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Original Article

Hepato-Protective Efficacy of Purified Anthocyanin from *Begonia* species on Albino Rats Intoxicated with Carbon Tetrachloride and Paracetamol

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ABSTRACT

Hepatic toxicity means drug or toxin driven liver injury and often leads to mortality. Search of plant based therapeutic molecule is an immediate challenge for liver health and also to cure liver prone disorders. Begonia malabarica and B. rex-cultorum ('Baby rainbow') are commonly used by the local people for curing many disorders and the species are rich in anthocyanin. No scientific validation has been carried regarding the protective efficacy of anthocyanin of the species to treat hepatic diseases. The objective of the present study is to analyze and compare the efficacy of purified anthocyanin from the two species against carbon tetrachloride and paracetamol induced damage in liver and kidney. MS liquid medium supplemented with 2, 4-D (0.1 mg L–1) + BAP (0.5 mg L–1) and 2, 4-D (0.5 mg L–1) and BAP (1 mg L–1) showed well established suspension cultures from the friable callus of B. malabarica and B. rex-cultorum ('Baby rainbow') respectively on the day 14 of culture. The in vitro suspension of cells showed remarkable level of anthocyanin as compared to in vivo plants. The anthocyanin from cell suspension was extracted by acidified methanol followed by Amberlite XAD-7HP column purification and eluted using 75% ethanol. The purified anthocyanin was used for hepatoprotective studies in the adult Wistar albino rats. The activities of transaminases, alkaline phosphatase, lactic dehydrogenase were increased in serum after 48 h of toxicants administration. Further, elevated level of lipid peroxidation with decreased reduced glutathione content were noticed. Concomitantly, a decline was seen in the enzymatic activities of adenosine triphosphatase, glucose-6- phosphatase, succinic dehydrogenase, superoxide dismutase and catalase. Administration of purified anthocyanin from B. malabarica and B. rex-cultorum ('Baby rainbow') significantly regained the values of the above parameters towards normal and also restored the histopathological damages of the liver and kidney. Thus, it may be concluded that purified anthocyanin of begonias can be used to reduce the hepatorenal damage and may serve as an alternative effective medicine.

Keywords: Anthocyanin, Begonia malabarica , B. rex-cultorum ('Baby rainbow') , hepatoxicity, CCl4, paracetamol.

1. INTRODUCTION

Liver is one of the unique organs in the body, performing multiple roles in the regulation of diverse processes like metabolism, secretion, storage, and detoxification of xenobiotics. Because of these roles, liver injuries are one of

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the major threat to human health throughout the world. In spite of advances in current medicine, no absolute effective drugs were reported that induce hepatic function, offer protection of the organ, or facilitate hepatic cells regeneration. Thus, it is need of the hour to identify pharmaceutical alternatives for the treatment of liver prone diseases, with the aim of efficacy and less toxicity. From time immemorial, the use of herbals and the consumption of different fruits, vegetables have played roles in rejuvenating human health. Many scientific validations have documented that the many medicinal plants were beneficial due to the presence of an array of diverse phytochemicals.

Oxidative stress (OS) and dysfunction of cellular immunity were the markers of hepatic disorders of hepatic diseases lead the liver induced by xenobiotics. Anthocyanins and proanthocyanidins were widely reported compounds in fruits, vegetables and seeds. Specifically, the proanthocyanidins were phenolic compounds that have proved a broad range of biological effects. Shin *et al*.,[1] investigated the protective effects of these compounds against toxicity induced hepatic lesions in rats. Treatment with synthetic drugs caused significant increase in the levels of serum ALAT, ASAT, ALP and bilirubin but, on administrating proanthocyanidins orally (20 mg kg^{-1} daily over 28 days), regained these parameters, as well as the normalization of serum albumin and total protein levels and a reduction in the hepatic MDA level. Similarly, accumulation of hepatic toxin leads to liver-renal damages as seen in the histological analysis and was reduced remarkably in rats treated with proanthocyanidins.

Literature analysis reveals the effect of different phytochemicals on health related issues. Among the most frequently cited examples, the vinca alkaloids such as vincristine, vinblastine, and vindesine, the betalain pigments (betanin and indicaxanthine), the anthocyanins (cranberries), and resveratrol, all these have been proved based on their chemoprotective properties against cancer.[2,3,4] Many of the phytochemical investigations have been directed towards sedative, analgesic, antipyretic, cardioprotective, antibacterial, antiviral, antiprotozoal and anticarcinogenic capacities without validating their hepatoprotective potential. In this juncture, present study was designed to compare the hepatoprotective power of the purified anthocyanin extracted from cell suspension culture of *Begonia* species using rat model.

2. MATERIALS AND METHODS

Plant materials

The fresh healthy plants of *Begonia malabarica* and *B. rex-cultorum* ('Baby rainbow') were collected from the wild habitat and the voucher specimens were deposited in the herbarium of University College, Trivandrum (UCB 1207, UCB 1208). Identity was confirmed by referring floras and confirmed by authenticating with herbaria of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Kerala.

***In vitro* callus and cell suspension culture**

The sterilized leaf explants of *Begonia malabarica* (BM) were inoculated on MS medium supplemented with various concentrations and combinations of 2, 4-dichlorophenoxyacetic acid (2, 4-D) (0.1- 1 mg L^{-1}) alone and in combination with Benzylaminopurine (BAP) (0.5 mg L^{-1}) for callus induction. Meanwhile, leaf explants of *B. rex-cultorum* ('Baby rainbow') (BR) were inoculated on MS medium supplemented with different levels of BAP (1mg L^{-1}) + 2,4-D (0.5 mg L^{-1}), BAP (2.0 mg L^{-1}) + IAA (1.0 mg L^{-1}), KN (2 mg L^{-1}) + IAA (1 mg L^{-1}) and KN (2 mg L^{-1}) + 2,4-D (1 mg L^{-1}) combinations. The cultures were maintained in continuous light/dark photoperiod with temperature 25 ± 2 ^{0}C. The friable calli emerged from the explants were subcultured every 2 weeks in liquid MS medium with the same plant growth regulators supplemented as for the callus induction.

