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Biochemical, Cytotoxicity and Hepatoprotectives of *Eichhornia crassipes*against *In vitro* and *In vivo* studies

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Abstract
The present study aims to evaluate the hepatoprotective activity of extract *Eichhornia crassipes*on carbon tetrachloride (CCl4) induced hepatoprotective in rats. The animals were daily treated with the extract of*Eichhornia crassipes*at a dose of 100, 200 & 400 mg/kg body weight for 21 days. Animals administrated orally with CCl_4 treated with Silymarin (20mg/ Kg BW) orally for 21 days. Atthe end of the experimental period, all rats were anesthetized to collect blood for the assessment of biochemical parameters andthen sacrificed to collect the liver for weighing& analysis. Hepatotoxicity was evaluated by measuring the serum levels of aspartateaminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (total and direct), malondialdehyde(MDA), total protein (TP), triglycerides (TG), total cholesterol, very low-density lipoprotein (VLDL-c), low-densitylipoprotein (LDL-c), high-density lipoprotein (HDL-c), urea, creatinine, and uric acid. Based on the results obtained in this study,*Eichhornia crassipes*before exposure to the administration of CCl4 conferred favourable hepatoprotective effect inrats. It exhibits a significant hepatoprotective effect by ameliorating CCl4induced alterationsof these biochemical parameters. Hence, *Eichhornia crassipes*could be a potential medicinal herb that can be used in the future to preventliver intoxication.
Keywords: *Eichhornia crassipes, Cytotoxicity, Hepatoprotectives.*

1. Introduction

Hepatic diseases are one of the major causes of morbidity and mortality all around the world. The drug induced liver injury is one of the most common causative factors for major clinical and regulatory challenge. The drug induced hepatotoxicity is highly variable, ranging from asymptomatic elevation of liver enzymes to hepatic failure. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations (Hikino and Kiso, 1988; Handa*et al*., 1989; Sharma *et al.,* 1991; Evans, 1996). Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. *Eichhornia crassipes*, commonly known as water hyacinth, aquatic, free-floating perennial plant which is native to tropical and sub-tropical of South America and native to the Amazon basin. This plant is known to cause major ecological and socioeconomic change. One of the fastest growing plants known, water hyacinth reproduces primarily by way of runners or stolon's, which eventually form daughter plants. Each plant additionally can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years [Sullivan*et al.,* 2012. Some water hyacinths were found to grow up to 2 to 5 meters a day in some sites in Southeast Asia. The leaves are 10–20 cm across, and float above the water surface. They have long, spongy and bulbous stalks. The plant is used as a carotene rich table vegetable in Taiwan, the flowers are used for medicating the skin of horses and leaves used for hepatoprotective activity in traditional therapy (Duke and Wain, 1981). In Chattisgarh, crassipes is being used as styptic. The fresh juice of this weed is used to treat fresh wounds as the tribes believe that it stops further spread of infection. Rice farmers consider this as a best first aid remedy for minor injuries. Along with vinegar, it is being used in treatment of septic wounds, but scientifically there are no valid reports available on hepatoprotective and curative activity of this plant. Hence, the present study was aimed to investigate the hepatoprotective properties of this plant against CCl4-induced hepatotoxicity in rats.

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2. MATERIALS AND METHODS

Plant Collection:

Domain: Eukaryota Kingdom: Plantae Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Monocotyledonae
Order: Pontederiales
Family: Pontederiaceae
Genus: Eichhornia
Species: Eichhornia crassipes

Preparation of the Plant extract

Apparently, disease free healthy aerial parts of *Eichhornia crassipes*were collectedfrom the botanical garden, University of Madras during the month ofAugust - October (2019). Taxonomic identification of the plant wasmade by Prof. K. Murugesan, University of Madras, Guindy Campusand Chennai, India. The plant materials were washed twice with tap water to remove soil and dust particles and finally rinse with distilled water. The collected plants were shade dried and pulverized in a mechanical grinder and stored in a closed vessel for further use.

Extraction

The air dried, powdered aerial parts of *Eichhornia crassipes*was extracted using a Soxhlet apparatus with 1L of methanol at room temperature for 8 h, the extraction procedure was repeated until the solvent become colourless. The obtained extract was condensed using a rotary evaporator (Lark Rotary Evaporator, Model RE 100-Pro).

Experimental Design

Group I – Normal Rats

Group II – Negative control - Animals will be administrated orally with CCl4(0.5 ml/150g of bw-v/v in olive oil) on 1st, 8th and 16th days.

Group III – Animals administrated orally with CCl4(0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th days) and treated with selected plant extract (100mg/ Kg BW) orally for 21 days.

Group IV - Animals administrated orally with CCl4(0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th days) and treated with selected plant extract (200mg/ Kg BW) orally for 21 days.

Group V –Animals administrated orally with CCl4(0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th days) and treated with selected plant extract (400mg/ Kg BW) orally for 21 days

Group VI –Animals administrated orally with CCl4(0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th days) and treated with Silymarin (20mg/ Kg BW) orally for 21 days

Maintenance of Cell

Human Breast cancer cell line MCF-7 was purchased from National Centre for Cell Science, Pune. They are squamous in nature and responsible for the diffusion of substances across the tissue. In vitro they grow adherently as a monolayer. The cells were grown in culture flask using Minimum Essential Medium supplemented with 3 % L-Glutamine, 10% Fetal bovine serum, Pencillin (100 IU/ml), Streptomycin (100 µg/ml) and Amphotericin B along with 7.5% sodium bicarbonate in a humidified atmosphere of 5% CO2 at 37C. The cells were subjected to passaging for required number of flasks for further studies.

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Media of Composition

Name of the Media	: Dulbecco's Modified Eagle Medium (DMEM)
Chemicals	: DMEM, FBS (Fetal Bovine Serum), Antibiotic, Sodium bicarbonate
Media Preparation	: DMEM – 9.6 g/L
FBS	: 10%
Antibiotic	: 20ml/L
Sodium bicarbonate	: 3.7 g/L, pH -7.4

Morphological analysis

Morphological observation of MCF-7 cells treated with plant extract was done to determine the changes. Cell line treated with plant extract shows Shrinkage, membrane blebbing, chromatin condensation, formation of apoptotic bodies was observed. Light microscopic examination of the cells was performed to observe the morphological changes after treatment. MCF-7 cells were grown in 6 well plates and treated with Plant extract at a concentration of 750 µg/mL for 24h. The morphological changes were observed under inverted light microscope (Soundarajanvijayarathna and Sreenivasan sasidharan, 2012).

In Vitro Cytotoxicity Assay

Trypan Blue (Unnikrishnan MC and Ramadasan K, 1998)

Short term in – vitro cytotoxicity was assessed using Breast cancer cell line (MCF-7) by incubating different concentrations of the plant extract at 37oC for 3 hours. The tumor cell line was cultured in culturing flask it was trypsined and transferred in centrifuge tube for centrifugation at 2500 rpm for 5 minutes. After centrifugation the cells are separated at the bottom and supernatant at the bottom and supernatant was discarded. Then added 2 ml of media and mixed well. The cell number was determined using a haemocytometer and adjusted to 1 x 10 4 cells /0.5 ml. For the cytotoxicity assay, 0.5ml of cell suspension was transferred into each 6 well plate and incubated for 24 Hrs at CO2 incubator. Then added 0.5 ml different concentrations of the extracts (100 – 1000 µg/ml) were added to each well and incubated at incubator for 3 Hrs. Added 0.5 ml trypsin to each well and incubated 5 minutes. Then add 20µl of 0.2 % Trypan blue dye in isotonic solution. Control well was maintained with the medium without the plant extract. The number of viable (unstained) and non – viable (stained) cells were counted using haemocytometer.

% Dead cells = Total cells counted – Total Viable Cells

Total cells counted x 100

MTT ASSAY (Mossman T, 1983)

Principle of the assay

The MTT cell Proliferation and Viability assay is a safe, sensitive, in vitro assay for the measurement of cell proliferation. When metabolic events lead to apoptosis or necrosis, there is a reduction in the cell viability. Cells were cultured in flat-bottomed, 96 well tissue culture plates. The cells are treated as per experimental design and incubation times are optimized for each cell type asystem. The tetrazolium compound MTT (3- [4, 5 – dimethylthiazol – 2- yl] -2, 5- diphenyltetrazolium bromide) is added to the wells and the cells are incubated. MTT is reduced by metabolically active cells to insoluble purple formazan dye crystals. Detergent is then added to the wells, solubilizing the crystals so the absorbance can be read using a spectrophotometer. The optimal wavelength for absorbance is 570 nm. The rate of tetrazolium reduction is proportional to the rate of cell proliferation.

