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Comparative Evaluation of Hepatoprotective Potential of roots of Blue and white flowered varieties of *Clitoria ternatea* Linn

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ABSTRACT

Pet Ether, Chloroform, and Methanol extracts of roots of Blue and White flowered varieties of Clitoria ternatea (CT) were studied for their hepatoprotective potential against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. The hepatoprotective activity was assessed using various biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP) and total bilirubin along with histopathological studies of liver tissues. The substantially elevated serum enzymatic levels of serum transaminases, alkaline phosphatase and total bilirubin were significantly restored towards normalization with the treatment of CT. The biochemical observations were supplemented with histopathological examination of liver sections. Liv-52 was used as standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. The results of this study strongly indicate that, methanolic extracts of blue and white flowered varieties of CT have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. Methanol extract of white flowered variety (MEWFV) effectively control SGOT, SGPT and ALP as compared to methanol extract of blue flowered variety (MEBFV). MEWFV of CT showed more significant hepatoprotective activity as compared to MEBFV of CT. This study suggests that possible mechanism of this activity may be due to free radical-scavenging and antioxidant activity, which may be due to the presence of phenolic compounds in the extracts.

Keywords: - *Clitoria ternatea*, Hepatoprotective, CCL_4, Antioxidant, Histopathology.

INTRODUCTION

Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney and liver disease, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders, inflammation and aging [1]. Liver damage is a widespread pathology which in most

cases involves oxidative stress and is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Various xenobiotics are known to cause hepatotoxicity one among them is carbon tetrachloride (CCl_4). Steroids, vaccines and antiviral drugs have been used as therapies for liver pathologies; have potential adverse side-effects, especially if administered chronically or sub-chronically. In the absence of reliable liver protective drugs in allopathic medicinal practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India [2].Therefore, herbal products and traditional medicines with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression. The use of antioxidants has been proposed as therapeutic agents, as well as drugs co-adjuvants, to counteract liver damage. A number of studies have shown that the plant extracts having antioxidant activity protect against CCl_4 hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity [3].

Clitoria ternatea L. (CT) a perennial twining herb, found throughout India in tropical areas. CT is commonly known as 'Butterfly pea' belongs to Family: Fabaceae. CT has two flowered varieties one is white flowered variety and second is blue flowered variety. CT has been traditionally used as a remedy for various disease like urinogenital disorder, bronchitis, purgative, diuretic, anthelmintic, rheumatism, demulcent, anticancer, antidote for animal stings [4, 5, 6, 7]. CT has been used as an ingredient in 'Medhya Rasayana' a rejuvenating recipe used for treatment of neurological disorders [8]. CT has been scientifically studied for various pharmacological activities like antioxidant [9,10], local anesthetic [11], anthelmintic [12,13], antipyretic, anti-inflammatory, analgesic [14], anxiolytic, antidepressant, anticonvulsant, sedative [15], hypoglycemic [16], anticancer [17] also enhances acetylcholine content in rat hippocampus [18]. Leaves of CT was studied for its hepatoprotective potential [19], still the comparative evaluation of hepatoprotective activity of roots of blue and white flowered varieties of CT has not been carried out. Aim of this study is to explore the hepatoprotective potential both varieties of *Clitoria ternatea* L.

MATERIALS AND METHODS

Plant material:- The roots of blue flowered variety and white flowered variety of *Clitoria ternatea* were collected from local habitat. The plant specimens were authenticated (Specimen No 9492, 9493) through Botany Department Rashtrasant Tukdoji Maharaj, Nagpur University, Nagpur.

Extraction of plant material :-

The roots of blue and white flowered varieties of *Clitoria ternatea* were cut into small pieces and dried at room temperature. The dried roots were subjected to size reduction to coarse powder by using dry grinder. This powder is packed into soxhlet apparatus and successively extracted with pet.ether ($60\text{-}80^0$c), chloroform, methanol [20]. The extracts were evaporated to dryness at 40^oC (White flowered variety yields 9%, 11%, 12% w/w respectively and Blue Flowered variety yields 9%, 11%, 10% w/w respectively). A phytochemical screening of residues revealed the presence of phenolic compounds, saponin, triterpennoid, sterol and protein [21, 22].