Extraction, purification and quantification of anthocyanin from cell suspension culture

Extraction of anthocyanin

The 25 g fresh cell suspension was homogenized with 50 ml of the extracting solvent (80 ml methanol + 0.1 ml concentrated HCl (0.5%) + 19.9 ml water) and kept overnight at 4°C. The extract was then filtered through a Buchner funnel using Whatman No. 1 filter paper and partitioned with ethyl acetate to free it from chlorophyll and stored in refrigerator. This experiment was carried out in methanol-HCl extracting system at different pH values (pH 1, pH 3, pH 4 and pH 5).

Purification of anthocyanin

The aqueous extracts obtained after the liquid-liquid partition was further purified using of Amberlite XAD-7 adsorption column chromatography.[5] Amberlite XAD-7 adsorbs the aromatic compounds including anthocyanins and other flavonoids in aqueous solutions, whereas free sugars and other polar non-aromatic compounds were removed by washing with distilled water until the eluted water has a neutral pH. Then the adsorbed anthocyanins were eluted using ethanol containing 7% acetic acid as mobile phase. [5]

Estimation of anthocyanin content

1g sample was homogenized in 3 ml methanol with 1% HCl and vortexed for 30 sec and kept in water bath at 60^o C for 20 min. Subsequently, the samples were centrifuged at 10000 rpm for 10 min. The supernatant was transferred to 10 ml volumetric flask. The residue was again mixed with 3 ml of methanol. The supernatant was again centrifuged and combined with the previous supernatant and made up to 10 ml. The final extract was kept at 0^{o}C for further analysis.

1 ml of extract was taken and transferred to 10 ml volumetric flask for preparing two dilutions of the sample, one adjusted with KCl buffer, pH 1.0 and the other with sodium acetate buffer pH 4.5. These dilutions were

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equilibrated for 15 min. The absorbance of each dilutions was read at 510 and 700 nm against distilled water as blank.[6]

Hepatoprotective analysis

Experimental animals

Studies were carried out by using adult Wistar albino rats weighing 150-200 g were purchased from Mahaveera agencies, Hyderabad, India with a prior permission from institutional animal ethical committee (CPCSEA Reg.No. 204/2017) and was used for the entire studies.

Animals were housed under standard conditions (25 ± 2 °C, 60-70% relative humidity and 12 h light and 12 h dark). The rats were fed on standard pellet diet (M/s Venkateshwara Feeds, Bengaluru) and water *ad libitum*. Animals were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India, Ministry of Environment & Forests (Animals Welfare Division), Chennai. Experiments were carried according to the Organization for economic co-operation and development (OECD) 420 guidelines.[7] The rats were randomly allocated to six animals in each group and treated as below.

Experimental design

The animals were administered paracetamol at a dose of (2 g kg^{-1}, p.o.) and CCl_4 at a dose of (1.0 ml kg^{-1}, i.p.) followed by therapeutic agents at effective doses for 24 h and silymarin treatment was considered as positive control. The whole set of experiment was divided into seven groups with six animals each.

Experiment No. 1: CCl_4

Group 1: Normal control (single daily dose of liquid paraffin1 mL kg^{-1} b.w p.o.) • Group 2: Positive control [30% CCl_4 in liquid paraffin (1 mL kg^{-1} b.w, i.p)] • Group 3 & 4: Sample BM (200/400 mg kg^{-1},b.w.) +[30% CCl_4 in liquid paraffin (1 mL kg^{-1} b.w, i.p)] • Group 5&6: Sample BR (200/400 mg kg^{-1},b.w.) + [30% CCl_4 in liquid paraffin (1 mL kg^{-1} b.w, i.p)] • Group 7: Standard group (Silymarin, 25 mg kg^{-1} p.o.) + [30% CCl_4 in liquid paraffin (1 mL kg^{-1} b.w, i.p)].CCl_4 was administrated simultaneously. Treatment duration was 7 days.

Experiment No. 2: paracetamol 2g/kg

Group 1: Normal control (only saline, p.o.) • Group 2: Positive control [2g kg^{-1} paracetamol] • Group 3 & 4: Sample BM (200/400 mg kg^{-1} ,b.w.) +[2g kg^{-1} paracetamol] • Group 5&6: Sample BR (200/400 mg kg^{-1},b.w.) + [2g kg^{-1} paracetamol]• Group 7: Standard group (Silymarin, 25 mg kg^{-1} p.o.) + [2g kg^{-1} paracetamol]. Paracetamol was given simultaneously with anthocyanin extract. Duration of treatment was 7 days. Rats were sacrificed 48 h after the last dose.

Biochemical assays

Blood samples of rats were collected by puncturing the retro- orbital venous sinus and serum was processed for the estimation of blood and tissue parameters.[8] Albumin, bilirubin, urea and creatinine concentration in serum was measured by the protocol prescribed by the diagnostic kits.

The blood was collected from the retro orbital plexus of the rats of all groups at the end of 7th day, 48 h after administration of CCl_4 and paracetamol under Isoflurane anesthesia. The blood samples were allowed to stand for 30 min at room temperature and then centrifuged at 3000 rpm for 15 min to separate the serum. The serum was analyzed for various biochemical parameters such as Aspartate aminotransferase (AST), Alanine amino transaminase (ALT), Alkaline phosphatase (ALP), Succinic dehydrogenase (SDH), Lactic dehydrogenase (LDH), Glucose-6-phosphatase, ATPase Total Bilirubin (TBL), Direct Bilirubin (DBL), Triglycerides (TGL), Cholesterol (CHOL), Glucose (GLU), Creatinine (CRT), Albumin (ALB), glycogen and Total Protein (TP). Similarly, superoxide dismutase (SOD), catalase (CAT), ascorbate, glutathione (reduced) and lipid peroxidation were also measured. RBC, WBC and Hb levels were also monitored. The body weight (before and after treatment), liver weight and pro-thrombin time were recorded. The animals were then dissected and the livers and kidneys were carefully removed and washed with 0.9% saline solution. A part of the liver and kidney sample was preserved in formalin solution (10% neutral buffered formalin) for histopathological light microscopic studies.

