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EVALUTION OF HEPATOPROTECTATIVE ACTIVITY OF TERMINELLA CATTAPA LEAVES ON PARACETAMOL-INDUCED HEPATOTOXICITY IN WISTAR RATS

Amna Ansari [1] , S.M.Ghufran Saeed[1], Seema Ashraf*[1,2], Nasreen Begum[2] , Zafar Saeed Saify[2] , Mehreen Asghar B.[4] , S.Asad Sayeed[1],

[1]Department of Food Science and Technology,
[2]HEJ Research Institute of Chemistry,
[3]Drug Research Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi, 75270, Pakistan
[4] Department of pharmacy Government college university, Faisalabad.

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***Author for Correspondence**
Seema Ashraf
Department of Food Science and Technology, Karachi, 75270, Pakistan.

ABSTRACT

Purpose: Whole plant of Terminalia catappa has been used for the treatment of different diseases including cancer, skin infections, diabetes and liver disorder that prompted us to investigate the hepatoprotective effect of chloroform and methanol extract (CEIF and MEIF) of leave part of Terminalia catappa using paracetamol-induced liver damage in rats. **Methods**: The chloroform and methanolic extracts of Terminalia catappa (CEIF and MEIF) were studied for their hepatoprotective and antioxidant effects on paracetamol (750mg/kg) induced acute liver damage on Wistar albino rats. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein. **Results**: CEIF and MEIF at a dose level of 250mg/kg and 500mg/kg produce significant ($P<0.05$) hepato protection by decreasing the activity of serum enzymes, bilirubin, and lipid peroxidation, in a dose dependent manner. The effects of CEIF and MEIF were comparable to that of standard drug Silymarin. **Conclusion:** From this study, it can be concluded that the chloroform and methanol extract of hepatoprotective agent is not only an effective hepatoprotective agent, but also possesses significant ($p<0.05$) antioxidant activity.

KEYWORD: Terminalia catappa, hepatoprotective agent, Silymarin.

INTRODUCTION

Liver is considered as one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it is also handling the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver plays an important role in digestion. Widely used analgesic and antipyretic drug has known to cause hepatotoxicity in experimental animals and human at very high doses (Raj et al., 2009). The laboratory features of hepatotoxicity induced by APAP resemble other kind of acute inflammation of liver disorder with prominent increase in level of GOT, GPT and ALP.

Epidemiological and in vitro studies strongly convinced that food containing different antioxidant phytochemicals has showed potential protective effects against many diseases, such as cardiovascular, cancer and diabetes (Senevirathne et al., 2006). Antioxidants have capability to protect biomolecules against the attacks of free radicals. Free radicals are very reactive species having short life and can cause many severe diseases. The oxidative damage due to free radicals might be prevented or limited by dietary antioxidants (Dewanto et al., 2002). Dietary antioxidants include vitamins, carotenoids and phytochemicals. Therefore, daily intake of fruits and vegetables has been recommended. Phytochemicals, such as phenolic compounds, are considered beneficial for human health, as they decrease the risk of degenerative diseases by reduction of oxidative stress and inhibition of macro-molecular oxidation (Luo et al., 2002; Pereira et al., 2007; Pulido et al., 2000; Silva et al., 2004). Anthocyanin are the largest group of water soluble natural pigment that give blue, red and violet colour to many fruits, vegetables and cereals grain (Choi et al., 2007; Chatha et al.,2006). Anthocyanin has been shown to have some beneficial effects on oxidative damage, detoxification enzyme, and the immune system (Manach et al., 2005; Shin et al., 2006). Anthocyanin not only scavenge free radicals and binds heavy metals such as iron, copper and zinc, but also appear to have a synergist effect on Vitamin C and other flavonoids (Dewell, et al., 2002; Manach et al., 2005).

Terminalia catappa L. belongs to Combretace family widely distributed in tropical and subtropical beaches. It is a medium size tree and branches showing layers of canopy. The leaves, fruit, bark and trunk of the tree have been used as a folk medicine for the treatment of dermatitis, antipyretic and homeostatic purposes. Leaves and bark extract the plant have been

reported as anti-HIV reverse transcriptase (Tan et al., 1991), hepatoprotective (Lin et al., 1997), anticancer, antioxidant (Masuda et al., 1999), anti-inflammatory hepatitis (Chen et al., 2000) and aphrodisiac (Ratnasooriya and Dharmasiri, 2000). Thin Layer Chromatography (TLC) methods for identifying authentic fruit and leaf raw material and commercial products, were developed to reduce the cost and to get accurate analytical method.