Animals:-
Healthy adult albino wistar rats weighing about 180-200gm were obtained from TVES HLMC College of Pharmacy, Faizpur. These animals were housed in polypropylene cages and fed on standard pellet diet and provided with water *ad libitum* during the experiment. The animals were housed under standard conditions (24-28^{o} c, relative humidity 60-70%, 12 h light and 12 h dark cycle). Ethical clearance for the handling of experimental animals was obtained from Institutional Animal Ethics Committee (Reg. No. 652 / 02 / a / CPCSEA).

Acute toxicity testing:-
Healthy Swiss albino mice of either sex, starved and divided into 5 groups. These animals orally fed the various extracts of CT in increasing dose level of 250, 500, 1000, 1500, 2000 mg/kg body weight. Mice were observed for 24 hours for any lethality [23, 24, 25]

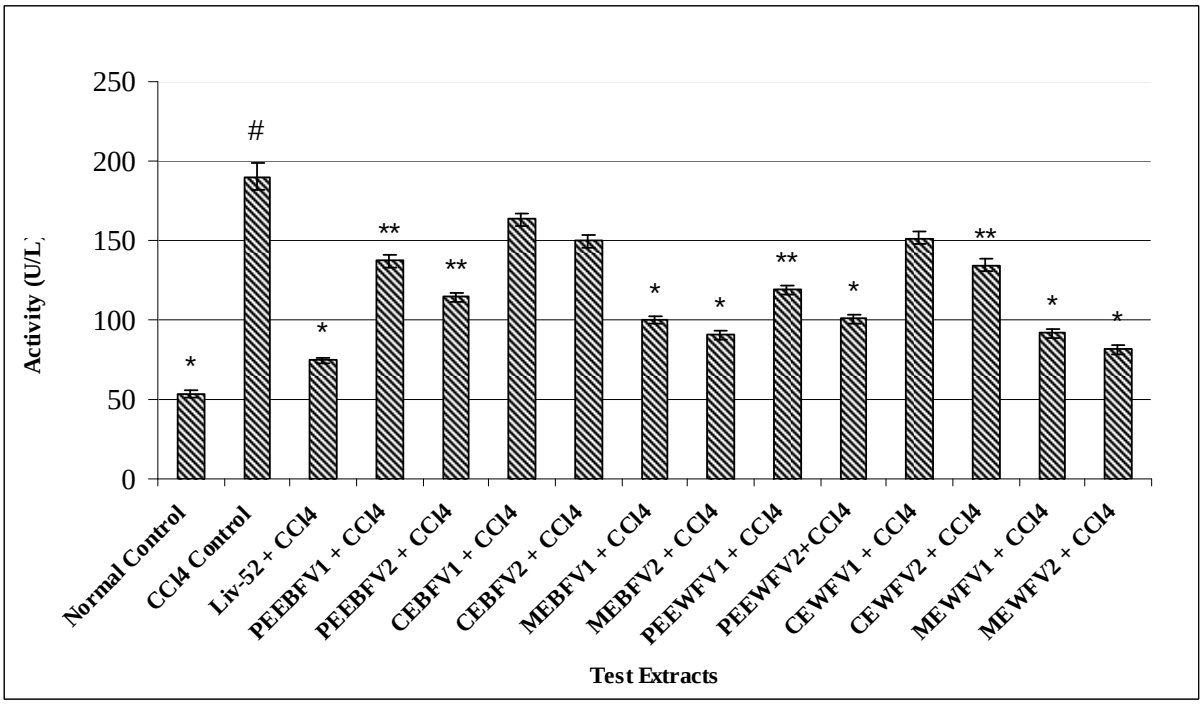
Evaluation of hepatoprotective activity:-
Animals were divided into following groups, each group consisting of six animals. Albino wistar rats were used for the study. and CCl4 was used to induce liver injury. Group I (Normal control) Received vehicle carboxymethyl cellulose (CMC-Na 0.3%, 1ml/kg orally) daily for 5 days and received olive oil (0.7 ml/kg, i.p.) on day 2 & 3.Group II (CCL_4 control) Received vehicle (CMC-Na 0.3%, 1ml/kg orally) daily for 5 days and received CCl4: olive oil (1:1, 0.7 ml/kg, i.p.) on day 2 & 3; 30 min. after administration of vehicle. Group III (Standard Control) Received standard drug Liv-52 (5ml/kg orally) daily for 5 days and received CCl4: olive oil (1:1, 0.7 ml/kg, i.p.) on day 2 & 3; 30 min. after administration of standard drug. From Group IV to Group XV received below mentioned doses of extracts daily for 5 days and received CCl4: olive oil (1:1, 0.7 ml/kg, i.p.) on day 2 & 3; 30 min. after administration of extracts. Group IV and V (Test groups) received pet ether extracts of root of blue flowered variety (PEEBFV) of CT (PEEBFV1-250mg/kg and PEEBFV2- 500mg/kg orally). Group VI and VII (Test groups): received chloroform extracts of root of blue flowered variety (CEBFV) of CT (CEBFV1-250mg/kg and CEBFV2-500mg/kg orally) Group VIII and IX (Test groups): received methanolic extracts of root of blue flowered variety (MEBFV) of CT (MEBFV1-250mg/kg and MEBFV2-500mg/kg orally). Group X and XI, received pet ether extracts of root of white flowered variety (PEEWFV) of CT (PEEWFV1- 250mg/kg and PEEWFV2-500mg/kg, orally). Group XII and XIII, received chloroform extracts of root of white flowered variety (CEWFV) of CT (CEWFV1-250mg/kg and CEWFV2- 500mg/kg orally). Group XIV and XV methanolic extracts of root of white flowered variety (MEWFV) of CT (MEWFV1-250mg/kg and MEWFV2 -500mg/kg, orally). On 6th day, animals were sacrificed under light anaesthetic ether. Blood was withdrawal from each rat through retro orbital plexus Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min for carrying out further biochemical investigations i.e. SGOT, SGPT, ALP and bilirubin estimation. Livers were dissected out and immediately transferred to 10% formalin for histopathological investigation.