STATISTICAL ANALYSIS

The data were expressed as mean ± SD. Statistical significance of difference between various treatments were analyzed by Students 't' test followed by one-way analysis of variance (ANOVA). *P* values ≤ 0.05 were considered as statistically significant.

3. RESULTS AND DISCUSSION

Cell suspension culture of *B. malabarica* and *B.rex-cultorum* ('Baby rainbow')

In the present investigation, cell suspension culture was established by culturing fresh friable calli from the leaf explants of *B. malabarica* in liquid MS media supplemented with definite combinations and doses of BAP, 2, 4-D and NAA (0.5, 0.1, 0.5 mg L^{-1} respectively). MS liquid medium supplemented with 2, 4-D (0.1 mg L^{-1}) and BAP (0.5 mg L^{-1}) showed well established suspension cultures i.e., suspensions without any aggregation or clumps of cells. The *in vitro* suspension of cells from *B. malabarica* revealed optimal and steady biomass accumulation on day 14. The same medium and growth hormone combination was used for the analysis of growth kinetics. After 16th day, cells in the suspension exhibited a negligible reduction in fresh as well as dry weight of cells. The maximum fresh weight (8.0 g) and dry weight (0.85 g) was noticed from the day 14 of culture in liquid MS medium complimented with 2, 4-D (0.1 mg L^{-1}) + BAP (0.5 mg L^{-1}). The time course of biomass accumulation was the typical sigmoid growth curves.

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The cell suspension cultures showed continuous and stable accumulation of biomass within 14 days at temperature 25±2°C having photoperiod of 16-8 h at 80 rpm in the liquid MS medium supplemented with the same combinations of the growth regulators as those used in callus culture for both the species. Further, the cell suspension culture was evaluated by culturing calli of *B.malabarica* in the liquid MS medium fortified with the combinations of BAP (0.5, 1, 1.5 mg L^{-1}) and NAA (0.1, 0.5, 1.0 mg L^{-1}). MS liquid medium supplemented with NAA and BAP showed less significant suspension cultures with aggregated or clumped cells. Generally, the cell growth was slow during the initial 4 days of cultivation. Thereafter, biomass accumulated rapidly, and reached the highest value on the 14th day. Then the culture entered the stationary phase and declined marginally. Some cultures continued to grow even up to 30th day. Similarly, suspension cultures were also initiated for *B. rex-cultorum* ('Baby rainbow') with 2 g of friable callus as an inoculum in the liquid MS medium supplemented with the different combinations of the growth regulators ie., BAP (1 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}), BAP (2.0 mg L^{-1}) + IAA (1.0 mg L^{-1}), KN (2 mg L^{-1}) + IAA (1 mg L^{-1}) and KN (2 mg L^{-1}) + 2,4-D (1 mg L^{-1}). Maximum growth was achieved in suspension culture supplemented with BAP (1 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}), followed by KN (2 mg L^{-1}) + IAA (1 mg L^{-1}). BAP (2.0 mg L^{-1}) + IAA (1.0 mg L^{-1}), KN (2 mg L^{-1}) + 2,4-D (1 mg L^{-1}) combinations showed only minimum outputs. The maximum fresh weight (7.4 g) and dry weight (0.70 g) was observed at 14th day of culture in liquid MS medium supplemented with 2, 4-D (0.5 mg L^{-1}) and BAP (1 mg L^{-1}). Here also, the cell cycle was sigmoidal.

Quantification and fractionation of anthocyanin content

Anthocyanin was isolated and quantified from the *in vitro* cell suspension and *in vivo* plants. The *in vitro* cells showed remarkable level of anthocyanin ie., 10.4 and 20.6g/100 ml for *Begonia malabarica* and *B. rex-cultorum* ('Baby rainbow') respectively as compared to the *in vivo* anthocyanin content noticed (5.7 and 9.8 mg g^{-1} for *Begonia malabarica* and *B. rex-cultorum* ('Baby rainbow')). From the given results it can be speculated that anthocyanin content may be effectively induced through *in vitro* culture by changing the culture parameters. The present results seem to be more effective and supported by *in vitro* cultures of phytochemicals from medicinal plants such as *Psoralea corylifolia*, *Coleus aromaticus* and *Lantana camara*.[9,10,11]

Purification of anthocyanin

25 g of fresh, homogenized cell suspension sample was extracted out in duplicate. Methanol-HCl system yields higher extraction due to their higher polarity and penetrability to diffuse into the cell membranes at pH 2.0 i.e., low pH may increase the permeability of the membrane leading to higher diffusion coefficient values for the extracting solvent.

The crude anthocyanin was purified initially by liquid-liquid partition using ethyl acetate followed by amberlite XAD-7HP adsorbent chromatography. The adsorbed anthocyanin was eluted using acidified ethanol. 50% and 75% acidified ethanol could elute around 70.4 and 72.5 % of anthocyanins from the column. There was not much difference between ethanol concentration of 50% and 75%. Meanwhile, in terms of anthocyanin elution the elution time periods were 78 and 50 min with ethanol concentration of 50 and 75% respectively. Therefore, considering all these reasons, acidified ethanol with the concentration of 75% was most efficient of the studied eluents for desorption of anthocyanins.

Subsequently, the amberlite column eluted anthocyanin from *B. malabarica* and *B. rex-cultorum* ('Baby rainbow') was fractionated using LC-MS/ MS analysis.