Chemicals

All the kits of SGOT, SGPT, TP, BD, and ALP were used and for lipid profile L-glyceride, L-cholestrol, LDL, were purchased from Sigma Chemicals (St. Louis, MO, USA). All reagents used were of analytical grade.

Extraction and Isolation

Dried and crushed leaves of T. catappa supplied by a company (Nakazen Co., Ltd.) in Okinawa were extracted with hot water (90°C) for 1 h (1 g/10 ml). The extract was dried by a spray-dry method and was used as a crude extract for in vitro and in vivo experiments. Anti-oxidantive components from T. catappa were isolated and identified by similar method as reported previously (Aniya et al, 2002). Briefly, 50% ethanol extract of dried leaves was chromatographed on a HW-40F column. Fractions eluted from the column were subjected to a centrifugal partition chromatography. The liver marker such as GOT, GPT, TP, BD, ALP were used and for lipid profile L-glyceride, L-cholestrol, LDL, HDL.

Sample:

1) Chloroform extract Died leaves
2) Methanolic extract

Treatment of animals

Wistar rats (n _ 42), with a mean weight of 120-200 g at the beginning of the experiment, were provided by International Center for Chemical and Biological Sciences (ICCBS) University of Karachi. They were housed in individual metal cages in an air-conditioned room (temperature 21–22°C, relative humanity 55–65%).The rats were divided into SEVEN groups of 6 animals each. These groups were designated as Control, positive control, Standard drug, G4/ CERd 250 mg/kg/Bwt, and G5/ CERd 500 mg/kg/Bwt, G6/ MERd 250 mg/kg/Bwt and G7/ MERd 500 mg/kg/Bwt oral diet. During 14 days of the experiment all rats were fed a normal diet (ND), which included wheat starch, casein, olive oil, vitamin and mineral mixtures. In the Control group 6 rats were fed ND only. To the BD of the four other groups were mentioned in table 1. All rats were fed once a day at 10 AM, and drinking

water. Food intake and body gains were monitored daily. It is generally accepted that the most reliable data on blood lipid metabolism can be obtained from fasting animals, 14–16 hours after their last feeding. Therefore, food was removed from the cages at 6 PM 1 day before blood samples were drawn, and the samples were collected at 9AM the next day. Before the experiment the blood samples experiment the rats were fully anesthetized by an intraperitoneal injection of verbutal and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests.

Animal model study

Total number of Rats: 16 [7 days]

Groups	Basal Diet	Std Drug	APAP
Group 1 (NC)	Basal diet	saline 5 ml/kg/B wt.	-
Group 2 (PC)	Basal diet	saline 5 ml/kg/B wt.	750 mg/kg on 7th day
Group 3 (DC)	Basal diet	Silimarin 25 mg/kg/Bwt	750 mg/kg on 7th day
Group 4 (C E)	Basal diet	CERd 250 mg/kg/Bwt	750 mg/kg on 7th day
Group 5 (C E)	Basal diet	CERd 500 mg/kg/Bwt	750 mg/kg on 7th day
Group 6 (ME)	Basal diet	MERd 250 mg/kg/Bwt	750 mg/kg on 7th day
Group 7 (ME)	Basal diet	MERd 500 mg/kg/Bwt	750 mg/kg on 7th day

Note: From day 1 to 6 sample will be given, on 7th day APAP induce. After 18 hr all animals sacrificed.

RESULT AND DISCUSSION

Administration of sub toxic dose of paracetamol has been used for preparing animal model hepatic failure in which apoptosis of hepatocytes is the major culprit and is caspase dependent (Nakama et al., 2001; Gujral et al., 2003). .The hepatoprotective activity of methanolic and cholorofom extract of Terminalia catappa L. was studied against APAP-induced acute hepatotoxicity in rats. APAP module of acetaminophen (N-acetyl-P-aminophenol paracetamol) widely as used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and human at very high doses (Raj et al., 2009). The laboratory features of hepatotoxicity induced by APAP resemble other kind of acute inflammation of liver disorder with prominent increase in level of GOT, GPT and ALP. When (N-acetyl-P-benzo-quinone imine) NAPQI covently binds to cysteine group on proteins to form 3- cystein-S-yl acetaminophen adducts. In the living system liver is considered to be highly sensitive to any toxic agents. Therefore the glutathione protects hepatocytes by combining with the reactive metabolites of APAP for preventing their covalent bonding of the liver proteins. The resulting study of the different enzymes activities

such as SGOT, SGPT, SALP, TP, BD, HDL, L-cholesterol and triglycerides etc. have been found to be valuable in assessment of clinical (Gomes et al, 2001).

