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**Research Article**

# Hepatic Regeneration by Basila Alba Fruit Extract against Chromium (VI) Induced Toxicity

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## ABSTRACT

**Objective:** The main purpose of this study is to analyze the hepato-regenerative and antioxidant capacity of Basila alba fruit extract (BFE) against Chromium (Cr) induced oxidative stress in albino mice (Mus musculus).
**Study Design:** Experimental Histopathological Study.
**Place and Duration:** That study was design in The Sargdha University and whole experimental work was completed from 2013 to 2016.
**Methods:** Thirty 3-4 months old male mice used that fed on standard diet and divided (n=10) as Con; Control, get drinking water, Cr; 50ppm Cr in drinking water (10 days), Cr-B; 50ppm Cr (10 day) followed by 0.2ml/12h BFE (5 days). All animals were recovered on 16$^{th}$ day for histpatholgical study of liver.
**Results:** Hepatic tissue cracks, derangements in hepatic cords, hepatic nuclear adduct formation with less no. of oval cells in Cr-exposure group. BFE enhanced number of regenerative cells oval and cholangiocytes evident to move towards the damage crack areas and establish the aggregations and leads liver regeneration.
**Conclusions:** The progenitor and cholangiocyte involve in rehabilitation of liver architecture with Basella alba consumption revitalizes apoptosis in hepatocyte and enhance hepatogenesis following exposure to environmental toxicant like Cr (VI).

**Keywords:** Chromium, histpatholgical, cholangiocytes, hepatic cords, oval cells

## INTRODUCTION

Heavy metals like Cr produce oxidative stress and its hexavalent compounds are mostly man-made and are much more toxic than trivalent compounds.[1]These compounds adversely affect the human health due to their carcinogenic, mutagenic and teratogenic aptitude. The blood parameters alterations, renal tubules atrophy, Bowman's capsules annihilation and basement membrane degeneration was evident by hexavalent Cr (VI). Hexavalent Chromium Cr (VI) induces morphological changes in rabbit brain along with effects on pituitary gland and hypothylamus of male rat. Their higher concentration damage DNA, cause cell death and tissue injury [1, 2]. The livers of workers in chrome-plating industry are badly affected and have elevated liver enzymes similarly derangement of sinusoidal spaces and

hepatocytes; necrosis and their cytoplasmic vacuolization are due to metal exposure [3, 4].

Fruits of many plants possess antioxidants like Basella alba fruit can remove oxidative stress. Basella alba is a wildly cultivated, cool season vegetable with climbing growth habit. Fruit is fleshy, stalk less, ovoid or spherical, 5-6 mm long, and purple when mature. Their leaves are traditionally used in medicine to bring sound refreshing sleep when it is applied on head about half an hour before bathing.[9]Their leaves and stem are used against melanoma, leukemia and oral cancer.[10] It has been used for the treatment of anemia, coughs, cold and cold related infections. Root and leaves has been used for the removal of after birth and increase the milk production [5].

They are also potent antibiotics, antihypertensives and have blood building agents to improve the fertility in females. Their anthocyanins pigments are reported to have many therapeutic benefits including vasoprotective and anti-inflammatory, chemoprotective and anti-neoplastic properties. The anthocyanins stabilize DNA triple helical complexes[6, 7]. One study that established higher testosterone secretion from the testes noted that estradiol was increased by Basella alba.[16] Betacyanin extracted from BFE exhibited excellent antioxidant activity, could be beneficial in scavenging free radicals[17]. On the basis of above mention beneficial effects, it was decided to investigate the ameliorative potential of Basella alba against Cr induced toxicity in mice [8].

## MATERIALS AND METHOD

The study was conducted on thirty young male albino laboratory mice weighing between 25 and 30g, born and nurtured in the Animal House, Department of Biological Sciences University of Sargodha, Pakistan. The prevailing housing conditions were at 23±3ºC, 45% relative humidity, and a 12-hr dark-light cycle. The animals were provided free access to standard laboratory mouse diet and water, strictly following the animal care and experimental guidelines of the University of Sargodha.