LC- MS was successful in identifying the major anthocyanins of *B. malabarica* and *B. rex-cultorum* ('Baby rainbow'). The major anthocyanin fractions from the two species were eluted between 4.7 to 5.4 min. Tandem MS of the *m/z* 655.3 peak was identified as the major anthocyanidin fraction namely, malvidin-3,5–diglucoside in *B. malabarica*. The other peaks identified were 3-Hydroxy phloretin-2'-O-xylosil glycoside (584.3), 3',5'-Bideoxy delphinidin-3-glycoside (459.2), Pelargonidin- 3-arabinoside (403.2) and 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-chromen-4-one (287.1). The others were sugar derivatives or minor fragments (m/z 234.2, 195.1 & 144.1). Similarly, the major anthocyanin fractions of *B. rex-cultorum* ('Baby rainbow') were similar to the anthocyanins of *B.malabarica* such as (655.3) malvidin-3,5-diglucoside, (584.3) Hydroxy phloretin-2'-O-xylosil glycoside, (468.4) 3',5'-Bideoxy delphinidin-3-glycoside, (403.2) Pelargonidin- 3-arabinoside, (286.2) 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-chromen-4-one and others (242.3, 195.1 & 144.1) may be sugar derivatives or insignificant fragments.

Hepatoprotective stuides

The ameliorative effect of CCl_4 and paracetamol induced hepatotoxicity in the rats by *Begonia malabarica* (BM) and *B. rex-cultorum* ('Baby rainbow') (BR) on organ weight showed variation. There were no significant difference between the weight of heart, liver, right kidney, left kidney, right testis and left testis in the positive control group and the negative control group (CCl_4 and paracetamol). Treating the hepatotoxic rats with BM and BR significantly ($P < 0.05$) increased the weight of heart, liver, right testis and left testis when compared with the positive control group, whereas the weight of right and left kidneys were non significantly changed. The CCl_4 intoxicated rats showed significant increase in prothrombin time (48.59 sec) and was regained to normal value with anthocyanin treatments.

Hepatospecific enzymes

Table 1 a & b displays the effect of purified anthocyanins from *Begonia malabarica* (BM) and *B. rex-cultorum* ('Baby rainbow') (BR) simultaneously administrated with CCl_4 or paracetamol on liver function tests. Administration of CCl_4 and paracetamol elevated the levels of hepatospecific

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enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) in serum indicating acute hepatocellular damage.

Pre-treatment with *Begonia malabarica* (BM), *B. rex-cultorum* ('Baby rainbow') (BR) and silymarin 25 mg kg^{-1} p.o recouped the level of all the parameters in their respective groups including the histopathological changes in the liver associated CCl_4 and paracetamol induced hepatic damages. Among them, percentage protection shown by BM with 200 and 400 mg kg^{-1} b.w.p.o. was remarkable than that of BR. Interestingly, the anthocyanin of *B. malabarica* (BM) results were well comparable to that of reference drug, silymarin (25 mg kg^{-1}).

Liver total protein, albumin, bilirubin and glucose (GLU)

Administration of CCl_4 and paracetamol caused an increase in the amounts of total bilirubin (TBL), direct bilirubin (DBL), glucose (GLU), and decreased of albumin (ALB) and total protein (TP) in serum, indicating acute hepatocellular damage. Pretreatment with BM, BR and silymarin restored their level more or less at par with control (Table 1 a & b; 2 a& b).

Glycogen and glucose-6-phosphatase

There was significant loss of the glycogen content and glucose-6- phosphatase activity in liver caused by CCl_4 and paracetamol exposure (Table 2 a & b). Parameters were recouped significantly with the BM, silymarin and BR ($p \leq 0.05$) however the effect was more pronounced with BM and silymarin in glycogen and G-6-Pase after the CCl_4 and paracetamol exposure. Significance at 5 % level ($P \leq 0.05$).

Urea, uric acid, creatinine, triglyceride and cholesterol

Table 2 (a & b) reveals profound rise in the triglycerides and cholesterol levels after CCl_4 and paracetamol toxicity ($P \leq 0.05$). Urea, uric acid, creatinine also showed a similar trend. Mean values of BM, silymarin and BR treated groups regained all the parameters. However, BM showed maximum protection and the values were almost at par with silymarin treated group. Analysis of variance showed significant recoupment at 5% level in all the blood biochemical indices.

Adenosine triphosphatase (ATPase) succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH)

Significant decline was observed in the activity of ATPase, SDH and LDH after CCl_4 and paracetamol administration as observed. Pretreatment with anthocyanin of BM, BR and silymarin restored the reduced values to normal and blocked the release of enzymes from the liver significantly. BM and silymarin showed better protective effect when compared with BR. The F values were remarkable with CCl_4 and paracetamol treated rats.

Superoxide dismutase (SOD), Glutathione reductase (GR) and catalase (CAT)

Administration of CCl_4 and paracetamol caused significant decrease in the hepatic SOD, GR CAT enzymatic activities after 48 h of intoxication (Table 3 a & b). Treatment with BM and silymarin showed significant improvement in the SOD, GR and CAT activities. BR showed less significant improvement in the enzymatic activity (Table 3 a & b).

Lipid peroxidation (LPX) and reduced glutathione (GSH)

The lipid peroxide level (MDA content) in the tissues (liver and kidney) from CCl_4 and paracetamol toxicity group was significantly ($P \leq 0.05$) higher than the respective control values. BM, silymarin and BR showed marked reversal in the lipid peroxidation level. Anthocyanin treatment displayed significant alterations by recouping the values in kidney and liver by reducing the LPX level. Reduced glutathione is an endogenous defense against peroxidative damage of cellular membranes. Thus, remarkable decline in the reduced glutathione level ($P \leq 0.05$) was seen with CCl_4 and paracetamol treated groups. Treatments with BM, silymarin and BR maintained the GSH level ($P \leq 0.05$), which has been substantially decreased the CCl_4 and paracetamol intoxication. Analysis of variance showed significant recoupment at 5% level in all the parameters.