In the present study it was observed that animals treated with acetaminophen resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The increase in the SGOT is usually accompanied by an elevation in the level of SGPT which play a vital role in the conversion of amino acids to keto acids (Gomes et al, 2001; Marina et al., 2009) as showed in figure no.1 to 2.

The entire sample reduced significantly the level of serum markers as compared to the positive group. The normalization of serum markers by samples suggests that they are able to condition the hepato cytes as to protect the cell membrane integrity against the acetaminophen induced leakages of marker enzymes in to the circulation. Changes can be considered as an expression of the functional improvement of hepatocytes which may be caused by an accelerated regeneration of pharenchyma cells. The other serum enzymes ALP, Bilirubin direct and total protein related to hepatic cell damage. Their increase in serum level due to elevated synthesis in presence of billiary pressure as shown in figure 1, 2 and 3. The most significant control of bilirubin direct level and alkaline phospahtase activities towards an early improvement in the secretory mechanism of the hepatic cell.

The lipid per oxidation has been leads to destructive process of liver injury due to acetaminophen administration. The result of total cholesterol, LDL-Cholesterol, HDL-cholesterol and T-glyceride as shown in figure no 4 to 8 attenuated elevated level of the serum cholesterol markers due to excellent profile of antioxidants may reduced the LDL-cholesterol level in serum and increases HDL –Cholesterol. The treatment with methanolic and cholorformic extracts Terminalia catappa showed a dose-dependent reduction in APAP--induced elevated serum enzyme activities with parallel increase in total proteins and bilirubin, indicating the extract could enhance the return of normal functional status of the liver comparable to normal rats.

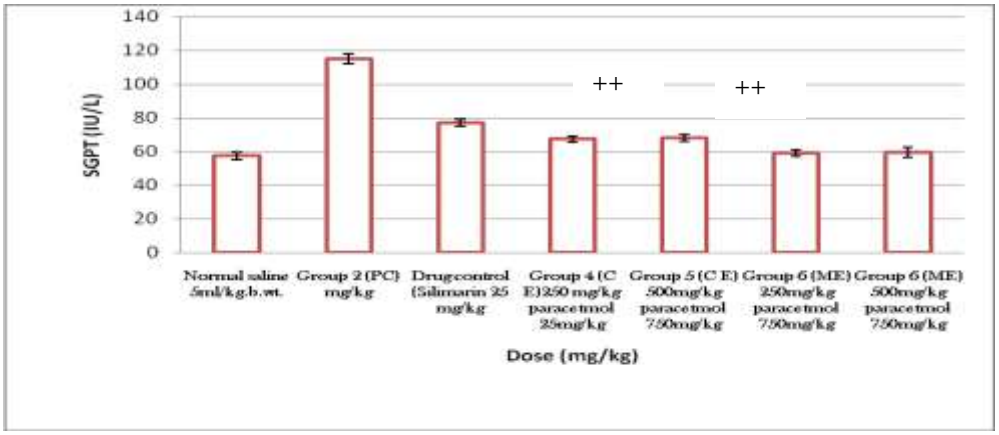
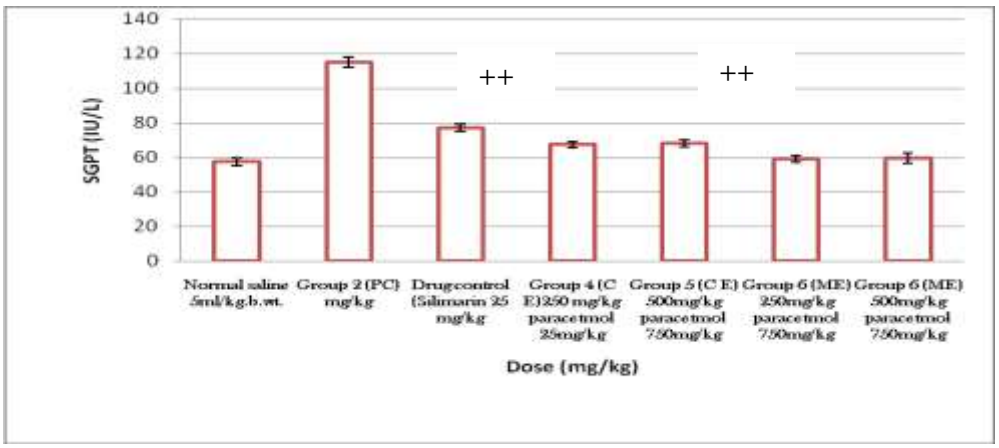