Materials required

Reagents

Phosphate Buffered Saline (PBS), sterile
Cell culture medium, sterile
MTT reagent: 5mg/ml
0.1% 0.1 N acidic isopropyl alcohol
DMSO (cell Culture grade)

Equipment

Inverted microscope
96-well plate(s), tissue culture grade, flatebottomed, sterile
5ml tubes, sterile
Serological pipettes, sterile
Pipette tips (1-200µl), sterile
Multichannel pipette
Cell culture facilities including Biosafety cabinet II hood and 370C, CO2 incubator
Plate reader with 570nm filters

Procedure

Cytotoxic activities of plant extract on MCF-7 cells were studied by MTT assay. Cells were seeded into 96 well plates, at a plating density of 5000 cells / well and 100µl media was added. After, the plate was

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incubated for 24 hours at 370C, 5% CO2 for attachment of cells. After 24 hours the spent media was removed and 100µl fresh media added in the seeded cells in the 96 well plates were treated with Nano Particle at various concentrations (15.62 - 250µg/0.1 ml in DMSO) and incubated again for 24 hours at 370C and 5% CO2. After 24 hours of incubation, added 20µl of MTT (5mg/ml) solution was added to the well plate and was incubated again for 3 hours. After incubation the medium was carefully removed without disturbing the formed formazan crystals and the crystals were solubilized in 100µl of DMSO and the absorbance was measured at 570nm using ELISA reader. The percentage of cell growth inhibition was calculated using the formula,

% of Growth Inhibition = (Control OD- Treated OD/ Control OD) X 100

LACTATE DEHYDROGENASE RELEASE ASSAY (Russo A et al., 2006)

Principle

LDH

Pyruvate + NADH + H + Lactate + NAD+

Lactate Dehydrogenase catalysis the conversion of pyruvate to lactate; NADH is oxidized to NAD in the process. The rate of decreases in NADH is directly proportional to the LDH activity and is determined photometrically.

Reagents:

Phosphate buffer Saline
50mM Tris HCL
20mM EDTA
0.5% SDS
1mM pyruvate
0.2mM NADH

Procedure

Cells were seeded into 96 well plates, at a plating density of 5000 cells / well and 100µl media was added. After, the plate was incubated for 24 hours at 370 C, 5% CO2 for attachment of cells. After 24 hours the spent media was removed and 100 µl fresh media added in the seeded cells in the 96 well plates were treated with plant extract at various concentrations (15.62 - 250µg/0.1 ml in DMSO) and incubated again for 24 hours at 370C and 5% CO2. Cells are lysed with 50mM 0.1ml Tris-HCL buffer, pH 7.4 + 0.1ml 20mM EDTA + 0.1ml of 0.5% Sodium Dodecyl Sulfate (SDS) and centrifuged at 13000 X g for 15 minutes. The precipitate obtained was added with 0.2 ml of 1mM pyruvate and 0.2ml of 0.2mM NADH. After 15 minutes incubation reduction in NADH was observed at 340 nm in UV spectrophotometer.

% of Growth Inhibition = (Control OD- Treated OD/ Control OD) X 100

Determination of body and organ weight changes in rats

Changes in the body weights of the experimental rats were monitored on weekly basis by weighing each rat daily using a compact scale weighing balance (FEJ-3000B, 3000 g capacity, China). Similarly, blood and tissue samples from the liver were collected from sacrificed rats daily, and were weighed using electronic weighing balance (SFE 300, Citizen's scale, Adams equipment company limited, China) to determine the organ-body weight relationship Shamaki et al., 2017.

Determination of haematological parameters

The Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France) was used for the determination of haematological parameters including red blood cells (RBC) and its related indices following manufacturer's instruction. These include haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RCDW). White blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelet were also analysed.

Analyses of Serum Samples

For estimation of different biochemical parameters, blood was withdrawn from the retroorbital plexus of the rats (fasted for 12 h) by sterilized capillary tubes under light ether anaesthesia on the final day of drug treatment period. The blood was collected in a clean test tube and allowed to coagulate for 30 minutes at room temperature and then centrifuged at 3000 rpm for 15 min. The serum, used as specimen, should be free from haemolysis and hence separated from the clot promptly. The resulting upper serum layer was collected in properly cleaned, dried, and labelled Eppendorf tubes and was stored at 2–8°C for further analysis of different parameters, that is, lipid profile and serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) and serums creatinine, urea, protein, superoxide dismutase (SOD), and malondialdehyde (MDA) [29–32].

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Blood Collection

After the study period, the animals were kept overnight fast and sacrificed under light chloroform anaesthesia. Blood was drawn from the ventricles and centrifuged. Serum was separated and analysed for various biochemical estimations. Liver, pancreas and kidney were excised immediately, washed with ice cold saline stored in 10% formalin and 0.9% saline, for histopathological and biochemical studies respectively.

Biochemical Estimations

Commercial diagnostic kits were used to estimate blood glucose level colorimetrically. (Sigma Diagnostics Pvt Ltd, Baroda, India). Lowry et al (1951) method was used for estimation of protein in serum and liver. Serum lipid profile, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), serum urea,creatinine, protein and albumin content were determined by standard procedures in an auto analyzer using Ecoline kits(E.Merck,Mumbai,India). Liver glycogen was estimated [Morales et al., 1973] and carbohydrate metabolic enzymes of liver like glycogen phosphorylase [Cornblath et al., 1963], Glucose-6-phosphatase [King, 1965] and Hexokinase [Brandstrup et al., 1957] were also analysed.

Blood Sampling and Organ Collection

Twelve hours after the last dose of CCl4 injection, all animals were anesthetized by light ethyl ether inhalation and sacrificed. Blood samples were collected from the carotid arteries and centrifuged at 3000 rpm for 10 min under cool temperature (4°C) to separate the plasma. The separated plasma was stored at −20°C for further assessments. Besides, the liver was weighed and conserved for the preparation of the liver homogenate (10% w/v) in sodium phosphate buffer (pH 7.0) and stored at −20°C for biochemical analysis. The liver index was calculated by the following formula [Su et al., 2014]: liver index (%) = weight of liver/weight of body x 100%.

Biochemical Parameters Determination

The biochemical parameters such as serum enzymes: aminotransferases (AST and ALT) [Reitman and Frankel, 1957], alkaline phosphatase (ALP) [Tietz et al., 1983], bilirubin (total and direct) [Malloy and Evelyn, 1937] total cholesterol [Allain et al., 1974], triglycerides (TG) [McGowan et al., 1983], high-density lipoprotein (HDL-c) [Burstein and Scholnick, 1973] low-density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c), total protein (TP), glucose, urea, uric acid, and creatinine were evaluated by using an autoanalyzer (Architect c-Systems, Hamburg, Germany) by using a commercial kit. All analyses were performed in triplicate for every sample.LDL-cholesterol was computed according to Friedewald et al., using the following equation: LDL-c = total cholesterol− [HDL-c + very low-density lipoprotein (VLDL-c)]. VLDL-c was calculated according to the formula as follows [Friedewald et al., 1972]: VLDL-c = triglycerides/5. The lipid profile of non-esterified free fatty acids [Falholt and Falholt, 1973], phospholipids [Zilversmit and Davis, 1950] (TBA)[Nichans WG and Samuelson, 1986]. catalase (CAT) [Sinha, 1972]. Glutathione Peroxidase (GPx) GPx activity was assayed using the method of Sharma[23]. The assay mixture consisted of 1.49 mL of sodium phosphate buffer (0.1 mol/L pH 7.4), 0.1 mL EDTA (1 mmol/L), 0.1 mL sodium azide (1 mmol/L), 0.1 mL 1 mmol/L GSH, 0.1 mmol/L of NADPH (0.02 mmol/L), 0.01 mmol/L of 1 mmol/L H2O2, and 0.1 mmol/L PMS in a total volume of 2 mL. Oxidation of NADPH was recorded spectrophotometrically at 340 nm and the enzyme activity was calculated as nmoles NADPH oxidized/min/mg of protein, using extinction coefficient of 6.22 × 103 mmol·L -1 ·cm-1. Glutathione-S-transferase (GST) Activity GST activity was assayed using the method of Haque [24]. The reaction mixture consisted of 1.67 mL sodium phosphate buffer (0.1 mol/L pH 6.5), 0.2 mL of 1 mmol/L GSH, 0.025 mL of 1 mmol/L CDNB and 0.1 mL of post mitochondrial supernatant in a total volume of 2 mL. The change in absorbance was recorded at 340 nm and the enzyme activity was calculated as moles of CDNB conjugates formed/min/mg protein using extinction coefficient of 9.6×103 mmol·L -1 ·cm-1.

Hepatic membrane-bound ATPases estimation

The sediment after centrifugation was resuspended in ice-cold Tris (hydroxymethyl) aminomethane (Tris)–hydrochloric acid (HCl) buffer (0.1 M) pH 7.4. This was used for the estimations of membrane-bound enzymes and protein content. The membrane-bound enzymes such as sodium ion (Na+)/potassium ion (K+)-ATPase, calcium ion (Ca2+)-ATPase and magnesium ion (Mg2+)-ATPase activities were assayed by estimating the amount of phosphorous liberated from the incubation mixture containing tissue homogenate, ATP, and the respective chloride salt of the electrolytes [Bonting and Wiley, 1970] [Ohnishi et al., 1982]. Total protein content was estimated by the method described by Lowry et al., 1951.