Histopathological analysis using LM on liver and kidney

Liver sections of control rats showed normal histological characters (Fig 1 and 2). Meanwhile, after CCl_4 and paracetamol administration revealed hydropic degeneration of liver cells with swelling and empty cytoplasmic areas (Fig.1 c,d and 2 c,d) followed by congestion in central veins, portal vessels, and sinusoids. Treatments with BM, silymarin and BR (400/25 mg kg^{-1}, p.o.) showed reversion. Angular hepatocytes with more or less normal nuclei, hepatic lobules appeared normal except marginal scattered degenerated cells (Fig.1 e-j and 2 e-j). Pretreatment showed significant hepatoprotection, exhibiting reduction in centrilobular necrosis and maintained the chord arrangements to normal (Fig. 1 and 2).

Kidney sections of control rats showed typical structural integrities (Fig.3 and 4). Meanwhile, the kidney exposed to CCl_4 and paracetamol showed diverse levels of degeneration. Most of the epithelium lining of the collecting tubules had been collapsed into the lumen revealing the toxicity of the compound (Fig. 3 and 4). Treatments with BM, silymarin and BR reduced the severe lesions induced by CCl_4 and paracetamol markedly by the 7 days administration (400/25 mg kg^{-1}, p.o.) group, which further substantiates the results of the biochemical parameters. Renal portions of rats showed the conspicuous recovery process with almost normal distal, proximal tubules and the lumen was clear. The renal corpuscles appeared almost normal. Glomeruli were intact with wide spacing in the Bowman's capsule (Fig. 3 and 4). Histopathological data strongly substantiates the liver serum and hematological results.

Rat experimental animal models have been employed to evaluate hepatoprotectivity against hepatotoxicants viz. CCl_4, paracetamol, rifampicin and acetaminophen. The liver dominates the role in metabolism with multiple functions in the body, including glycogen storage, decomposition of

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RBCs, plasma protein synthesis, and detoxification. Many clinically proven synthetic drugs can cause cellular dysfunction through metabolic induction of highly reactive molecules like free radicals, ROSs, carbenes and nitrenes.[12] Thus, the aim of the present study was to explore whether or not anthocyanin of *Begonia malabarica* (BM), *B. rex-cultorum* ('Baby rainbow') (BR) could prevent the hepatic damage caused by CCl_4 and paracetamol, a model hepatotoxicants. Hepatic cells were involved in a wide array of metabolic events due to the presence of enzymes like AST, ALT, ALP, LDH. AST and ALT found in the cytoplasm. AST also occur in mitochondria whereas ALT is unique in liver cells. ALP mainly arises from the lining of the canaliculi and sinusoidal surface of hepatocyte.[13] The enzymes ALT, AST and ALP showed decrease in activity with increase in concentration of anthocyanin. Rats treated with BM (400 mg kg^{-1}) produced ALT (40.4 U L^{-1}), AST (39.5 U L^{-1}) and ALP (65 U L^{-1}) activities at par with the values of the standard silymarin (ALT - 41.59 U L^{-1}, AST - 43.8 U L^{-1} and ALP - 65.9 U L^{-1}). But, rats treated with BR at the same concentration showed an elevated level of enzyme activities. Thus, the treatment with *Begonia malabarica* (BM), *B. rex-cultorum* ('Baby rainbow') (BR) recalled the activities of transaminases and alkaline phosphatase thus recouped them towards normal values depicting the functional status of the liver and thus substantiating the hepatoprotective role. Rats treated with BR/BM showed no visible hepatocellular necrosis. Marginal ballooning and binucleate cells were noticed in rats treated with BM (400 mg kg^{-1}).[14] Binucleate cells in liver indicate the regeneration of hepatic cells. Remarkable increase in urea and creatinine levels by the toxicants indicates the dysfunctional and dystrophic changes accounted in the liver and kidney. In the present study the creatinine levels decreased with the administration of BM (0.65 and 0.71 mg dl^{-1}) and BR (0.68 and 0.76 mg dl^{-1}) to the rats at concentrations 200 and 400 mg kg^{-1} respectively which was comparable to the standard silymarin. Due to acute renal malformation, excretion of urea fails and its serum concentration increase rapidly. Lipid peroxidation (LPX), an autocatalytic reaction leads to membrane peroxidation and subsequent cell death. It involves alterations, degeneration and leads to the development of other disorders of lipoprotein metabolism in the liver and also in the peripheral tissues.[15] Reduced glutathione (GSH) is one of the abundant tripeptide (y-glutamyl cysteinyl glycine) distributed actively in cells. Reduction of GSH was noticed in the toxicant treated hepatocytes and RBCs. Liver is the major site of GSH synthesis. The detoxification of drugs and other xenobiotics in the liver takes place via GSH due to their intra-cellular reductant and play role in catalysis, metabolism and transport. Treatment with *Begonia malabarica* (BM), *B. rex- cultorum* ('Baby rainbow') (BR) boosted the GSH levels. The present results were supported by the administration of active principles isolated from *Polygonum bistorta* on albino rats intoxicated with carbon tetrachloride and paracetamol.