Figure no. 1: Effect of Terminalia catappa extracts on serum enzymes SGPT level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**



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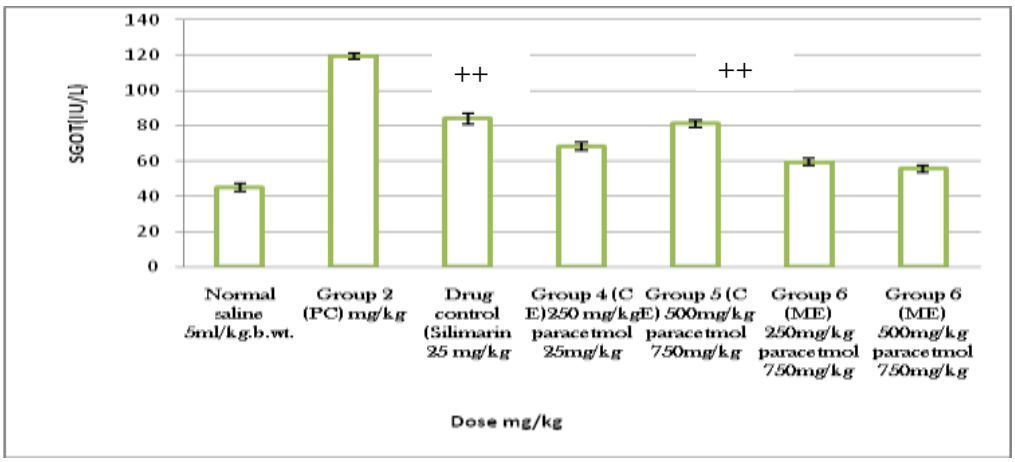

Figure no. 2: Effect of Terminalia catappa extracts on serum enzymes SGOT level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**

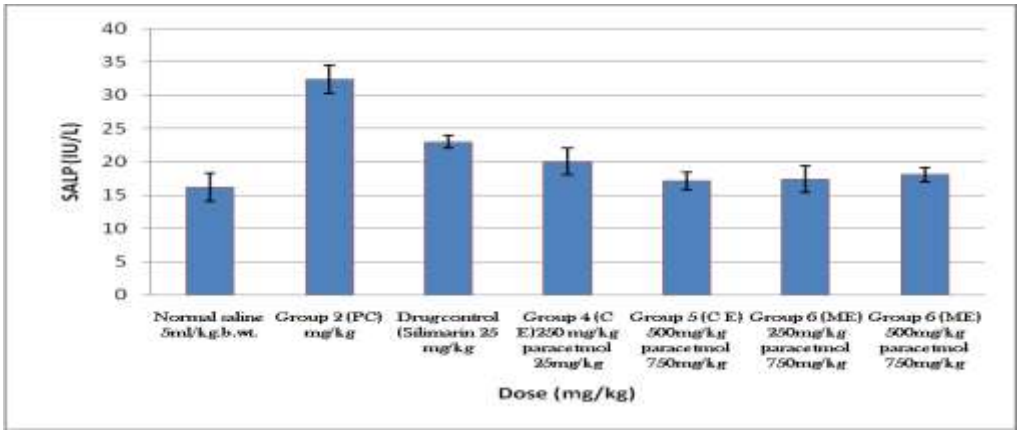

Figure no. 3: Effect of Terminalia catappa extracts on serum enzymes SALP level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**