Ripe fruit of Basella alba was obtained from local residential areas from which fully ripe berries were carefully selected. These were washed thoroughly in cooled boiled drinking water for 5min, air dried, and finally the pulp was softened and separated by means of vigorous shaking in a tightly closed sterilized wide mouth glass jar. 100g of the pulp was blended with an electric juicer in 100mL of cooled boiled drinking water for 5min. The resulting juicy material was centrifuged at 500rpm for 10minutes to separate the supernatants from the bottom-settled fibrous pulpy mass. The supernatants were immediately placed at –30ºC in sterilized 5mL ice-cube dishes. The frozen cubes (one each) were then placed in sterilized (airtight) plastic bags and stored at –30ºC. For each treatment, BFE from a freshly thawed (at room temperature) cube was used .

Potassium dichromate stock solution (1000ppm) was prepared by dissolving 2.82g $K_2Cr_2O_7$ per liter water and then further diluted to prepare 50ppm solution. Mice were randomly divided into three groups (n=10) as, Con: Control group; without any treatment. Cr: Cr (VI) group; provided 50ppm Cr in drinking water (ad-libitum) for 10days followed by withdrawal and supply simple Cr-free water for the next 5days. Cr-B: Cr (VI)-Basella alba group; maintained as Cr group but followed by 0.2mL/12h BFE for next 5days. The animals were euthanized by cervical dislocation on the day 16$^{th}$ and liver was removed through a medial abdominal incision, placed in alcoholic Bouin's fixative for 48h and then processed for wax embedding for microtomy. Histological sections of 5µ thickness were affixed on albumanized glass slides for H&E staining. Digital photographs of hepatic sections (100 and 400x) were obtained to highlight histpatholgical outcomes and to obtain histometric data. The cross-sectional area (CSA) of the hepatocytes, their nuclei size and number of progenitor (oval) cells and cholangiocytes were measured as standard protocol.[18]The histometric data of liver, group means±SEM were further subjected to the analysis of variance and post-hoc comparative

analysis of group means (Duncan's multiple range tests).

## RESULTS

Histopathological study of liver in control group showed rounded shaped hepatic central vein, hepatic cords are arranged normally in proper alignment around central hepatic vein. Hepatocytes have proper nucleus along with arranged around central hepatic vein (Figure 1A).

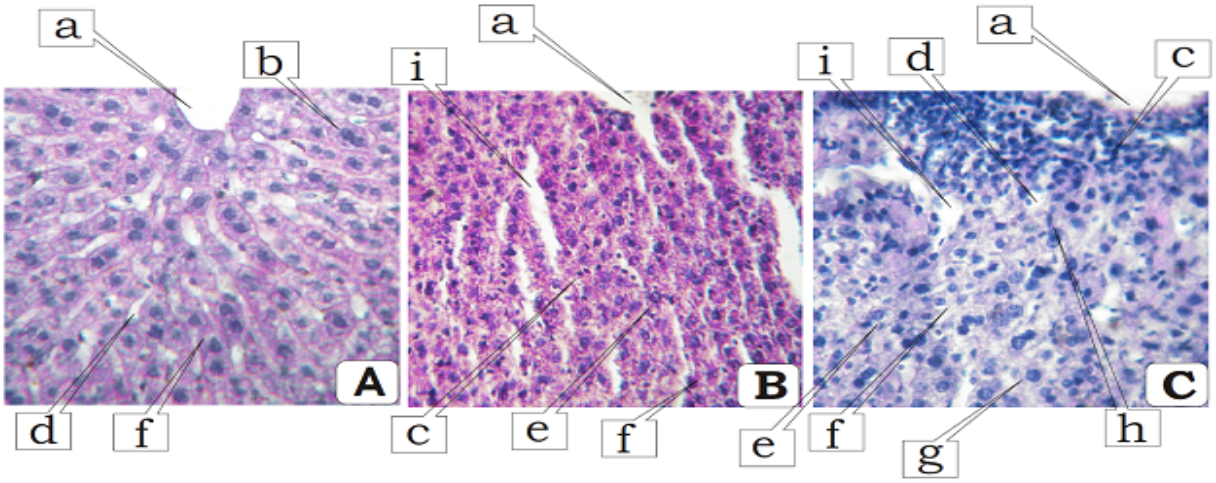


**Figure 1:** Selected section of liver (400X), A: Con, Vehicle Control group, B: Cr (VI) exposure group, C: Cr (VI)-B, Basila treated group a: Central hepatic vein, d: Sinusoids, e: Uni-nucleated hepatocytes, h: Epithelial cells, g: Oval hepatoblast, c: Oval nuclear progenerator cells, b: Bi-nuleated Hepatocyte, f: Hepatic cord, i: Hepatic tissue crack.

