana chamelea , when contrast with different concentrates treated gatherings.

Catalase (CAT) is an enzymatic cancer prevention agent broadly disseminated in every single creature tissue, and the most elevated movement is tracked down in the red cells and liver. It catalyzes the deterioration of hydrogen peroxide to water and oxygen. It is a vital protein in shielding the cell from oxidative harm by receptive oxygen species (ROS) and safeguards the tissues from profoundly responsive hydroxyl radicals49. Hence decrease in the degree of CAT might bring about various pernicious impacts because of the absorption of superoxide extremist and hydrogen peroxide50,51. It was seen that catalase catalyst levels had diminished altogether in creatures which were given CCl4 when contrasted with ordinary gathering. Treatment with Silymarin showed a critical expansion in the degrees of catalase. The different concentrates of stem bark of Sebestiana chamelea causes a critical expansion in hepatic Catalase level showing a decrease of responsive free revolutionary prompted oxidative harm to liver. The rise was more in the gathering treated with the ethanolic concentrates of Sebestiana chamelea , when contrast with different concentrates treated gatherings.

From the benchmark group, Liver area showing ordinary histological appearance. CCl4 initiated gathering of Liver segment showed diffuse areas of vacuolar degeneration, lobular irritation, entrance to gateway fibrosis and centrilobular putrefaction with mononuclear cell invasion. The liver segment of

standard medication silymarin treated bunch was showing gentle hepatocyte vacuolation. The liver part of CCl4 alongside ethanolic concentrate of Sebestiana chamelea 400 mg/kg: treated bunch was showing gentle vacuolar degeneration and gentle hepatocyte enlarging. The liver segment of CCl4 alongside ethyl acetic acid derivation concentrate of Sebestiana chamelea 400 mg/kg treated bunch has shown vacuolar degeneration, lymphocyte present in gateway lot and mononuclear cell penetration in parenchyma and entrance regions. The liver segment of CCl4 alongside pet ether concentrate of Sebestiana chamelea 400 mg/kg treated bunch has shown gentle vacuolar degeneration and gentle hepatocyte enlarging.

Histopathological liver segments likewise uncovered that the hepatic engineering was modified by hepatotoxin in Carbon tetrachloride bunch, though in the liver areas of the rodent treated with the different stem bark concentrates of Sebestiana chamelea and inebriated with CCl4, the hepatic design was not changed and was tantamount with the standard medication Silymarin. The histopathological study affirms the critical hepatoprotective impact of ethanolic concentrate of stem bark of Sebestiana chamelea.

CONCLUSION:

The various concentrates of the leaves of Sebestiana chamelea had promising hepatoprotective movement against CCl4 instigated hepatic harm. The hepatoprotective action of Sebestiana chamelea is found on a mission to be more in ethanolic extract.The action could be because of the improvement in the cell reinforcement catalyst level and a diminishing in free extreme levels. The presence of phytochemicals, for example, flavonoids has been demonstrated to be liable for hepatoprotective activity. Further studies can be completed in the future to explain the system of activity of the ethanolic concentrate of leaves of Sebestiana chamelea, which may then be followed and clinical examinations to lay out its adequacy in people**.**

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