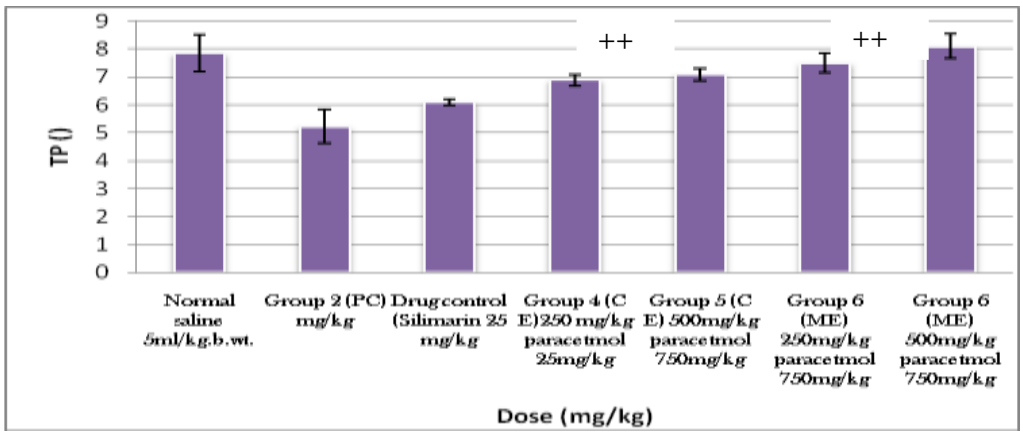

Figure no. 4: Effect of Terminalia catappa extracts on serum enzymes TP level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**

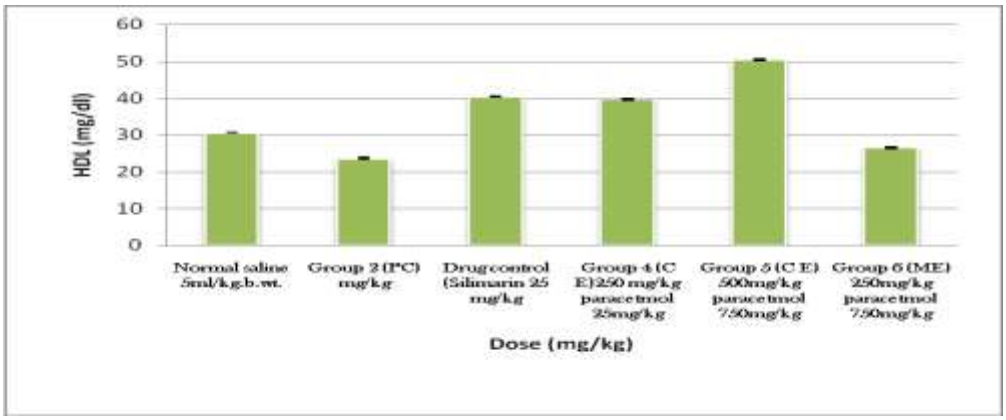

Figure no. 5: Effect of Terminalia catappa extracts on HDL level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**

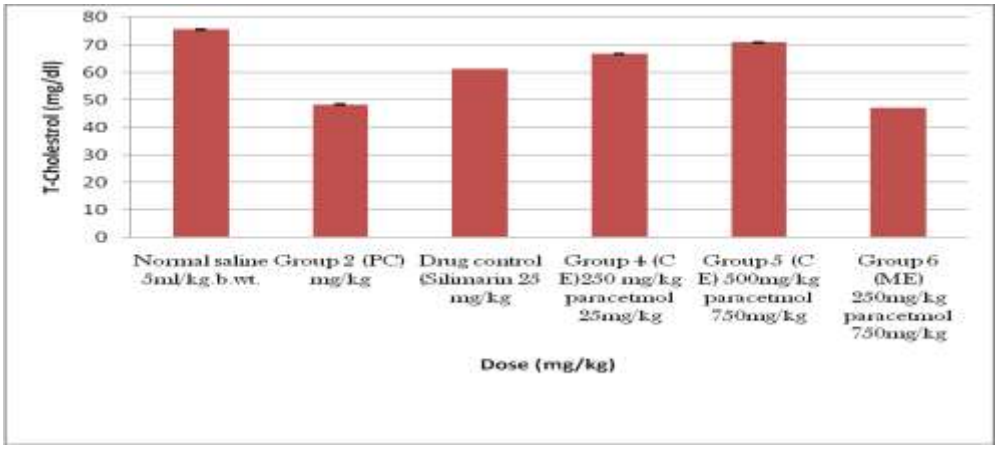

Figure no. 6: Effect of Terminalia catappa extracts on T-Cholesterol level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**