Determination of Hepatic Carbohydrate Enzymes

Hexokinase, phosphofructokinase, glucose-6-phosphatase, and fructose-1,6-bisphosphatase activities were assayed in the liver by the methods [New et al., 1998], [Javelaud and Mauviel, 2004], [Nolan et al., 2005], and [Fujita et al., 2003], respectively. Glycogen content was determined according to the procedure described by Ong and Khoo [Singh, 2000]. Estimation vitamin C content the vitamin C content of the sample

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was determined as described in AOAC (1993). The sample (5 g) was extracted by 100 ml H2O and 10 ml of the extract was mixed with 25 ml of 20% glacial acetic acid and titrated against standardized 2,6-dichloroindophenol (0.05 g/100 ml) solution. Ascorbic acid was used as standard and the result was expressed as mg/g of the sample.

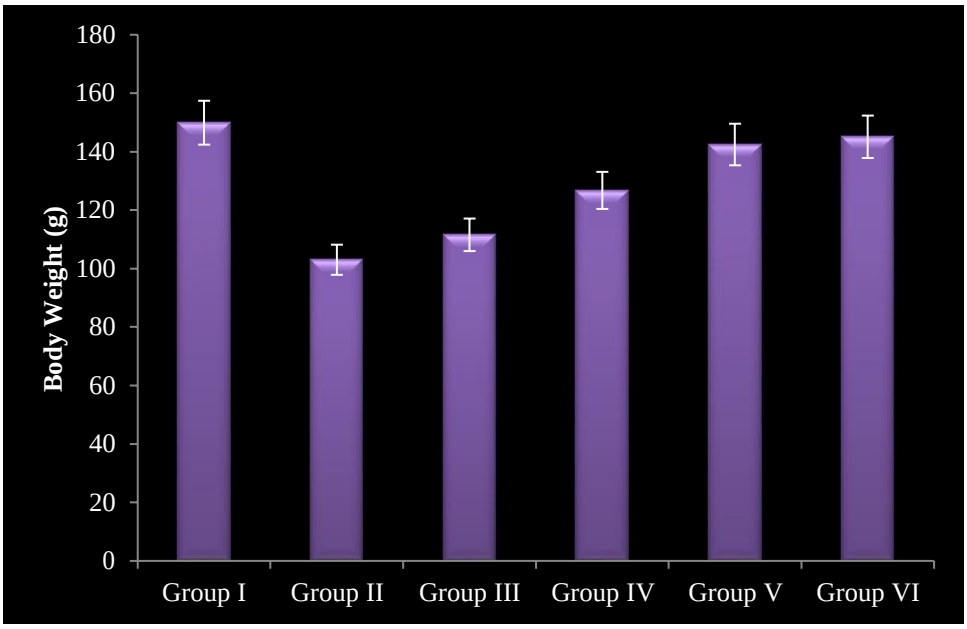
Estimation of vitamin E content A modified method of Kivcak and Akay (2005) was used for vitamin E determination. Extracts dissolved in CHCl3 (1 mg/ml) or different volumes of vitamin E dissolved in CHCl3 (10 lg/ml) were pipetted into test tubes. To each tube was added 1 ml of 2,2-dipyridyl reagent (0.125 g of 2,2-dipyridyl in 25 ml of 95% ethanol) and 1 ml of Ferric chloride reagent (0.2 g of Ferric chloride hexahydrate in 100 ml of 95% ethanol). Absorbance of the resulting solution in each test tube was taken at 522 nm. Vitamin E content (mg/ml) of the sample was interpolated from the prepared standard curve.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by STD with error bars.

3. Results and Discussion

Liver disease is a metabolic disorder, which is the most common cause of mortality and morbidity worldwide. Hence, medicinal herbs with hepatoprotective properties have received considerable attention from researchers. Recently, medicinal herbs have been utilized by researchers in experiments to investigate their hepatoprotective properties on animals (Okaiyeto et al., 2018). In this study, we aimed to investigate the hepatoprotective effect of Eichhornia crassipes on liver damage by measuring serum levels of aminotransferases (AST and ALT) activities, as enzyme markers of hepatocellular damage [Kharchoufaet al., 2020]. Liver injuries are induced by carbon tetrachloride in rats' models. CCl4 is a commonly used model for the investigation of hepatoprotective activity on various experimental animals [Bouhrim et al., 2018]. The liver damage caused by CCl4 is similar to that produced by viral hepatitis [Ponmari et al., 2014]. The elevated serum enzyme levels of AST, ALT, and ALP have been attributed to the damaged structural integrity of the liver because they are cytoplasmic in origin and are released into the blood after hepatic damage [Sharma and Shukla, 2011]. Our findings showed that AST, ALT, and ALP activities were increased in rats with the CCl4 treatment alone in comparison with the normal control group. This elevation in hepatic markers has been attributed to the cells damaged or cell membranes became leaky and they are released into the circulation [Kharchoufa et al., 2020;Ko and Lim, 2006].



Group I – Positive Control
Group II – Negative Control
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200 mg/kg
Group V – Plant extract/CCl4/400 mg/kg
Group VI – Silymarin/CCl4/20 mg/kg

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In our study the Cytotoxic potential of Plant extract against MCF-7 Cell Lines - Trypan Blue dye exclusion Method shows that the various concentrations were used during the assay ranging from 100 – 1000 µg/ml. The IC50 value was obtained at the concentration of 750 µg/ml. The dead cells increased while the concentration of plant extract Eichhornia crassipes is increased with the various concentrationwith the various concentration which is shown in Table 1. The cell viability assay conducted by trypan blue dye exclusion method showed that there was a highly significant ($p< 0.001$) decrease in viability with an increase in time and concentration in compound treated Daudi cells as compared to untreated controlled cells (Felisa Parmar, et al., 2016) The anti-leukemic effect of the 10 selected plant-derived drugs was investigated against the different stably-transformed leukaemia cell lines, OM10.1, HL-60 and NB4, using a trypan blue exclusion assay following exposure for 24 h. Mevinolin and honikiolexhibited the most potent anti-leukemic effects, while salicin showed the least promising response. The anti-leukemic effect of these plant-derived drugs, in descending order, was: mevinolin, honikiol, L-ascorbic acid 6-palmitate, cerulenin, resveratrol, cholecalciferol, retinyl palmitate, (S)- (−) limonene, chrysin and salicin. The most responsive cell line to the natural products under investigation was NB4 cells, while the most resistant cell line was OM10.1 (Mourad A. M. AboulSoud et al., 2015).

Table: 1. Cytotoxic potential of Plant extract against MCF-7 Cell Lines - Trypan Blue dye exclusion Method

Concentration (µg/ml)	Death cells (%)
Control	-
100	11.90
250	18.18
500	27.36
750	43.33
1000	63.59

In this study, the various concentrations of plant extract were tested in the MCF-7 cells. For this, the concentration was used range from 15.62 µg/ml to 250 µg/ml. In this, the IC50 value was obtained at the concentration of 125 µg/ml. While the concentration was increased the dead cells also increased which is shown in Table 2. The result of cytotoxicity assay by MTT method showed that CfME did not exhibit significant cytotoxicity in HepG2 cells after incubation for 24 and 48 h. Even though CfME was at concentration of 100 µg/mL and incubated for 48 h, the inhibitory rate of cell viability was only 10.6%, which meant the IC50 value of CfME was greatly higher than 100 µg/mL. This result demonstrated that CfME had no significantly cytotoxic activity in HepG2 cells (YajiePenga et al., 2019). Hepatoprotective activity of the Turmesac® on HUH-7 cells from Human Liver cells was investigated in vitro 3-(4) 5-Dimethyl-thiazol-Zyl) - 2,5 biphenyl tetrazolium bromide (MTT) assay. In MTT assay, 25 to 400 µg/mL different concentrations were used by stimulating the cells with 200 µM of H2O2 for 24 h. The hepatoprotective activity of Turmesac® was evaluated using well-maintained HuH7 cells. H2O2 was used as hepatotoxicant and silymarin was used as a standard positive control. The hepatoprotective activity of silymarin and Turmesac® were found to be IC50 value 8.7 and 150.8 µg/mL, respectively (Sadashiva et al., 2019)

Table 2: Cytotoxic potential of Plant extract against MCF-7 Cell Lines – MTT Assay Method