In Cr (VI) treated group hepatic cell size reduced and their nuclear size elevated. Hepatic cord show distortion with prominent tissue cracks. Central hepatic vein damage and few numbers of oval progenitor cell and cholangiocyte were observed in Cr treated group as compared to control. In the Cr-B group hepatic structure seems to be re-established with sub-populations and massive aggregations localized in different damaged cracked portions indicate the progenitor cells growth towards the damaged cracked areas. Hepatic cell size regains to some extent and the number of hepatocytes cell per unit area come close to control. Basella alba activate oval progenitor cell and rescue the damage hepatic cracks (Figure 1C).

**Tab 1:** Ameliorative effects of Basella on Cr induced anomalies in Liver of mice at micrometric level.

| Parameters | Con | Cr | Cr-B |
|---|---|---|---|
| Hepatocytes/area ** | †2.30±0.127[a] | 3.75±0.190[b] | 2.40±0.133[a] |
| Hepatocyte CSA ($\mu^2$) *** | 431.70±17.773[a] | 64.45±2.310[b] | 194.08±8.922[c] |
| Hepatocyte nuclear CSA ($\mu^2$) *** | 59.79±1.555[s] | 43.97±1.461[b] | 26.93±0.905[c] |
| Hepatocytes relative cytoplasmic index *** | 371.91±17.732[s] | 22.23±2.186[b] | 167.15±9.181[c] |
| Progenitor cells/area ** | 1.450±0.153[a] | 2.250±0.239[a] | 13.850±0.599[b] |
| Cholangiocytes/area *** | 0.95±0.184[a] | 1.95±0.198[b] | 4.15±0.254[c] |

**Con:** control. **Cr:** chromium exposure, **Cr-B:** chromium-Basella treated group. CSA; cross sectional area at (400×), n=10, per unit area 6400 $\mu^2$, Statistical analysis (ANOVA: two factors without replication). **: $p \leq 0.001$; ***: $p \leq .0001$, † group means ±SEM, [a b c]: any two groups not sharing a lower case letters differ significantly from each other (Duncan's Multiple Range comparison- post hoc analysis).

**Hepatic Histometry:** The CSA (cross-sectional areas) of the hepatocyte cell size, hepatic nuclear size, number of progenitor cells and hepatic cell per unit area showed a trend towards approaching significant variation among the groups. Post-hoc analysis of the data (Duncan's Multiple Range Test) indicated significant variations of the Cr (VI)

group compared to the data for the Con group and the Cr(VI)+ Basella alba group (Table 1).

## DISCUSSION

Chromium break DNA strand and form stable complexes and adducts of Cr(VI)+Proteins, proteins+Cr(VI)DNA and DNA+Cr(VI)DNA self-adduct.[19] In Cr (VI) it is observed that size of nucleus is enhanced due to adduct formation of Cr (VI) proteins and DNA. After adduct formation Cr (VI) stop genes transcriptional processes.[20] Cr (VI) targets the specific inducible genes promoters found on chromatin. Cr (VI) adducts exist in nuclear matrix portion.[21] In Cr (VI) exposure group cytoplasmic index reduced and nuclear content enhance having Cr (VI) protein and DNA adducts. As the result of adduct formation all cellular mechanisms like DNA replication, transcription and RNA processing inhibit.[22] Cr (VI) adduct inhibit activation of *Cyp1a1* and *Nqo1* gene expression important in signaling pathways.[23] Treatment with Basella alba fruit pulp extract has shown massive oval cell aggregations at the tissue cracked areas, along with oval cells large number of BEC travel from bile duct and moved towards the damaged cracks. Basella alba fruit pulp extract show potent histologic, histometric and regenerative signs of hepatic recovery from Cr (VI) exposure-related deteriorations hepatic cells in mice indicating its ameliorative potentials in liver related complications. All above mention results are accordingly our recent publications about Cr (VI) exposure-related anomalies and amelioration by Jambul.[24, 25]

## CONCLUSIONS

In this study we report reclamation and regeneration of hepatic tissue after Basella alba extract treatment in mice. Our findings clearly indicate regeration in liver by progenitor and cholangiocyte involvement and a rehabilitation of liver architecture with Basella alba fruit-pulp extract. Based on these findings, we propose Basella alba consumption revitalizes apoptosis in hepatocyte and enhance hepatogenisis in liver following exposure to environmental contaminants like Cr (VI). This treatment can be recommended for liver patients in jaundice and liver cirrhosis.

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