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A SCIENTIFIC EXPERIMENTAL STUDY TO ASSESS THE MFE (MORUS FRUIT EXTRACT) EFFECTS ON HEPATIC DISORDERS IN THE MICE

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Abstract:

Objective: *The research was conducted to find out the anomalies created by Chromium (Cr) spread by microscopic evaluation and effect of Morus Fruit Extract (MFE) on the Hepatic anomalies in mice.*

Methods: *This scientific experimental study was carried out at Services Hospital, Lahore from February 2016 to August 2017. A total of 30 mice (male) were selected and distributed into 3 categories. Each category consisted of 10 mice. The categories were assigned according to the experimental requirements except for Control group (C). The other 2 categories were Chromium (Cr) and Chromium Morus (CrM). Both groups' mice were given 50ppm chromium solution for 10 days whereas CrM were given 0.2 ml Morus Fruit Extract after 12 hours for 5 more days to judge the effect of MFE on mice.*

Results: *The Cr treated groups were seen with the anomalies in liver cords which resulted in the pathological indications of damaged liver the architecture and hence developed necrosis and fibrosis. Other clinical findings revealed that the No of hepatocytes, cross-sectional area (CSA) of hepatocytes and Nuclei size and width were different from the control group. The Cr group mice were seen with decreased values for the parameters such as lymphocytes, monocytes, eosinophil and haemoglobin but increased liver weight, Red Blood Cells, neutrophils and platelets. The histopathology findings in the CrM group shthe owed that the hepatic cords were recovering gradually and other clinical parameters were tending to normal after the 5 days treatment of MFE.*

Conclusion: *Our research concluded that MFE contains nutritional ingredients which help to recover the toxicants, especially heavy metals like chromium (Cr).*

Keywords: *Histopathological, Amelioration, Hepatocyte, Necrosis, Chromium, Morus Fruit Extract (MFE)*

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INTRODUCTION:

Chromium (Cr) constituents in the underwater in Pakistan are likely to pollute the water and hence Cr is included in the food chain and bioaccumulation. Cr is also used in drugs, tonics without scientific testing which is bad for health. Many Pakistani sweets namely Gulab-Jaman, Jalaibi and Zarda contain Potassium dichromate for colourant purposes. According to the National Research Council, Cr used in a diet is 50 – 200 pg/day and its reduced consumption can cause diabetes [1]. Hexavalent Cr is considered a pollutant which destroys the cells and damages the DNAs very easily [2, 3]. The Cr impurities damage the defence system of antioxidants and change the composition of cytoplasm and blood characteristics and also affect the liver [4 – 7]. Presentation to Cr (VI) results in the development of hepatocellular apoptosis, necrosis, lysis of cores and atrophy [8 – 10]. The sinusoid space of hepatocytes, irritation of hepatocytes, lipid peroxidation, oxidative stress and vacuolization widens with the exposure of Cr [11, 12]. In Pakistan, researchers have identified numerous plants with free radical's scrounger and can react with metals by creating additional bonds [13 – 15]. Curative plants such as Mulberry has cancer prevention agent because of the essence of anthocyanin. Anthocyanin shield from lipid peroxidation and repress the LDL oxidation [16]. Utilization of Mulberry organic product (Morus nigra) has proven to be effective for liver and kidney issues, fortifies the joints, enhances visual perception in old age and improves lipid profiles [17, 18]. The current research was conducted to judge the effects of MFE on mice's livers due to use of Cr (VI).

MATERIALS AND METHODS:

This scientific experimental study was carried out at Services Hospital, Lahore from February 2016 to August 2017. Mulberries fruit was bought from market and berries were processed (washed, dried and mixed in a 100-millilitre water with the help of blender). The pulps from the Morus Nigra fruit was separated with the help of centrifuge. The extract was further treated with ice to obtain 3D crystals of MFE.

A Cr (IV) stock was prepared in liquid form and 50 ppm diluted solution was prepared for the experiment. The sample was composed of 30 Swiss Webster mice weighing between 25-30g and 3-4 months of age. The sample was further divided into three categories each containing 10 mice. The three groups were named as a control group, Cr (IV) group and CrM group. The control group was provided with Cr free water, 50ppm Cr (VI) solution in drinking water was provided to Cr (IV) and CrM groups for 10 days while CrM group was additionally given Morus Nigra Fruit Extract for next 5 days. Livers were removed by means of dissection after the experiment and weighed to ascertain Hepatosomatic Index (HSI) – the ratio of liver weight with respect to the weight of the body. Livers were then made ready for wax implanting. Serial areas (5-6 q) were treated with histopathological techniques and results were captured with a high-resolution camera. The images were processed in Corel Draw for better results [11]. Multiple parameters including CSA of hepatocytes, cores, focal hepatic vein, sinusoidal spaces, Kupffer cells etc. were calculated and statistically analyzed by means of ANOVA and Duncan's Numerous Range Test [19, 20].