Concentration (µg/ml)	% Of Cytotoxicity
15.62	6.20
31.25	19.86
62.5	31.87

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125	41,10
250	52.88

In this, MCF-7 Cell Lines for LDH assay exclusion method is carried out to find the leakage in MCF-7 c ells exposed to plant extract, CCl4 and silymarin. The various concentrations were used for this study, while the concentration was increased the cytotoxicity was also increasedwhich is shown in Table 3. The impact of different solvent fractions and a crude ethanol extract of Eichhornia crassipes flowers on LDH leakage in BRL 3A cells exposed to H2O2 induced oxidative stress was investigated. LDH leakage into medium was significantly increased (p0.001) in H2O2 treated cells relative to control cells. When compared to control cells, which had a percentage release of 11.97 2.68, the percentage increase was found to be 74.70 0.57. In a group of cells pre-treated with benzene fraction at various concentrations and untreated H2O2 treated cells, there was no substantial reduction in LDH leakage. 71.00 1.2, 74.20 3.34, 72.97 1.44, 68.50 0.83, and 70.27 1.38 percent LDH release was observed in cells pre-treated with benzene fraction of Eichhornia crassipes flowers at different concentrations (0.01, 0.1, 1, 10, and 100 g/ml). In comparison to H2O2 control cells, pre-treatment with chloroform fraction of Eichhornia crassipes flowers at different concentrations (0.01, 0.1, 1, 10, and 100 g/ml) resulted in percentage LDH release of 74.13 5.35, 72.63 3.32, 62.23 4.35, 60.2 5.11, and 47.8 1.23 percent. The percentage LDH release in cells pre-treated with the n-butanol fraction of Eichhornia crassipes flowers at different concentrations (0.01, 0.1, 1, 10, and 100 g/ml) was 78.20 3.48, 72.82 3.21, 62.24 2.45, 59.45 1.74, and 55.32 1.26, respectively. Pre-treatment of BRL 3A cells with ethanol extract at concentrations of 0.01, 0.1, 1, 10, and 100 g/ml resulted in percentage LDH release of 74.57 3.51, 72.70 3.05, 60.40 3.00, 44.4 0.15, and 35.77 1.56 percent, respectively. In comparison to H2O2 control cells, BRL 3A pre-treated with ethanol extract at concentrations of 10 and 100 g/ml significantly decreased (p0.001) LDH leakage into medium. Pre-treatment with chloroform and n-butanol fractions at concentrations of 1 g/ml, 10 g/ml, and 100 g/ml resulted in a substantial difference (p0.5) in LDH release percentage when compared to H2O2 control cells, but the significance is poor when compared to ethanol extract and H2O2 control (p0.5 to p0.001) (Rajarajan et al., 2015). The cell membrane damage of Caco-2 cells after the treatment with RFRA extract was measured by the release of LDH by following CytoTox961 assay. The control cells and cells treated with DMSO showed lesser LDH release when compared to cells exposed to plant extracts. The RFRA treatments provoked a higher release of LDH activity than the control nontreated cells. There was a significant ($P < 0.01$ with 5 μg/mL and $P < 0.001$ with 10, 20, and 40 μg/mL of RFRA) dose dependent increase in the LDH release observed at increasing concentrations of RFRA (BlassanPlackalAdimuriyil George et al., 2015)

Table 3: Cytotoxic potential of Plant extract against MCF-7 Cell Lines – MTT Assay Method

Concentration (μg/ml)	**% of Cytotoxicity**
15.62	6.20
31.25	19.86
62.5	31.87
125	41,10
250	52.88

In the present study, the treated group VI (Silymarin/CCl4/200mg/kg) animals exhibited significantly ($P< 0.05$) higher mean body weight compared to the control and other group animals at the end of the 21 days during the induced period. It was found that the loss of body weight in other five groups was faster when compare to control and VI group rats treated during the drug period. The reduction of body weight of CCl4 and

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Eichhornia crassipes extract treated animals was found to be more when compare to Silymarin treated group animals respectively. Animals treated with 100,200 and 400mg/kg of CCl4 with plant extract had reduction in body weight on the 21st day, respectively as shown in Graph 1. The treated group animals exhibited significantly ($P< 0.05$) higher mean body weight and abdominal girth compared to the diet control group animals at the end of the fourth week of diet manipulating period as well as during diabetes induced period. It was found that the loss of body weight of diabetic control group rats was faster compared to MESG and metformin treated animals during drug treatment period. The percentage reduction of body weight of diabetic control and metformin treated animals was found to be 16% and 7.41%, respectively, on 28th day of drug treatment. Animals treated with 200 and 400mg/kg of MESG had 6.07% and 4.87% reduction of body weight on the 14th day, respectively, and further gained the body weight by 2.76% and 3.25% on 28th day of drug treatment period, respectively. The metformin and MESG treatment significantly ($P< 0.05$) reduced the abdominal girth during drug treatment period in comparison to diabetic control group (Ghanshyam Panigrahiet al., 2016). The significant decrease in the body weights (28-33 g) of diabetic animals was observed 10 days after induction of streptozotocin into the animals. The oral administration of plant extract markedly increased the body weight of the animals but the effect was not dose related. The percentage increase in the body weight at 200 mg/kg was 8.82% while that of 100 mg/kg did not show any significant difference as compared with the initial body weight (Oyedemi et al., 2011).

Table: 4. Cytotoxic potential of Plant extract against MCF-7 Cell Lines – LDH assayexclusion Method

Concentration (µg/ml)	**% Of Cytotoxicity**
15.62	12.34
31.25	25.28
62.5	36.79
125	47.35
250	56.25

In in vivo study, normal rats treated with laboratory food and water (200 mg/kg) did not affect the liver weight. The animals treated with CCl4+plant extract shows decrease in the liver weight in group III, IV and V indicating that the dose may have liver toxicity in rats. After administration of CCl4+Silymarin, the liver weight and liver index significantly increased in rats ($p < 0.001$), indicating serious hepatomegaly that was markedly suppressed by a dose of silymarin ($p < 0.001$ and $p <0.001$; respectively) shown in Graph 2. The normal rats treated with AECe (250 mg/kg) did not affect the liver weight and liver index compared with those of the normal control rats, indicating that the dose of AECe may have no liver toxicity in rats. After CCl4 administration, the liver weight and liver index significantly increased in rats ($p < 0.001$), indicating serious hepatomegaly that was markedly suppressed by a dose of AECe (250 mg/kg) and silymarin ($p < 0.001$ and $p < 0.001$; respectively) (Hayat Ouassouet al., 2021). The hepatomegaly was the other toxicity of STZ injection and the liver index of experimental mice could reflect the changes. The liver index of diabetic mice was significantly augmented compared to normal control mice. After treatment with metformin and 250 mg/kg CfME for weeks significantly ameliorated hepatomegaly of STZ-induced diabetic mice by decreasing the liver index 27% and 21%, respectively, compared to the diabetic control group (YajiePenga et al., 2019).

In the present study, Glucose, Glycogen, protein and Urea levels of both normal and experimental rats after 21 days of treatment was taken for study. Oral administration of CCl4, Eichhornia crassipesplant extract and Silymarin into the rats causes significant increase in the blood sugar levels when compared with normal rats. The glucoselevel was increased from 118.33±0.67mg/dl to 140.17 ± 1.25mg/dl. Following oral administration of CCl4, Eichhornia crassipesplant extract and Silymarin causes increase in glycogen levels in the liver tissue from 47.79±2.31to 66.91±2.21significantly. There is a gradual decrease in protein levels from group III to group VI rats from 8.20±0.10g/dl to 10.88±0.33 g/dl. The analysis of urea in serum samples shows fluctuation in the experimental rats shows reduction in positive control and increase in negative control rats but

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shows decrease in experimental rats from 54.68±0.33to 23.01±0.47 respectively which is shown in Graph 3. The levels of blood sugar as compared with normal rats. The blood glucose level was increased from 5.60 mmol/L to 28.30 mmol/L. Following oral administration of extract at the dose of 200 mg/kg the blood glucose level was significantly reduced ($P<0.05$). Meanwhile, the dose at 100 mg/kg also had significant effect on the blood glucose level as compared with diabetic untreated rats. The data obtained at 200 mg/kg compared favourably well with that of glibenclamide treated group (10.43± 3.30 mmol/L) (Oyedemi et al., 2011). The administration of CCl4 alone to the animals resulted in a marked increase in the plasma glucose, triglycerides, and VLDL levels ($p < 0.001$; $p < 0.01$ and $p < 0.01$, respectively) when compared to the normal control group. The rats treated with AECe (250 mg/ kg) and the standard treatment silymarin (40 mg/kg), showed a significant reduction in all of the parameters that were increased in the CCl4-treated group. Overall, the results observed after administration of AECe at 250 mg/kg were comparable to those of silymarin at 40 mg/kg. On the other hand, no significant differences were detected in total cholesterol, LDL, and HDL levels in the CCl4-treated group compared to the normal control group (Hayat Ouassou et al., 2021). In this, the Direct Bilirubin, Total Bilirubin, Total Cholesterol, Phospholipids, Triglycerides and Free fatty acids were tested in liver tissue of rats. The DB, TB, TC, Phospholipids, Triglyceride's level was1.35±0.05, 1.79±0.05, 415.00±3.42, 146.71±1.66, 379.00±5.79 but the free fatty acids level was 132.96±0.95. The free fatty acid level was high in Group III when compare to other groups whereas the other parameters was high in Group II except fatty acids. The least level was observed in group VI in all the parameters. From this we conclude that, Animals administrated orally with CCl4with Silymarin shows high hepatoprotective activity.The administration of CCl4 to the rats induced a significant ($p < 0.001$) increase in total and direct bilirubin levels, indicating the impaired excretory function of the liver shown in Graph 4. On the other hand, treatment with AECe at a dose of 250 mg/kg and silymarin (40 mg/kg) produced a highly significant ($p < 0.01$; $p < 0.05$) fall in the total and direct bilirubin levels compared to the CCl4-treated rats (Hayat Ouassou et al., 2021).