Statistical Analysis:-
All values are expressed as mean ± SEM. The data obtained were statistically analyzed by One way analysis of variance (ANOVA) followed by Tukey`s multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

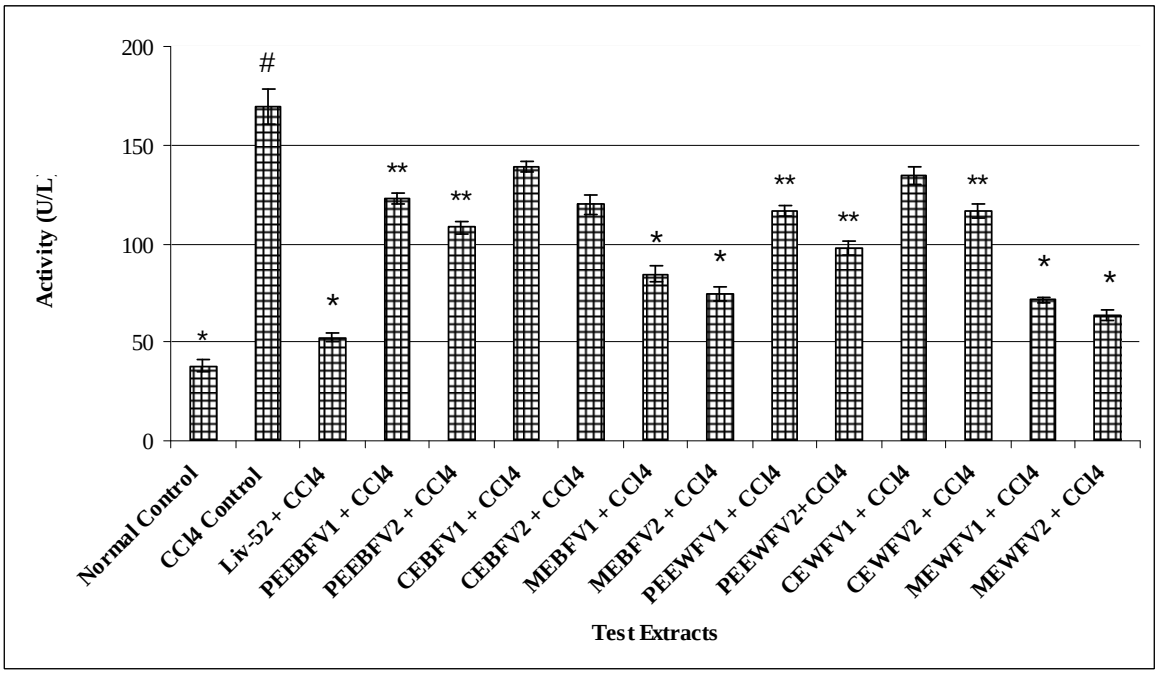
Acute toxicity study revealed the non toxic nature of extracts of *Clitoria ternatea.* There was no lethality or any toxic reactions found at any of the doses selected. A phytochemical screening of residues revealed the presence of phenolic compounds, tannins, triterpennoid, sterol, saponin and protein. Hepatotoxicity induced by CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver disease. $CC1_4$ produces an experimental damage that histologically resembles viral hepatitis [26]. The hepatotoxic effects of CCl_4 are largely due to generation of free radicals. CCl_4 is biotransformed by the cytochrome P450 system to produce the trichloromethyl free radicals, which in turn covalently bind to cell membranes and organelles to elicit lipid peroxidation. Further it has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of lipid peroxidation induced by CCl_4 [27].