RESULTS:

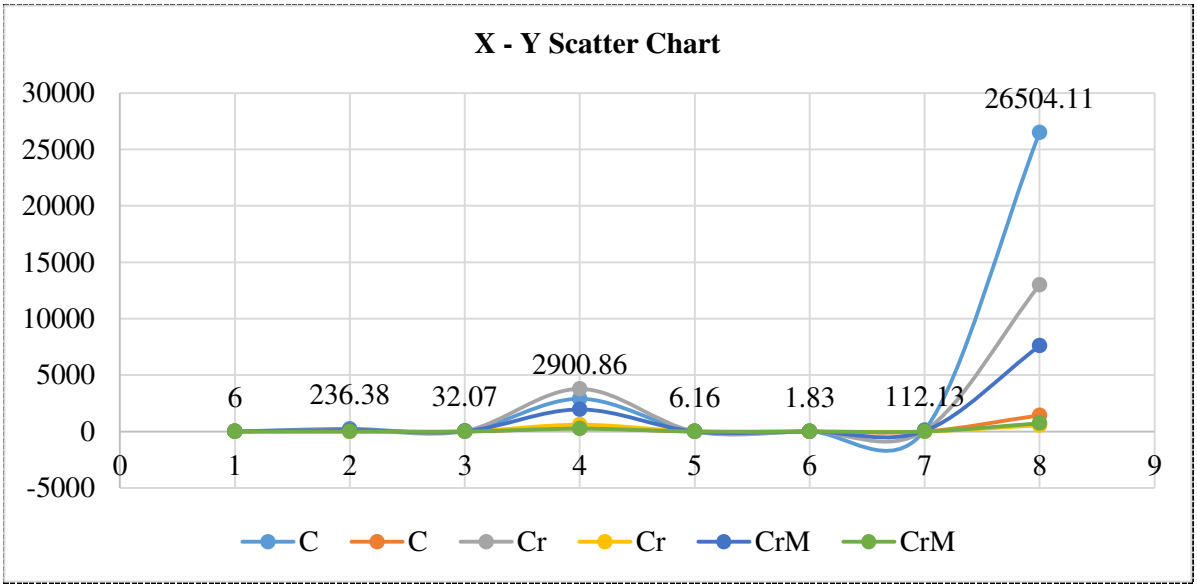
The histological indications in the control group (C) showed hepatic lobules (hepatocytes arranged in hepatic cords) starting from the main vein and linking with Kupffer cells. The lobules showed sinusoidal spaces. Damaged liver lobules were noticed in the Cr group. Unequal liver cords, damaged hepatocytes, widening of intracellular spaces and progressive connective tissues were noticed in this regard.

Liver necrosis (de-shaped nuclei, uneven cell margins and cytoplasmic abnormalities) was observed.

The indications of the rot of hepatocytes incorporate deformed cores, unpredictable cell edges and cytoplasmic combinations between balanced hepatocytes. Invade endothelial cells from the lobular vein into the lobules was also observed.

Table – I: Micrometrical and Anatomical amelioration

Parameters	C		Cr		CrM	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Mean fractional weight of Liver	6	0.33	11.54	1.25	5	0.139
Mean CSA of hepatocytes (μ^2) (400×)	236.38	9.82	161.18	13.01	133.81	4.04
Mean CSA of hepatocytic nucleus (μ^2) (400×)	32.07	1.19	41.94	1.53	28.03	1.09
Mean CSA of central vein(μ^2) (40×)	2900.86	296.49	3779.05	605.2	1956.3	256.32
Mean width of Sinusoidal spaces (μ) (400×)	6.16	0.28	7.15	0.35	5.17	0.31
Mean number of Kupffer cells in 14400μ^2 area (400×)	1.83	0.34	15.5	0.85	11.42	0.48
Mean number of hepatocytes in 46225μ^2 area (100×)	112.13	6.08	80.63	3.46	56.75	5.47
MRA of hepatocytes in 46225μ^2 area (100×)	26504.1	1437.5	12995.1	558.3	7593.7	732.36