In this study, the Plant extract on HDL, LDL, VLDL, AST, ALT and ALP of experimental animals shown high increase in group VI when compare to negative control. In other groups the variation is slightly increased from group III to group V which is shown in the Graph 5. Effect AECe on ALT, AST, and ALP. ALT, AST, and ALP are sensitive markers of the liver, and their elevated levels are indicative of liver damage. The marked changes of AST, ALT, and ALP levels were detected in normal control rats and the AECe group, which confirmed the safety of AECe at a dose of 250 mg/kg. The injection of CCl4 to the rats induced liver injury, which represented markedly elevating activities of AST, ALT, and ALP serum levels compared with the normal control group. However, the AECe treatment (250 mg/kg) induced a significant ($p < 0.05$, $p < 0.001$) decrease in the CCl4-induced elevation of serum enzymes AST, ALT, and ALP compared to the CCl4-treated group. The effect of AECe is comparable with that of the silymarin treatment. These results indicated a protective effect of AECe on CCl4-induced liver injury in rats (Hayat Ouassou et al., 2021). Administration of isoniazid in rats by oral route caused liver damage as indicated by a significant increase in serum enzymes ALP, SGOT, SGPT activity, and creatinine, bilirubin contents while decrease in hemoglobin level was observed as compared with control rats. Co-administration of rats with S. podophyllum and E. crassipes extracts with isoniazid accounted for altered levels of serum biochemical markers. Administration ofS. podophyllum aqueous extract showed elevation in drug induced serum biomarkers indicating liver damage. Liv-52 and E. crassipes leaf aqueous extract restored the hepatic marker levels in serum. E. crassipes extract (400mg/kg body weight) restored ALP (69.22%), SGOT (29.91%), SGPT (62.31%), creatinine (108.80%), bilirubin total (48.95%), bilirubin direct (40.22%), and haemoglobin (65.69%) level towards normal values (Shashank Kumar, et al., 2014)

In this study, The Oral administration of CCl4and Silymarin into the rats causes significant increase in the LDH, GGT, LPO, GSH, SOD andCATof experimental animals when compare to negative control. The group VI shows low level of LDH, GGT, LPO, GSH, SOD and CAT which is similar to positive control. From this result, the silymarin shows good result when compare to plant extract treated groups in Graph 6. LPO concentration end product MDA was significantly increased in the liver ($P<0.01$) in rats treated with KBrO3. Vitamin C (Group IV) effectively prevented the oxidative damage induced by KBrO3, which decreased MDA concentration significantly in comparison to KBrO3 treated group. KBrO3 treatment decreased the levels of SOD and GSH significantly ($P<0.05$) in the liver. By contrast, increased levels of GSH and SOD were observed in Vitamin C plus KBrO3 treated group (Naglaa, et al., 2016).

In the present study, plant extract on GPx and GST of experimental animals shows increase in group VI but it shows decrease in group II. But there is a slight variation was observed in other groups. The CCl4 treated with silymarin showed good result and it is similar to positive control shown in Graph 7. The activities of GR, GPx, SOD, and GST in liver tissue of CCl4-induced rats are shown in Table 2. In liver tissue, CCl4 treatment caused reduction of GR (2.03±0.11), GPx (2.08±0.12), SOD (13.0±0.77 U/mg protein) and GST (3.98±0.94 U/mg protein) activities, as compared with those in the control group, showing values of 31.22, 33.39, 32.07, and 15.67 U/mg protein respectively. Enhancement of GR activity was observed in the groups treated with 20 mg/kg-day of P. hexandrum (9.07±0.37), 30 mg/kg-day of P. hexandrum (12.86±1.99) and 50

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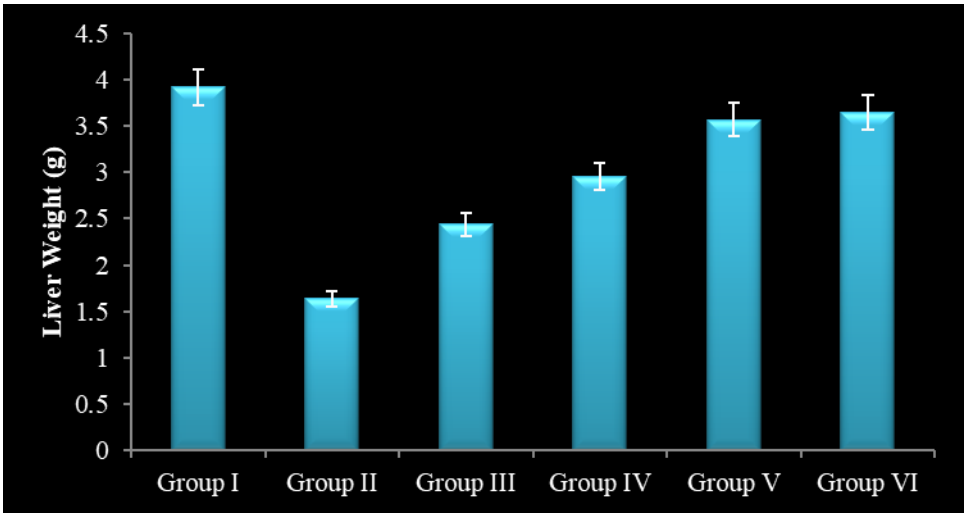
mg/kg-day of P. hexandrum (22.13±1.22) compared with those administered with CCl4 alone. Additionally, the groups treated with P. Hexandrum at respective doses of 20 mg/kg-day (9.40±0.57), 30 mg/kg-day (15.09±0.30), and 50 mg/kg-day (24.98±3.52) were found to cause significant increase in GPx activity, as compared with the CCl4 group. Enhancement of SOD activity was observed in the groups IV, V, and VI treated with P. Hexandrum hexane extract at the concentration of 20, 30, and 50 mg/kg-day to 15.95, 19.80, and 23.74 U/mg protein respectively as compared with that of the CCl4 treated group. Similar results were observed with GST treatment with 20, 30, and 50 mg/kg-day of P. hexandrum increased the activity of GST significantly in a dose dependent manner as compared to the CCl4 treated group. In group III, continuous treatment with vitamin E a known antioxidant (50 mg/kg bw-day) increased the GR, GPx, SOD, and GST activity significantly in all experimental animals as compared to CCl4 treated group Showkat Ahmad Ganie et al., 2013. There was significant increase in the GPx and GSH of GBN treated animals but these enzymes level remained similar in both diabetic control and polyphenol-treated animals Mutiu Idowu Kazeem et al., 2013

In Graph 8, the Hb, RBC, WBC, Platelets, ESR and PCV of experimental animals showed decrease in the group II while in group VI showed increase in the above parameters. It clearly shows that the silymarin treated with CCl4 shows hepatoprotective activity. The significant decrease in the levels of RBC, Hb, PCV, MCH, MCV, RCDW and MCHC observed in the diabetic animals was drastically increased to near normal level as well as glibenclamide treated group after administration of extract especially at the dose of 200 mg/kg body weight. The levels of serum WBC, basophils, neutrophils, eosinophils, lymphocyte and monocytes. The level of WBC was slightly increased after oral administration of the extract at 100 mg/kg while the dose of 200 mg/kg did not have any effect as compared with the diabetic groups. The plant extract significantly increased the level of lymphocyte, eosinophils, monocytes and platelet at both dosages while the best result was observed at the lower dose mg/kg compared favourably well whereas that of 200 mg/kg significantly boosted the level of neutrophils (Table 3). The extracts at both dosages did not have any beneficial effect on the level of basophils (Oyedemi et al., 2011). Assessment of red and white blood cell count, hematocrit and hemoglobin concentration is useful in determining the effect of some chemical substances on hematopoietic system. The number of RBC increased significantly in thetreatment of P1, P3 and P4 compared with the controls. P2 and also P5 treatment did not increased in RBC count but just same as the control. Hemoglobin values in all of the treatment significantly higher ($p < 0.05$) compared to the control treatment (P1). There is a tendency of increase in hemoglobin at treatment P2, P4 and P4. The significant inceased in the levels of WBC observed in the P5, P4 and P3 treatment. The number of WBC in the three treatments is significantly higher ($P < 0.05$) compared to the controls. WBC on P1 and P2treatmentsalmost same as control (Melva Silitonga1 and Pasar M Silitonga, 2017) Increased in total WBC count has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue [Das and Mukherjee, 2003]. The profile of the WBC count reflects the balance between the rate of granulocyte production and that of WBC.