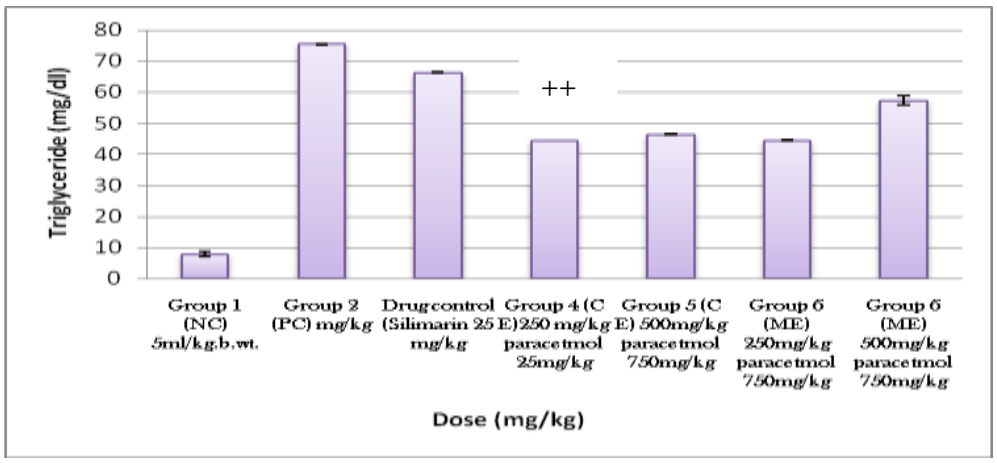

Figure no. 7: Effect of Terminalia catappa extracts on serum Triglyceride level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**

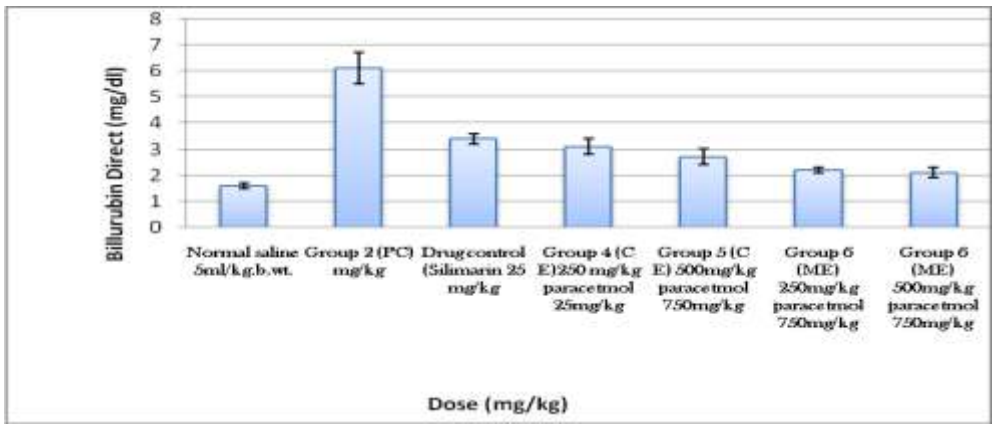

Figure no. 8: Effect of Terminalia catappa extracts on serum BD level in paracetamol-induced liver damage in rat (mean ±SEM; n=6);*p<0.05 is considered significant when compared with normal saline group;P<0.01 is considered significant when compared with paracetamol group.**

CONCLUSION

Alcoholic and choloroform extracts CEIF and MEIF of the *Terminalia catappa* L. was evaluated in APAP- induced hepatotoxicity in rats and levels of SGPT, SGOT, SALP HDL, T- Cholesterol level, Serum triglyceride, Serum BD level, total proteins, total and direct bilirubin.At (250mg) dose level the chloroform extract (CEIF) significantly ($p<0.05$) decreases the activity of enzymes (SGPT, SALP, and Serum BD level) that was competent with that of silymarin revealing its heptoprotective effect and showed lowest activity against total protein and serum glyceride level. At a dose level of 250 mg/kg, the methanolic extract (MEIF) showed less significant activity in SGPT and Toptal protein level while non significant in HDL. When the dose level was increased to 500 mg/kg the methanolic extract (MEIF) showed significant activity against HDL and serum glyceride level while showed potent activity in total protein level to that of silymarin indicating enhancement to the normal function of the liver and open the door for pharmacist as well as natural product chemists for further research.

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