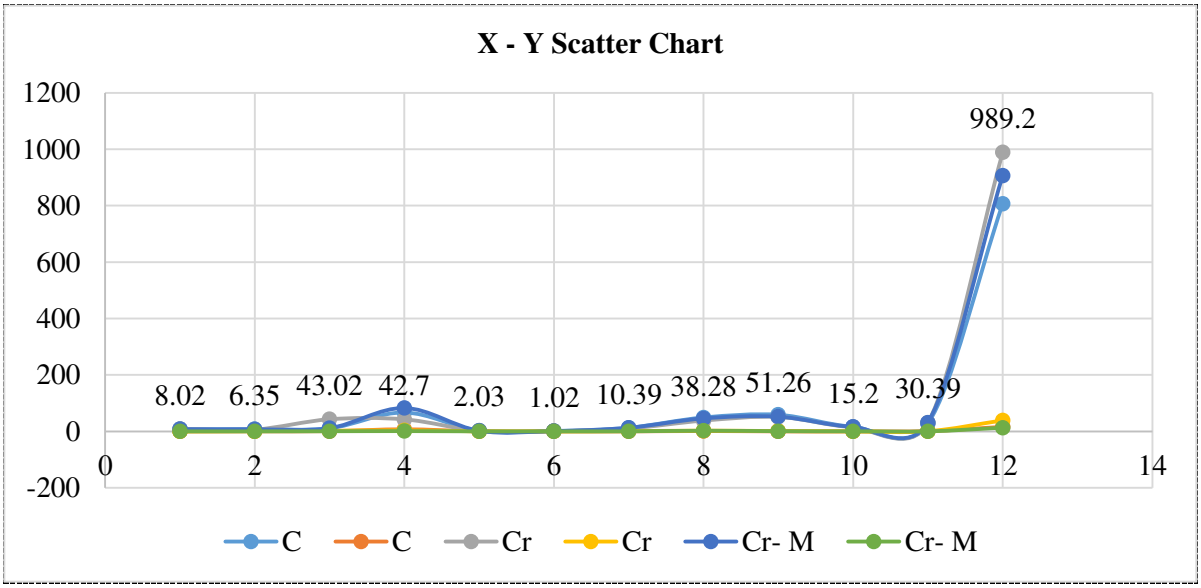


The ratio of liver weight to body weight was different in all groups. Moreover, the mean value of cross-sectional areas of hepatocytes and nuclei were found to be considerably different in each group. The details of these observations are summarized in the tabular data.

The impact of Cr (IV) toxicant on the haematology of mice was also studied. Blood parameters such as bilirubin, total protein and globulin were measured.

Table – II: Moruson Cr induced anomalies on Blood Profile

PARAMETERS	C		Cr		Cr- M	
	Mean	± SD	Mean	± SD	Mean	± SD
RBC ($\times 10^6$/ul)	7.97	0.16	8.02	0.01	7.84	0.08
TLC ($\times 10^3$/ul)	8.06	0.42	6.35	0.74	7.47	0.07
Neutrophil	11.05	0.86	43.02	2.09	11.45	0.61
Lymphocytes	67.06	7.39	42.7	3.9	83.04	1.04
Monocytes	2.07	0.15	2.03	0.15	2.06	0.22
Eosinophil	1.55	0.14	1.02	0.01	0.95	0.14
Hb(g/dl)	13.06	0.16	10.39	0.95	12.86	0.29
PCV	49.23	1.35	38.28	2.65	46.16	2.37
MCV fl	58.93	0.09	51.26	1.17	52.05	0.87
MCH (pg)	16.58	0.03	15.2	0.18	16.46	0.28
MCHC(g/dl)	27.65	1.01	30.39	0.37	31.32	0.24
Platelet($\times 10^3$/ul)	807	16.1	989.2	39.1	907	13.1



DISCUSSION:

Cr intake in frequent doses is toxic especially in its hexavalent structure [21]. The MFE, on the other hand, is used as a cure for many infectious and pathological situations [22]. The researchers have delivered that the Cr intake resulted in the loss of body weight and use of MFE helped to regain the weight. The liver weight in Cr user has increased causing liver inflammation as compared to the control group. With the 5 days use of MFE, the decrease in liver weight and size was noticed due to natural capabilities of MFE. Microscopic results showed that the mice livers were severely affected by Cr intake. The livers were seen with necrosis, lysis of nuclei, disturbed sinusoidal spaces etc. The results of our research are comparable to the findings of available studies on this topic. Histological results after MFE treatment revealed a decrease in the CSA of hepatocytes, per unit quantity of hepatocytes, improved structure of cords, vein and nuclei. MFE treatment has a positive effect on red blood cells, neutrophil, MCHC and platelets and reduces TLC, lymphocytes, monocytes, eosinophil, Hb, PCV, MCV and MCH [23]. Cr exposure results in loss of plasma in blood and causes water reduction, which in turn increases RBC related to fibrosis [24]. Chromium also causes the damage to cellular walls, vacuolation and

inflammation in mononuclear cells [26]. The livers becomes damaged by the use of chromium and fail to purify the blood completely.

The hepatocytes characteristics (CSA, per unit quantity and hepatocytes area) were improved after MFE treatment. However, these results are analyzed with the microscopic examination to ascertain the true picture of the characteristics of the hepatocytes. The liver function has improved after Morus Nigra Fruit Extract treatment despite the smaller number of operational hepatocytes in CrM group as compared to the Cr group. Sinusoidal spaces and the lobular vein rehabilitation were also noticed after the use of MFE.
The average size of RBC is usually increased with the use of herbal ingredients from plants. The use of MFE was also noticed to bear this property in cases of Cr group [27]. MFE not only stopped the MCV but also placed a barrier on the toxicity of hexavalent chromium. The chromium-induced fibrosis was controlled by the use of MFE which nourishes the RBC showing the positive results for the detoxification caused by the metals such as Cr (IV) [15].

Mulberry is identified as a plant with free radical's scrounger which can react with metals by creating additional bonds [28]. Blueberry contains ingredients such as carnitine which boosts energy and activates the metabolism. This effect certifies the pleasant effect of MFE on the microbial features of liver and lipid profiles [30].

CONCLUSION:

Our research concluded that MFE contains nutritional ingredients which help to recover the toxicants, especially heavy metals like chromium (Cr).

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