Group I – Positive Control
Group II – Negative Control
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200 mg/kg
Group V – Plant extract/CCl4/400 mg/kg
Group VI – Silymarin/CCl4/20 mg/kg

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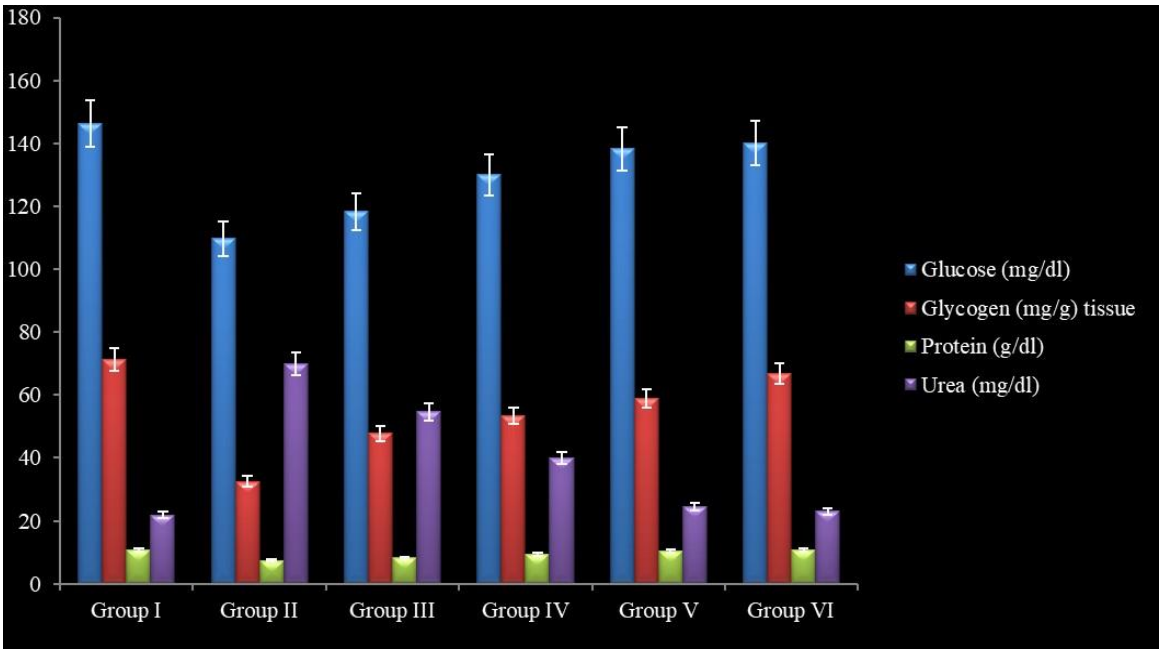
The raised WBC count may also reflect low-grade inflammation Platelets are significantly higher in all treatments compared with controls. Platelet highest value at T5 treatment. AEP significantly increased platelets. Methanol extract of Plectranthusamboinicus leaves increase platelet in mice [Pillai et al., 2011]. Platelet value of this research is still in the normal range is 638 - 1177 (103 / mL) [Das and Mukherjee, 2003] and 500-1000 (103 mL) [Cox et al., 2011]. Platelets, or thrombocytes is one of the haematological parameters are often used to determine the occurrence of agglutination and attack foreign substances. Platelets contribute to innate immunity in various ways, namely [Khumar et al., 2012] the platelets have a basic activity as antibacterial and fagositotik and show interactions with bacteria, viruses and parasites. Bacterial interactions with platelets induce platelet activation and secrete antimicrobial peptides, [Morera and MacKenzie, 2011] platelet containing several proinflammatory cytokines (eg IL-1) that modulate the inflammatory response / immune [Giknis et al., 2008]. This shows the good functioning AEP in maintaining platelet function, in which case a very large increase in the MVP can cause some diseases such as coronary artery, acute inflammatory diseases, stroke and so on [Pass dan Freeth, 1993]. The significant increase in platelets indicates that the plant compounds present in the extract promote blood clotting. Hematocrit percentage is the ratio of erythrocytes to the total blood volume. The percentage of hematocrit between 45-47% [Laurence and Bacharach, 1964].Hematocrit and MCHC, did not show significant difference at all doses of administration. MCV increasing significantly in treatment P1, whereas the other treatments did not different significantly as compare with the control. MCH or mean cospuscularhemoglobin was to measure the color indexes erythrocytes in the blood. In this study there were a significant increase MCH in treatment P1, P2, P3 and P4 compared to the control and P5. Provision of 200 and 400 g / kg methanolic extract in mice no significant effect on MCV, MCH and MCHC [Pillai et al., 2011]. But AEP significantly increase levels of MCH in this study. There was no significant difference in the levels of MCHC, although there was a trend increase in treatment P1, P2, P3, P4, and P5. MCHC levels were highest in treatment P1. Normally MCHC in male rats aged 17 months or more was 35.1 g / dl [Das and Mukherjee, 2003] whereas in this study the levels of MCHC mice treated EEP, the EEP + SDMD and SDMD are respectively 38.60, 37.67 and 36.00 g / dl. Erithrocyte Sediment Rate (ESR) of blood is a non-specific indicator that can be used to diagnose several diseases with a wide spectrum. ESR tends to be associated with the presence of inflammation or infection, but may also help monitoring immune abnormalities, diabetes, tuberculosis, anemia, and even cancer [Brigden and Malcolm, 1999]. In this study there is a significant difference between ESR in AEPtreatment and control. AEP decreased ESR significantly with compare to the control so ESR profile profile is maintained.



Group I – Positive Control
Group II – Negative Control
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200

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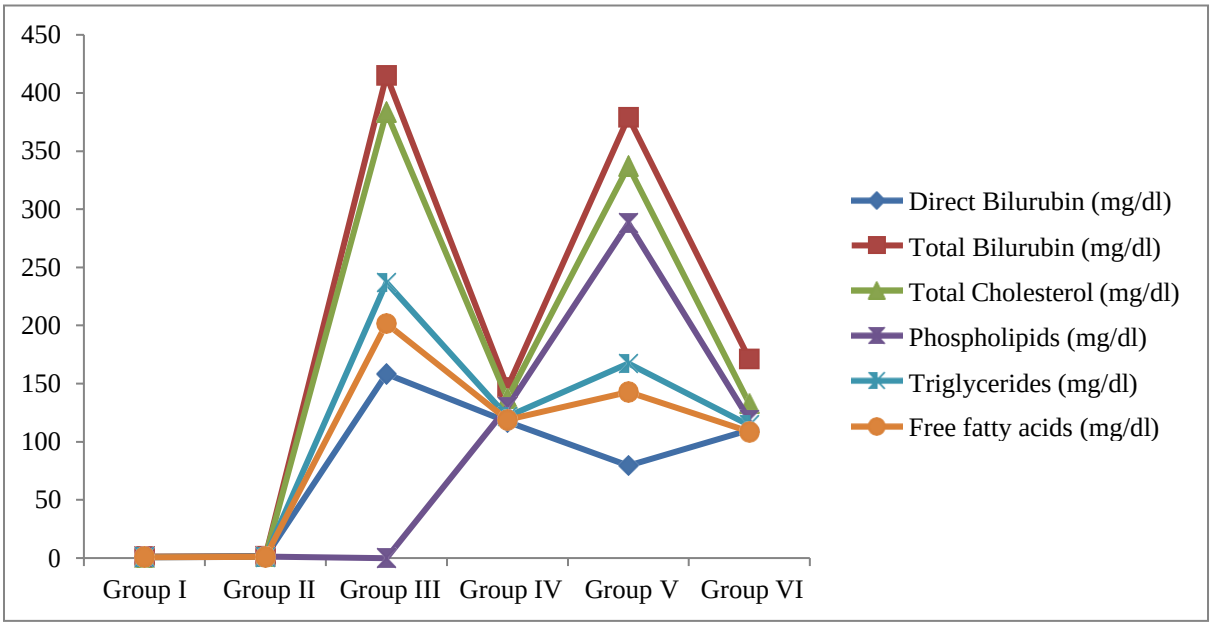
In this study, NA+K+ATPase, Ca2+ATPase, Mg2+ATPase, G-6-P Dehydrogenase and Hexokinase of experimental animals showed increase in the parameters ranging from 84.75, 78.83, 93.68, 247.25 and 90.20 in group VI but in group II shows decrease in the parameters. But the group VI values are similar to positive control shown in Graph 9. The effect of administration of polyphenols from Z. officinale on the activities of carbohydrate metabolic enzymes in the liver of normal and streptozotocin-induced diabetic rats. There were fluctuations in the activities of hexokinase and phosphofructokinase in all the groups of animals studied but was not significantly different ($P > 0.05$) from one another. The activities of fructose-1,6-bisphosphatase andglucose-6-phosphatase, significantly reduced ($P < 0.05$) in the diabetic control rats compared to the normal control. The 28-day administration of polyphenols especially free polyphenol from Z. officinale significantly increased fructose- 1,6-bisphosphatase and glucose-6-phosphatase activities in the diabetic rats Mutiu Idowu Kazeem et al., 2013. The activity of fructose 1,6 bis phosphatase and glucose-6-phosphatase was found to increase significantly in STZ induced diabetic rats when compared to normal control rats. However, diabetic rats treated with ELBD (500 mg/kg b.w.) resulted in significant decrease of this enzyme, which was similar to glibenclamide treatment (Vasundhara and Gayathri Devi, 2018) Serum minerals and electrolyte assessment in BRD affected calves showed a significant decrease of Ca, P, Mg, Na and Cl when compared to healthy calves. On other hand diseased calves revealed a significant increase in K level when compared to healthy calves (Sarhan et al., 2019).