Proteins were synthesized in liver and its reduction suggests liver damage. CCl_4 and paracetamol application leads to significant protein loss in liver. Treatment with *Begonia malabarica* (BM), *B. rex- cultorum* ('Baby rainbow') (BR) ameliorates CCl_4 / paracetamol induced alterations in LDH, SDH and ATPase activities in the liver of rats. The ameliorative effect of therapy might be due to its flavanoid nature having antioxidative potentiality. Flavanoids were antioxidants and protect membrane lipids and unsaturated fatty acids against oxidative damages. Crude *Nigella sativa* oil safe guarded the male rats against CCl_4 induced hepatotoxicity.[16] The crude extracts of *Marsilea minuta* [17] was also proved as hepatoprotecants. Glycogen was the auxiliary storage source, tapped and degraded into glucose during energy need. The maintenance of glycogen reserve was a specific attribute of the liver cell. Many enzymes were involved in the anabolism and catabolism of glycogen. Deviation of carbohydrate metabolism was one of the biochemical lesions noticed during liver toxicity. G-6-Pase, a crucial enzyme of glucose homeostasis and plays an important role in the regulation of the blood glucose level. Liver glycogen phosphorylase functions as the sensor of liver i.e., glycogenolysis whenever the level of blood glucose falls. Subramanian and Selvam[18] reported that CCl_4 caused significant decline in the G-6-Pase activity. This has been used for analysis of genotoxicity and for monitoring DNA damages.[19] Paracetamol was mainly metabolized in liver and excreted as conjucates of glucuronide and sulphate. Meanwhile, the paracetamol hepatotoxicity has been due to the formation of toxic xenobiotics through hepatic cytochrome P-450 into a toxic reactive N-acetyl- P-benzoquinoneimine (NAPQI). NAPQI is initially removed by GSH in to mercapturic acid. But, when the rate of formation exceeds the rate of detoxification, it oxidizes biomolecules like lipid or –SH group of protein and alters the homeostasis of calcium protein components.

The experimental intoxication induced by CCl_4 was widely used for remodeling liver injury in animals using plant based compounds like the methanol extract of *Oldenlandia umbellata*.[20] Free radicals or ROS/RNS initiate the lipid peroxidation, which was generally caused by the impairment of enzyme activity. It is generally known that the hepatotoxicity is the result of reductive dehalogenation, which is carried by P-450, and forms reactive trichloromethyl free radicals. This interacts with oxygen to form the trichloromethyl peroxy radicals. Both these free radicals are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating peroxidation, liver damage and indirectly to pathogenesis. Bilirubin, a metabolic by-product of hemoglobin, conjugates with glucuronate in hepatocytes to enhance its water solubility. Serum bilirubin represents a marker for measuring the hepatic function, and any

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abnormality in the levels of serum bilirubin refers hepatobiliary disorders and severe hepatocellular malfunction. Decreased serum bilirubin level following anthocyanin treatment indicated the effectiveness of the plant in restoring normal liver functional status. Crude extract of *Homalium letestui*, *Luffa acutangula*, *Cedrelopsis grevei* and *Xylopia aethiopica* were previously documented as hepatotonic against liver prone disorders.[15,14,13,12] Thus, the entire data in the present study provides amble evidence for the local uses of *Begonia malabarica* and *B. rex-cultorum* ('Baby rainbow') in the treatments of hepatic damages. Further studies are warranted at large scale on pharmacological evaluation of the efficacy of anthocyanins from *Begonia* species.

Table 1a: The hepatoprotective effect of purified anthocyanin from *Begonia malabarica* (BM), *B. rex- cultorum* ('Baby rainbow') (BR) against CCl_4 induced hepatotoxicity in rats on liver enzymes, total proteins, albumin and total bilirubin

	G I	G II	G III	G IV	G V	G VI	G VII
ALT (U L^{-1})	32.7	69.1	43.4	40.4	45.6	40.5	41.5
AST (U L^{-1})	36.3	65.2	45.7	39.5	49.8	42.3	43.8
ALP (U L^{-1})	60.8	114	67.8	65	70.2	66	65.9
TP (g dL^{-1})	7.65	4.81	10.6	12.3	11.0	14.5	12.61
Albumin g/ 100 mL	4.50	2.55	7.8	6.5	9.2	7.6	4.29
Total Bilirubin mg dL^{-1}	0.42	1.38	0.59	0.48	0.64	0.5	0.53
Direct BL (mg dL^{-1})	0.321	1.42	0.45	0.4	0.49	0.44	0.39
F	287.44**	253.04*	357.39*8	363.55**	379.57**	385.71**	380.42**
SE-T	0.81						
SE-V	0.60						
CD-T	3.881						
CD-V	2.258						

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes.

G I: Normal control (only saline, p.o.) • Group II: Control [CCl_4/paracetamol] • Group 3&4: Sample BM (200/400 mg kg^{-1},b.w.) +[CCl_4/paracetamol] • Group 5&6: Sample BR (200/400 mg kg^{-1},b.w.) + [CCl_4/paracetamol]• Group 7: Standard group (Silymarin, 25 mg kg^{-1} p.o.) + [CCl_4/paracetamol]

Table 1b: The hepatoprotective effect of purified anthocyanin from *Begonia malabarica* (BM), *B. rex- cultorum* ('Baby rainbow') (BR) against paracetamol induced hepatotoxicity in rats on liver enzymes, total proteins, albumin and total bilirubin

	G I	G II	G III	G IV	G V	G VI	G VII
ALT (U L^{-1})	32.7	128.1	39.6	40.5	40.3	42.6	50.55
AST (U L^{-1})	36.3	159.46	40.1	45.3	44	50.1	49.65
ALP (U L^{-1})	60.8	375.68	65.5	69.6	66.7	71.2	70.2
TP (g dL^{-1})	7.65	4.51	10.3	11.6	10.9	12.5	8.08
Albumin g/ 100 mL	4.50	1.95	4.9	5.1	5.0	5.4	3.98
Total Bilirubin mg dL^{-1}	0.42	2.74	0.51	0.57	0.53	0.60	0.51
Direct BL (mg dL^{-1})	0.321	0.59	0.34	0.38	0.35	0.4	0.30
F	297.56**	276.30*	352.72**	345.22**	369.55**	357.03**	345.75**
SE-T	0.91						
SE-V	0.38						
CD-T	2.821						
CD-V	1.580						

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes.