Fig. No: 01 Effect of Various extracts of CT on CCl_4-induced elevation of SGOT levels in rat.



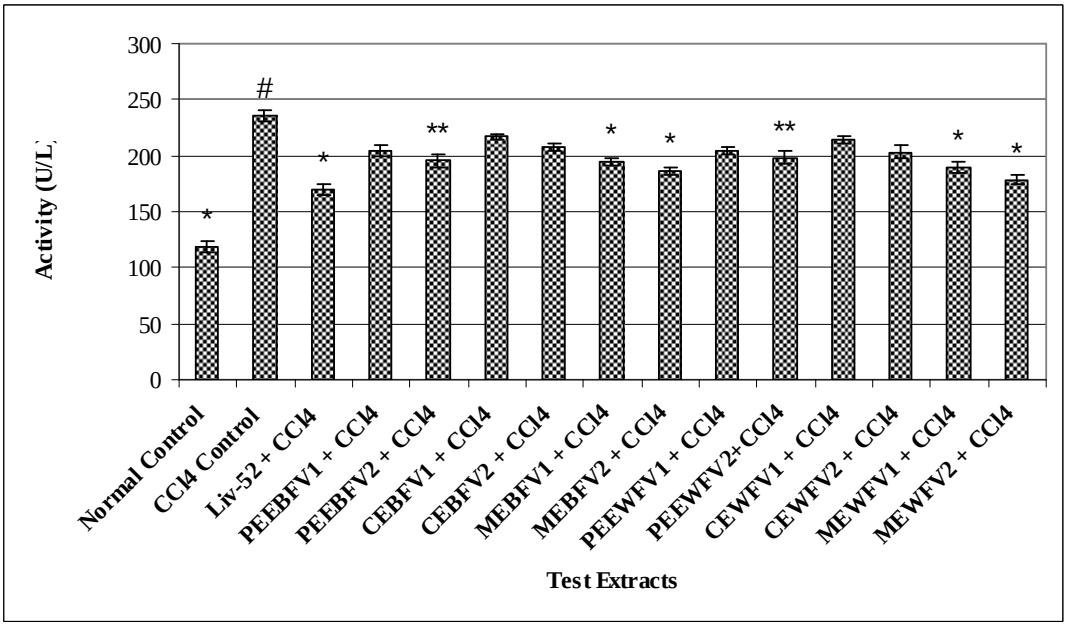
Values are expressed as a mean ±standard error of mean of 6 observations
Statistical test was performed using One way ANOVA and comparison done by Tukey test.
 # Represents statistical significance: $p < 0.001$, when compared with normal control, n = 6.
** Represents statistical significance: $p < 0.001$, when compared with CCl_4 control group, n = 6.*
*** Represents statistical significance: $p < 0.05$, when compared with CCl_4 control group, n = 6.*

Fig. No: 02 Effect of Various extracts of CT on CCl_4-induced elevation of SGPT levels in rat.



