Original Research Article

Ameliorative Activities of Morus and Jamun against Cr inducedAndro-hepatic Anomalies

Abstract:

Chromium (Cr) is a vital micronutrient for sugar metabolismwhile its unauthorized use may agonize health fatalities. This study was conducted about the noveltyof Cr^{+6} histopathological potentials along with Mulberry Fruit Extractand Jambul Fruit Extractto definethe bio-chelation and ameliorative aptitude.

The histological findings in Cr exposure specify; cessation of testicular seminiferous tubules (ST), the annihilation of smooth muscles in ~~the~~basement membrane, Leydig's cell and spermatids. The ST have halted spermatogenesis, with irregular ~~boundary~~ boundariesand dead spermatogonia and Leydig's cells are disorganized. The sperm CSA, tail lengthand middle piece diameter ($p \leq 0.001$) significantly reducedfindingsalong with significant elevation of hepatocytes nuclei, central vein CSA and mean widthof Sinusoidal Spaces as compared to control. Theprotuberant marks of steatosis, fibrosis, dehydration and atrophy werenullified by designated fruit extracts and their bio-chelating effects wasobvious due to the presence of anthocyanin, β-sitosterol and phytochemicals.

Keywords: Chromium, steatosis, fibrosis, dehydration and atrophy.

1. Introduction:

Theunhygienic water frequently used to irrigate crops which produce inevitable anomalies(Asmatullah *et al*., 1998).Metals such as lead, mercury, chromium, arsenic, copper, cadmium, and iron in contaminated water and food supplements have potential to generate reactive oxygen species (Stohs *et al*., 2001), affect immune system and body organs such as testes, kidneys and liver (Jayabarath *et al.,* 2009). The adequate amount of chromium (Cr) as supplement used to enhance endocrine system and alteration of behavioral-related activities (Molinero and Marquez, 2009) but their ridiculous use produce severe injuries; block the androgen receptors and excess androgen produce hepatic, renal and hematologic anomalies (Thompson and Bannigan, 2008). Theelevated androgen inhibit the production of endogenous androgens by Leydig cells, which indemnities liver and testicular seminiferous tubules (Coss *et al.,* 2012).

Hepatocyte metabolizes, detoxify and inactivate the ROS by antioxidant (Keren *et al.,* 2013), and many herbs from Pakistan have been investigated as source of antioxidants to augment free ~~radicals~~radical's scavenger and metal chelating activities (Hussain *et al.,* 2012), so the plants extracts have ameliorative competency against heavy metals (Batool *et al.,* 2010; Swami *et al.,* 2012; Okon*et al.,* 2013).Antioxidant may be synthesized in the body or obtained in the diet; have capability of β-carotene-linoleate and β-sitosterol with reducing power, superoxide, nitric oxide-scavenging capacity and ferrous ion chelating potency (Rout and Banerjee, 2007) and their anthocyaninprevent lipid peroxidation (Ozsahin *et al.,* 2012).The flavonoids and polyphenols in *Trianthema triquetra* (Chitra *et al*., 2007), *Pisonia aculeata* (Palanivel *et al*., 2008), *Benincasa hispida* and Castor oil (Shetty *et al.,* 2008) are very beneficial in scavenging the free radicals (Alia *et al*., 2008) by regulating aspartate aminotransferase, alanine aminotransferase, total serum bilirubin and malondialdehyde in hepatocytes (El-Sayed *et al.,* 2011).

Table: 1; Plants phytochemical compounds used for bio-chelation.