Table 2a: Hepatoprotective potential of purified anthocyanin from *Begonia malabarica* (BM), *B. rex- cultorum* ('Baby rainbow') (BR) against CCl_4 induced hepatotoxicity in rats on renal functions

	G I	G II	G III	G IV	G V	G VI	G VII
GLU(mg dL^{-1})	86.9	189.4	91.2	99.6	90.7	112.3	97.06
TG (mg dL^{-1})	80.32	132.51	83.6	90.2	85.3	93.6	88.61
CHOL (mg dL^{-1})	90.32	133.55	92.4	97.5	96.2	100.3	92.1
Prothrombin time (sec)	18.95	48.59	20.1	21.3	18.6	21.9	21.74
Glycogen mg g^{-1}	609	350	515	600	522	589	607
F	987.45**	874.56**	901.31**	1049.71**	995.37**	1053.87**	1054.34**
SE-T	1.62						
SE-V	1.77						
CD-T	4.839						
CD-V	2.672						

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes.

Table 2b: Hepatoprotective potential of purified anthocyanin from *Begonia malabarica* (BM), *B. rex- cultorum* ('Baby rainbow') (BR) against paracetamol induced hepatotoxicity in rats on renal functions

	G I	G II	G III	G IV	G V	G VI	G VII
GLU(mg dL^{-1})	86.9	201.93	89.2	91.3	90	94	102.81
TG (mg dL^{-1})	80.32	125.70	83	89	85	92	78.63
CHOL (mg dL^{-1})	90.32	84.43	92.5	97.6	95.2	100	73.63
Prothrombin time (sec)	18.95	48.59	20.2	18.4	21.2	19.2	21.74
Glycogen mg g^{-1}	608	322	499	623	510	592.7	611
F	874.34**	852.59**	985.34**	991.83**	990.42**	996.21**	987.43**
SE-T	1.46						
SE-V	1.84						
CD-T	4.881						
CD-V	2.693						

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes.

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Table 3a: The effect of treating CCl_4 induced hepatotoxicity in rats with purified anthocyanin from *Begonia malabarica* (BM), *B. rex- cultorum* ('Baby rainbow') (BR) on CAT, SOD and GSST in serum and liver tissue homogenate

	G I	G II	G III	G IV	G V	G VI	G VII
Serum Catalase (S.CAT) U L^{-1}	2.77	0.13	3.3	4.39	3.1	5.2	5.0
Serum Superoxide dismutase (S.SOD) U mL^{-1}	626.11	215.31	644.30	682.5	629.5	710	694.5
Serum Glutathione reductase (S.GSST) U mL^{-1}	717.81	246.68	731.6	769.7	725	810.2	720
CAT U g^{-1}. Liver tissue	5.53	0 .18	5.59	7.4	6.2	7.7	5.8
SOD U g^{-1}. Liver tissue	815.06	213.81	845.80	892	825.3	910	868.6
F	585.29**	529.38**	578.21**	582.48**	581.20**	595.99**	593.19**
SE-T	1.66						
SE-V	0.71						
CD-T	2.739						
CD-V	1.181						

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes

Table 3b: The effect of treating paracetamol induced hepatotoxicity in rats with purified anthocyanin from *Begonia malabarica* (BM), *B. rex-cultorum* ('Baby rainbow') (BR) on CAT, SOD and GSST in serum and liver tissue homogenate

	G I	G II	G III	G IV	G V	G VI	G VII
Serum Catalase (S.CAT) U L^{-1}	2.77	0.13	2.9	3.1	3.0	3.5	3.0
Serum Superoxide dismutase (S.SOD) U mL^{-1}	626.11	215.31	631.50	667	638	692	640
Serum Glutathione reductase (S.GSST)U mL^{-1}	717.81	246.68	727	810	720	846	729
CAT U g^{-1}. Liver tissue	5.53	0 .18	6.5	7.4	6.6	8.2	5.9
SOD U g^{-1}. Liver tissue	815.06	213.81	845.80	890	869	902	829
F	518.77**	429.27**	518.34**	538.52**	529.17**	547.01**	540.19**
SE-T	1.59						
SE-V	0.88						
CD-T	2.182						
CD-V	1.320						

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes.

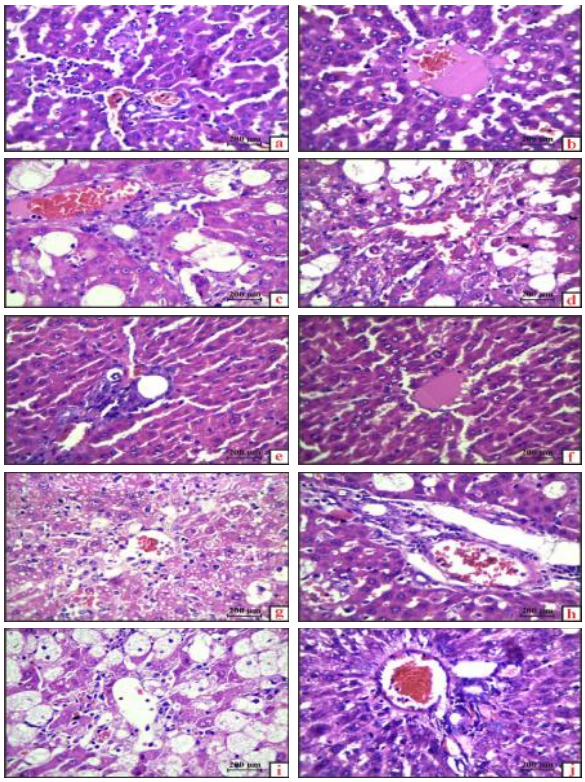

Fig 1: a - j. Hepatoprotective activity of *Begonia malabarica* (BM) and *Begoniarex-cultorum* ('Baby rainbow') (BB)

a & b. Normal Control; c & d. Positive control [CCl_4 + Liquid paraffin]; e & f. Standard treated [Silymarin]; g & h. Anthocyanin of BM and BB – 200 mg kg^{-1}; i & j. BM and BB – 400 mg kg^{-1}