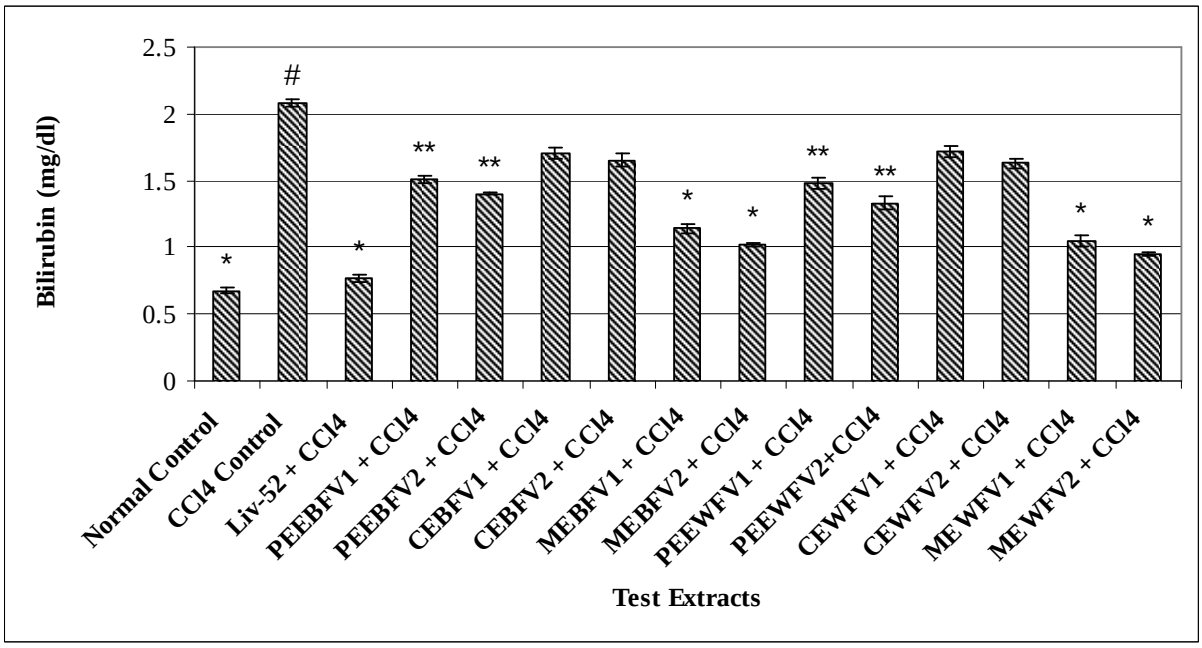
Values are expressed as a mean ±standard error of mean of 6 observations
Statistical test was performed using One way ANOVA and comparison done by Tukey test.
 # Represents statistical significance: p < 0.001, when compared with normal control, n = 6.
** Represents statistical significance: p < 0.001, when compared with CCl_4 control group, n = 6.*
*** Represents statistical significance: p < 0.05, when compared with CCl_4 control group, n = 6.*

Fig. No: 03 Effect of Various extracts of CT on CCl_4-induced elevation of ALP levels in rat.



Values are expressed as a mean ±standard error of mean of 6 observations
Statistical test was performed using One way ANOVA and comparison done by Tukey test.
 # Represents statistical significance: p < 0.001, when compared with normal control, n = 6.
** Represents statistical significance: p < 0.001, when compared with CCl_4 control group, n = 6.*
*** Represents statistical significance: p < 0.05, when compared with CCl_4 control group, n = 6.*

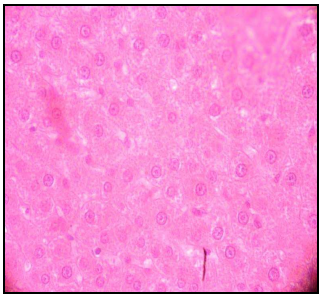
Fig. No: 04 Effect of Various extracts of CT on CCl_4-induced elevation of Total Bilirubin levels in rat



Values are expressed as a mean ± standard error of mean of 6 observations
Statistical test was performed using One way ANOVA and comparison done by Tukey test.
 # Represents statistical significance: $p < 0.001$, when compared with normal control, n = 6.
** Represents statistical significance: $p < 0.001$, when compared with CCl_4 control group, n = 6.*
*** Represents statistical significance: $p < 0.05$, when compared with CCl_4 control group, n = 6.*