Group I – Positive Control
Group II – Negative Control
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200 mg/kg
Group V – Plant extract/CCl4/400 mg/kg
Group VI – Silymarin/CCl4/20 mg/kg

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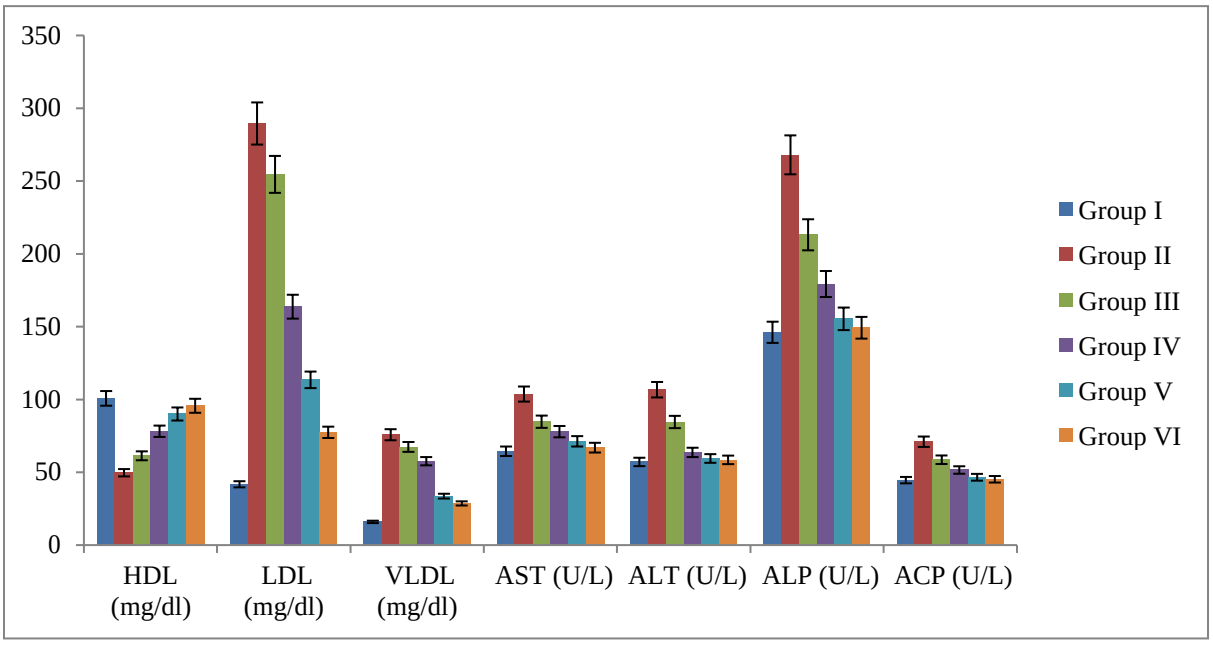

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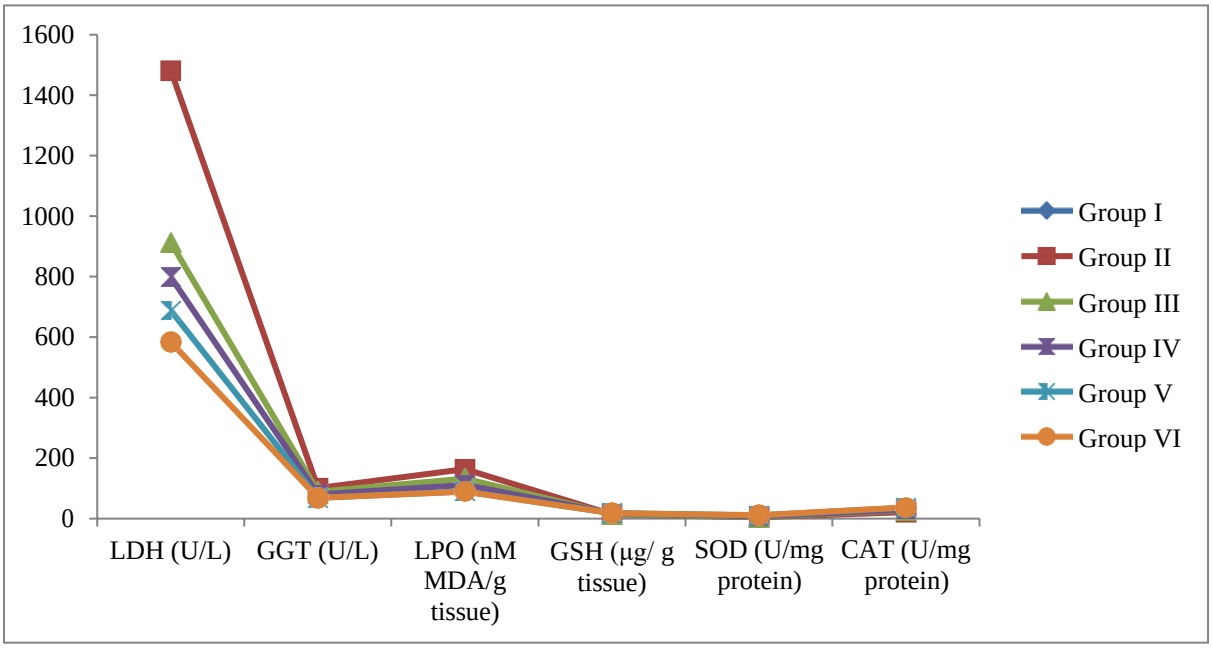
Group IV – Plant extract/CCl4/200 mg/kg
Group V – Plant extract/CCl4/400 mg/kg
Group VI – Silymarin/CCl4/20 mg/kg



Group I – Positive Control
Group II – Negative Control
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200 mg/kg
Group V – Plant extract/CCl4/400 mg/kg
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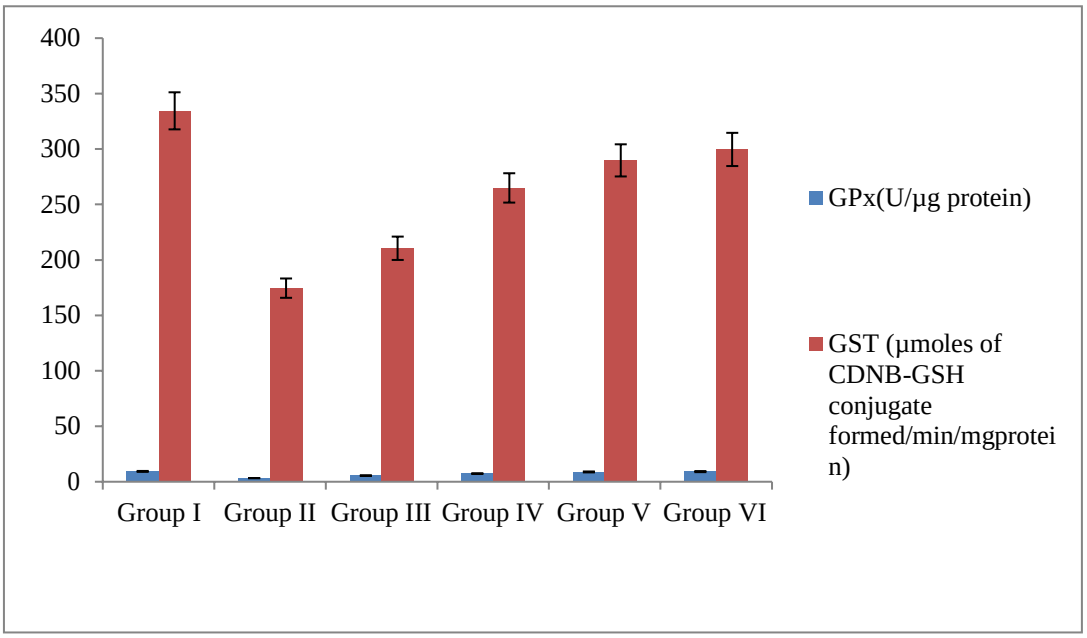