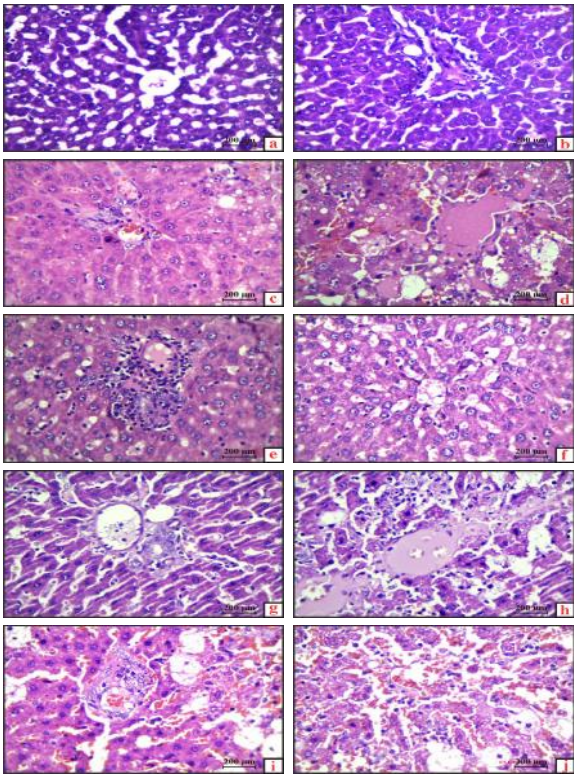

Fig 2: a - j. Hepatoprotective activity of *Begonia malabarica* (BM) and *Begoniarex-cultorum* ('Baby rainbow') (BB)

a & b. Normal Control; c & d. Positive control [Paracetamol + Liquid paraffin]; e & f. Standard treated [Silymarin]; g & h. Anthocyanin of BM and BB – 200 mg kg^{-1}; i & j. BM and BB – 400 mg kg^{-1}

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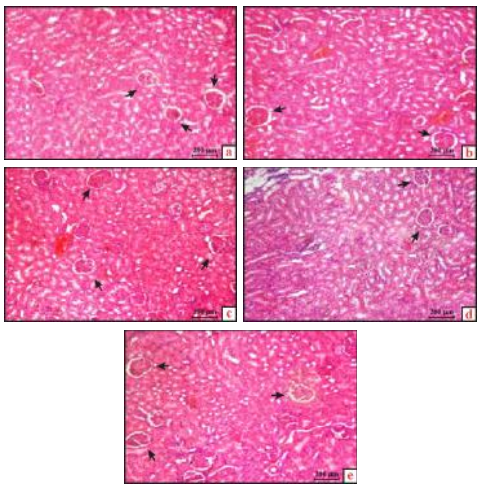

Fig 3: a-e: Photomicrographs of kidney sections from
a. Control rats showing normal histological features. The section indicates a detailed cortical parenchyma and the renal corpuscles appear as dense rounded structures.
b. Rats treated with CCl_4 only showing severe distortion and disruption in microanatomy of the renal cortex, including queried edema. The kidney sections were stained with H/E and observed with a 10X objective.
c. Rats treated with 200 mg kg^{-1} bw. BM and CCl_4 showing some distortion and disruption in micro anatomy of the renal cortex, including mild queried edema, although with prominent renal corpuscles.
d. Rats treated with 200 mg kg^{-1} bw BB and CCl_4 showing some recovery in micro anatomy of the renal cortex.
e. Rats treated with 400 mg kg^{-1} b.w BM and CCl_4 showing some mild degree of distortion and disruption in microanatomy of the renal cortex

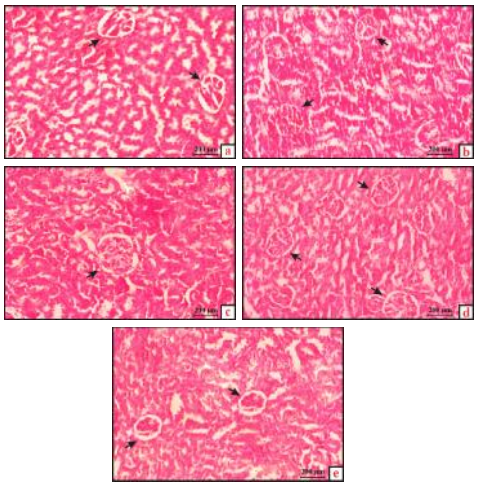

Fig 4: a-e: Histological appearance of the kidney (HE staining)
a. Section of control kidney showing a normal histological appearance glomeruli and tubules (arrows) appear normal (× 100), with higher magnification section (× 200);
b. Renal cortex in paracetamol group. Vacuolization, atrophy and detachment of tubular epithelial cells (arrows) seen (× 100).
c. Renal cortex in paracetamol (24 h) group. Segmental glomerular necrosis (arrows) seen, tubular dilation (arrows) and detachment of tubular epithelial cells also visible (× 100);
d. Renal cortex in paracetamol + BM group, glomeruli and tubules (arrows) appear normal in cortex (× 200);
e. Renal cortex in paracetamol + BB group, glomeruli and tubules (arrows) appear normal seen in cortex (× 100)

4. CONCLUSION

The present study substantiated the liver hepatotoxicity by CCl_4 and paracetamol by the rise of liver specific parameters (enzymic and other analytical) and the histopathogical damages in the liver and kidney tissues of rats. Administration of purified anthocyanin from *B. malabarica* and *B. rex- cultorum* ('Baby rainbow') on hepatotoxic rats protected the liver structure against CCl_4 and paracetamol induced toxicity positively. This hepatoprotective activity may be attributed to the biologically active anthocyanins that scavenge free radicals. So, the current study substantiates that the usage of the coloured extracts from the species for curing liver prone disorders. Future studies are warranted to identify the active fraction(s), to evaluate the efficacy and toxicity in various models with the mode of action for developing it as a safe and effective herbal hepatoprotective compound.

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