Liver damage was assessed by biochemical studies (SGOT, SGPT, ALP and Total Bilirubin) and histopathological examinations. Due to the liver injury caused by the hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum bilirubin [27]. In this study significant (P<0.001) increased level of SGOT (Fig No.1), SGPT (Fig No.2),ALP (Fig No.3) and total Bilirubin content (Fig No.4) activities in the CCl_4 control group could be taken as an index of liver damage. Treatment with extracts of *Clitoria ternatea* showed inhibited CCl_4 induced increase in SGOT (Fig No.1), SGPT (Fig No.2),ALP (Fig No.3) and total Bilirubin content (Fig No.4) as compared with CCl_4 control group which is statistically significant (P<0.001). Treatment with methanolic extract of white flowered variety of CT at dose 250 and 500mg/kg b. w. were showed significant reduction in levels of SGOT and SGPT as compared to methanolic extract of blue flowered variety of CT. A high concentration of bilirubin in serum is an indication for increased erythrocyte degeneration rate. Methanolic extracts of roots of blue and white flowered varieties at dose 250 and 500mg/kg b. w. were showed significant (P<0.001) reduction in the serum TB level (Fig No.4). The decreased in the level of TB was found to be greater in standard drug followed by MEWFV and MEBFV of CT. The white flowered variety of CT showed much more reduction in TB level as compared to blue flowered variety of CT. In this view, the reduction in levels of SGOT and SGPT by the extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by $CC1_4$ which was further confirmed by the reduced amount of histopathological injuries.

Histopathological photomicrographs:-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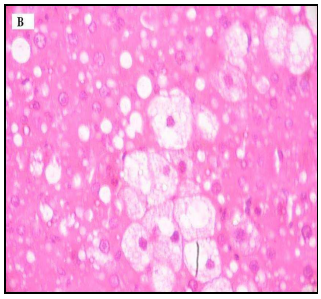
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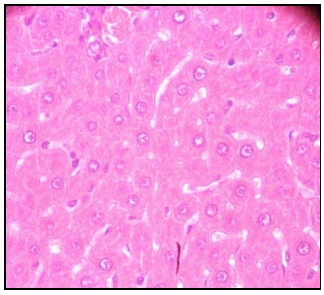
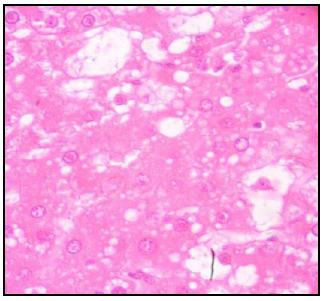
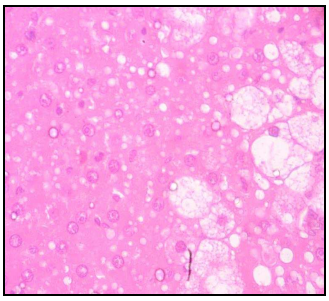

(C)


(D)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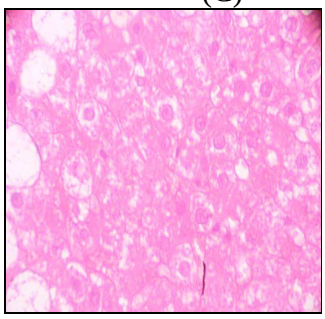
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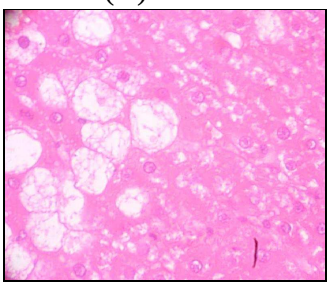
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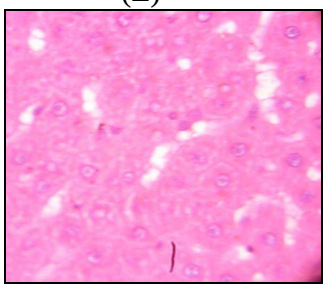
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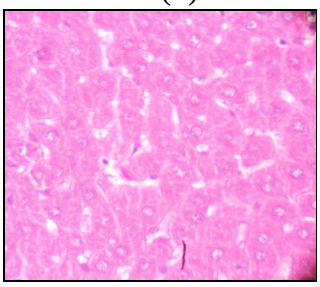
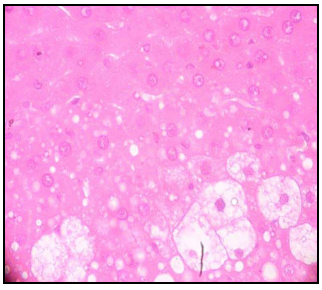
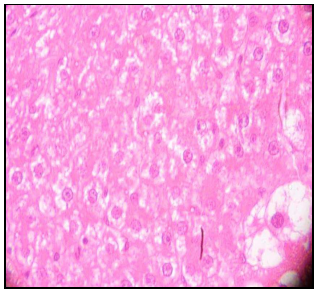

(I)