Plant	Compound	Bio-chelation	References
Fruit extract	Anthocyanin	Fe	Veigas *et al.*, 2007, 2008
Mulberry extracts	phenolics, β-sitosterol and anthocyanins	CCl_4 and heavy metals	Jude and Catherine, 2011; Awasthi, 2012
Morus alba	Phytochemicals	CCl_4	Ali, 2010
Syzygium cumini	Antioxidant, anthocyanin	Nitric oxide, Fe, CCl_4	Ki-Tae *et al.*, 2005; Benherlal and Arumughan, 2007
	Flavonoid	Fe, methylmercury	Abalea *et al.*, 1999
Moringa oleifera	Phytochemicals	Cr	Akunna *et al*., 2012
Hippophae rhamnoides			Geetha *et al.*, 2003
Plants, fruits, vegetable	Vitamins E/C	Heavy metals	Mittal and Flora, 2007
		Pb	Rendon-Ramirez *et al.*, 2007
Mangifera indica	Phytochemicals	Fluoride	Narasimhacharya *et al*., 2011
Red cabbage		Heavy metals	(Glinska andGabara, 2011
Plants	L-carnitine	CCl_4	Demirdag *et al.*, 2004
Salvia plebeia	Flavonoid		Xiao-feng, 2011
Plants	Ascorbic acid, tocopherol	Arsenic	Ramanathan *et al.*, 2005
Plants derived drug	Deferiprone	Fe, Al	Albina *et al.*, 2000

Mulberry has antioxidants used as cytotoxic protective activity to reduce edema (Chuanguang *et al.,* 2010), with ameliorative effect on hyperlipidemia, lipogenesisand fatty acid oxidation(Volpato *et al*., 2011). *Morus nigra* have anti-cancerous, anti-inflammatory, antibacterial and anti-fungal, antimicrobial and radical rummaging activities (Hamdy, 2012). Their flavonoids quercetin protects and prevents from lipid peroxidation(Pereira *et al.,* 2013).Pharmacologically*Syzigium cumini* fruit extracts protect the cultured human peripheral blood lymphocytes from DNA damage (Jagetia and Baliga, 2003) also used to treathuman breast cancer (Goyal *et al*., 2010), repair hepatocyte from iron damages (Abalea *et al.,* 1999), protect from hydrogen peroxide injuries and gamma-irradiations (Jagetia *et al.,* 2008).Their anthocyanins are anti-spasmodic (Das and Sarma, 2009),ameliorate hepatic enzymes (Hossain *et al*., 2010**)** normalizes serum ALT and AST levels and have antagonistic behavioragainst methylmercury (Ayyanar and Subash-Babu, 2012).The flavonoids in litchi (*Litchi chinensis*) used against cancer (Wen *et al.,* 2014), *Viola odorata* for asthma (Qasemzadeh *et al.,* 2015), red clover for hot flashes/menopausal symptoms (Ghazanfarpour *et al.*, 2016), green tea for the risk of prostate problems (Guo *et al.,* 2017), cactus (*Opuntia ficus-indica*) cladodes on methotrexate-induced oxidative damage (Amira *et al.,* 2018) andchamomile (*Matricaria recutita*) as

antioxidant and anticancer activities (Bayan *et al.*, 2019).The enzymatic changes/gene expression are almost same in humans and rodents(Zhang*et al*., 2013) so the mammalian model mice was selected for *vivo*study, and the cheapest Pakistan cultivated economical fruit extracts were selected to probe the poisonous effects of heavy metals and shieldagainst andro-hepatic anomalies at micrometric level.

2. MATERIAL AND METHODS:

The study was conducted on 25-30g/3-4months old 50 male albino laboratory mice (*Mus musculus*) kept at 26±4 C˚/45% relative humidity with 12-hr L/D cyclesthroughout experimentalduration.

2.1 Preparation of Solution and Fruit Extracts:Dose for various groups were prepared from pure Potassium dichromate (K_2 Cr_2 O_7), stock solution of 1000ppm anddiluted up-to 50ppm required solutions.Ripe black fruit of *Morus nigra* and *Syzygium cumini* were washed, and 100g of their pulp was blended in an electric juicerin 100ml cooled boiled drinking water for 5minutes and finally centrifuged at 500rpm for 10minutes. The supernatant was immediately placed in sterilized 5ml capacity ice-cube dishes, store in sterilized plastic bags at -30^{o}C and a fresh thawed cube was used for each experiment at room temperature.