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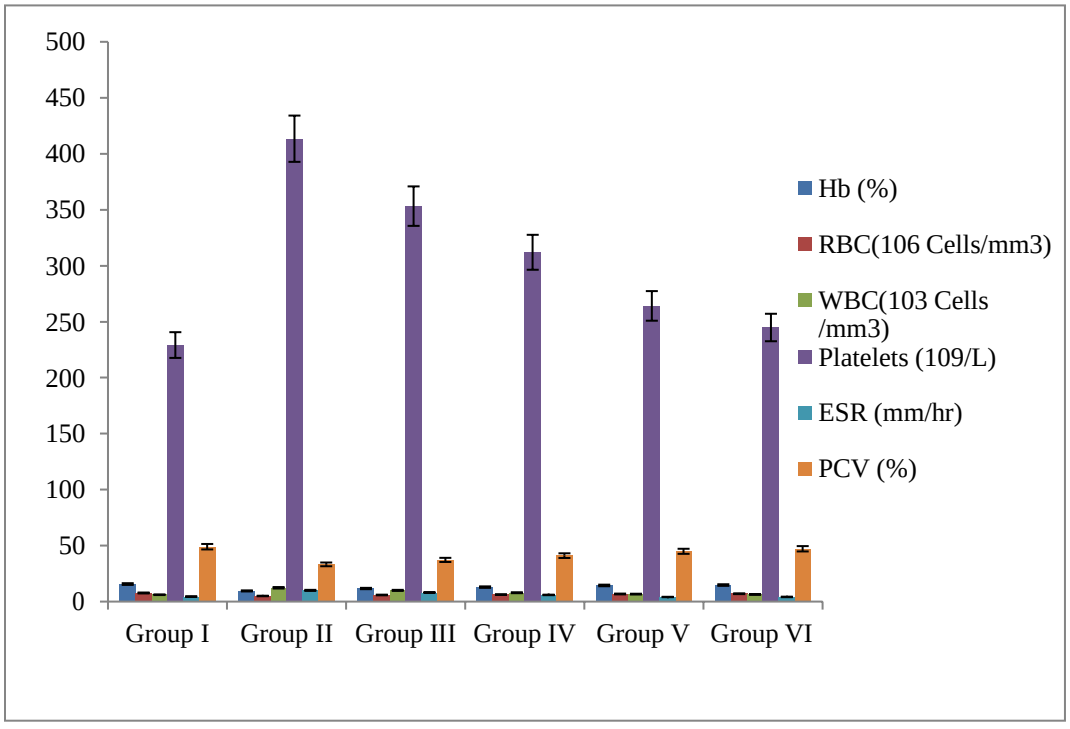
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200 mg/kg
Group V – Plant extract/CCl4/400 mg/kg
Group VI – Silymarin/CCl4/20 mg/kg



Group I – Positive Control
Group II – Negative Control
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200 mg/kg
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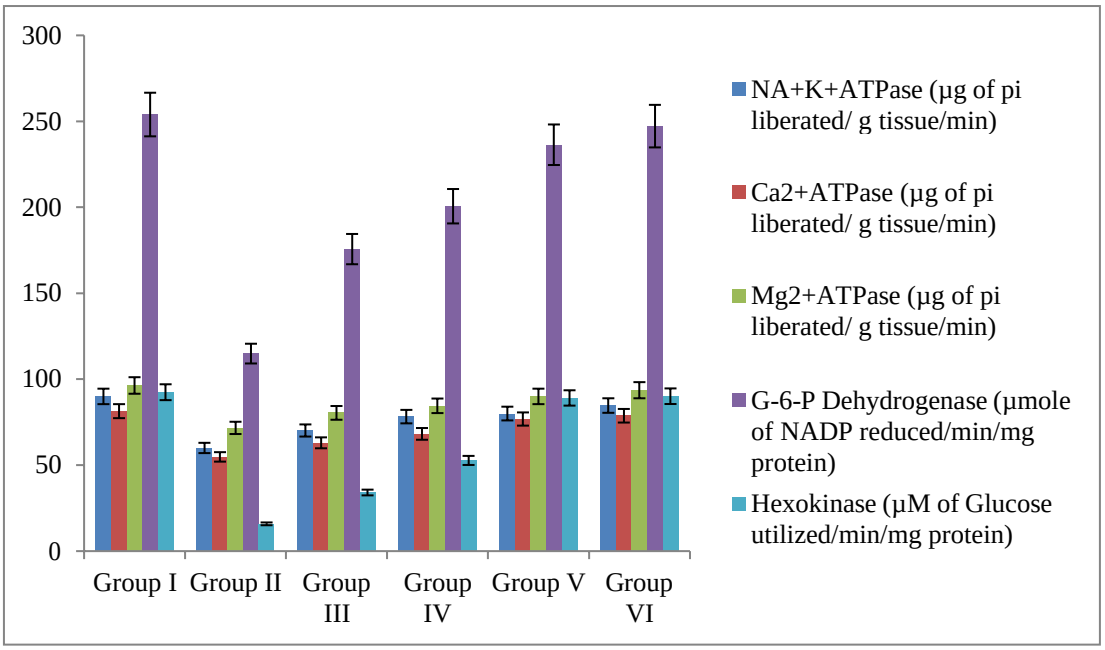
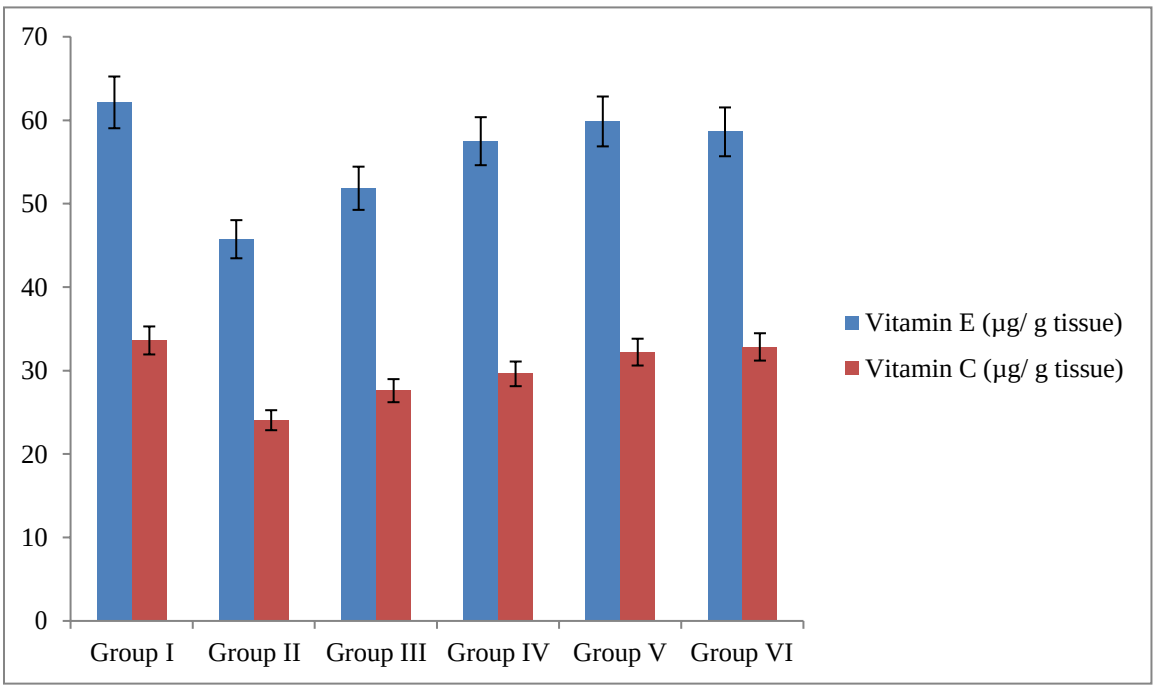


Group I – Positive Control
Group II – Negative Control
Group III – Plant extract/CCl4/100 mg/kg
Group IV – Plant extract/CCl4/200 mg/kg



Group I – Positive Control
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In this, the vitamin C shows increase in group VI parameters as 32.82 µg/g in tissue but in group II shows decrease in tissue sample. The vitamin E shows increase in group V and VI but there is a decrease in group III and IV, the least no was observed in group II. From this study, it is clear that there is increase in vitamin C and E which clearly shows the hepatoprotective activity. Vitamin C (Group IV) effectively prevented the oxidative damage induced by KBrO3, which decreased MDA concentration significantly in comparison to KBrO3 treated group. KBrO3 treatment decreased the levels of SOD and GSH significantly ($P<0.05$) in the liver. By contrast, increased levels of GSH and SOD were observed in Vitamin C plus KBrO3 treated group (Naglaa, *et al.*, 2016).

Conclusion

The present investigation indicates that *Eichhornia crassipes* shows promising properties for cytotoxicity and hepatoprotective activities against MCF-7, respectively. The cytotoxic and hepatoprotective role of Eichhornia crassipes is due to its polysaccharides. The study serves as a scientific data for the use of Eichhornia crassipes with CCl4 and Silymarin had exhibited significant activity of cytotoxic and hepatoprotective drug.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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