(J)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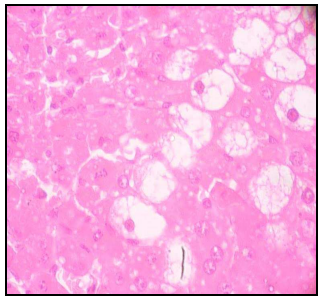
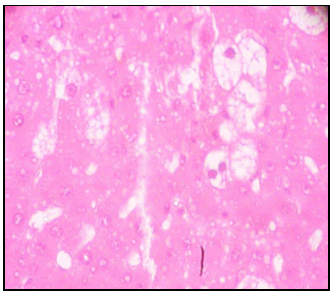
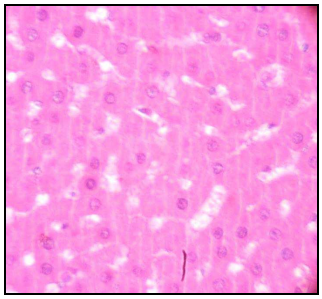
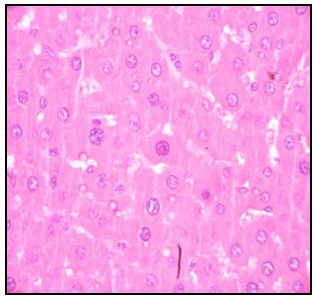

(L)



(M)



(N)



(O)

Photomicrograph: (A) Liver sections of normal control, (B) Liver section of CCl_4 treated rat, (C) Liver section of rats treated with Std Liv-52+CCl_4 (D) Liver section of rats treated with PEEBFV1 + CCl4, (E) Liver section of rats treated with PEEBFV2 + CCl_4, (F) Liver section of rats treated with CEBFV1 + CCl_4, (G) Liver section of rats treated with CEBFV2 + CCl_4, (H) Liver section of rats treated with MEBFV1 + CCl_4, (I) Liver section of rats treated with MEBFV2 + CCl_4, (J) Liver section of rats treated with PEEWFV1 + CCl_4, (K) Liver section of rats treated with PEEWFV2+CCl_4, (L) Liver section of rats treated with CEWFV1 + CCl_4, (M) Liver section of rats treated with CEWFV2 + CCl_4, (N) Liver section of rats treated with MEWFV1 + CCl_4, (O) Liver section of rats treated with MEWFV2 + CCl_4

Liver sections of normal control rats (A) showing: normal hepatic cells with well-preserved cytoplasm; prominent nucleus. (B) Liver section of CCl_4 treated rats showing: massive fatty changes, necrosis, vacuolation, degeneration of hepatic cells and broad infiltration of the lymphocytes and the loss of cellular boundaries. (C) Liver section of rats treated with CCl_4 and std drug showed no of hepatocyte, well-preserved cytoplasm which is comparable to normal control. (H and I) and (N and O) showed much more number of hepatocyte as compared to ccl_4 control. In histopathology, treatment with extract of White flowered variety of CT showed no of hepatocyte much more as compared to Blue flowered variety of CT.

The hepatprotective index of a drug can be evaluated by its capability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been induced by a hepatotoxin. CT was confirmed to have free radical scavenging and antioxidant activities in a concentration-dependent manner [10]. It appears, therefore, that the hepatoprotective activity of CT against CCl_4-induced hepatic damage is likely to be due to its antioxidant capacity and free radical scavenging activity. Additionally, the phenolic compounds are the constituents in this plant, which had been verified to be good antioxidants may play a significant role in the hepatoprotective effect.

CONCLUSION

The methanolic extract of *Clitoria ternatea* Linn. (MECT) could effectively control the AST, ALT, ALP and TB levels. The protective effect of MECT may be attributed due to improved defence of the hepatocytes against the reactive oxygen species. The histopathological studies also substantiate the activity of the drug. Therefore the study scientifically supports this plant may be useful in various Ayurvedic preparations and traditional medicine for treatment of liver disorders and as a tonic. Methanolic extract of white flowered variety of root of CT showed good hepatoprotective potential as compared to blue flowered variety of CT. In earlier reports, plants are found to have hepatoprotective activity due to presence of antioxidants, flavonoid, terpenoid, tannin and steroid nature. Hepatoprotective activity may be due to presence of

phenolic compounds and triterpenoids present in MECT. However, the exact components responsible for the Hepatoprotective activity of MECT are not clear. Therefore, further work is necessary to isolate and characterize those constituents responsible for hepatoprotective activity.

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