2.2 Experimental Groups:Animals were randomly divided into 5 groups (n=10) as:Control group (C);provided boiled cooled mineral water for 15days, Cr-group (Cr); provided 50ppm Cr-solution for first 10days at *ad libitum* followed by simple mineral water for next 5days, Cr +Mulberry group (Cr-M); as Cr group but the last 5days they were givenMFE, similarly Cr +Jambul group (Cr-J)given JFE and Cr +Mulberry+Jambul group (Cr-MJ)were given equally mixed MFE and JFEthrough gavage as post-treatment 0.25ml/12hrs for next 5 days.

2.3 Histological and Micrometric Analysis:The animals were euthanized by cervical dislocation and body organswere dissected out on day 16th for histological HE staining, testicular smear formation and micrometric analysis following Khawja Raees Ahmad (2011) protocol. Photograph at 100×, 400× and 1000× with 7.2 MP digital camera (Sony) were processed in CorelDRAW11 for micrometry. The results were expressed as mean ± SD and data was analyzed through ANOVA (two factors, without replication) and Duncan's Multiple Range Test (post hoc

analysis).

3. RESULTS:

In control group testes of animals indicate the clear boundaries of seminiferous tubules (ST) with integral basement membranes, spermatogonia (Sp.g) are evenly disseminated and multiple whirls of spermatocytes (Sp.c), secondary spermatocytes (Se.c), spermatozoa as parrot beak headed sperm (PBH) and club headed sperms (CH) with prominent elongated tail (Sp.T) were visible.

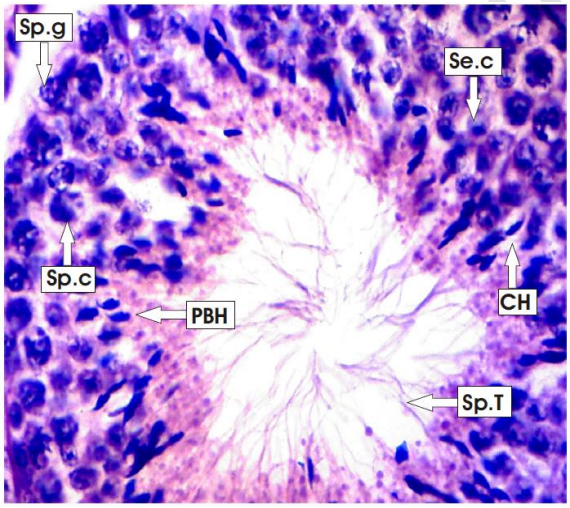

Fig 1: Mice Seminiferous Tubule, control group. Sp.g; spermatogonium, Se.c; sertoli cell, Sp.c; spermatocyte, PBH; parrot beak headed spermatozoa, CH; club headed spermatozoa,Sp.T; Spermatozoa Tail

The ST in the animals without Cr treatment were symmetrical equally distributed rounded with prominent Leydig's cells (Fig 2-a), spermeogenesis (Fig 2-b) and spermatozoa with elongated tails directed towards the lumen (Fig 2-c). The spermatogonium have prominent dark stain nuclei without vacuolation and cracks (Fig 2-d). In Cr treated group there was breakdown of basement membrane, destruction of smooth muscles, Leydig's cell (Fig 2-e) and spermatid without spermeogenesis (Fig 2-f). The tailless sperm (Fig 2-g) and debris filled in ST lumen move like vortex (Fig 2-h). The ST have halted spermatogenesis, with irregular boundary and basement membrane loses intertubular junction (Fig 2-i). The dead spermatogonia and Leydig's cells are disorganized (Fig 2-j, l) and whirls of primary and secondary spermatocytes

break and lose their regular symmetry (Fig 2-k).

The Morus as post-treatment reorganized the basement membrane (Fig 2-CrM-m), with prominent Sertoli cells (Fig 2-CrM-n), spermatogenesis (Fig 2-CrM-o) with regular whirls of Sp.g, Se.c, Sp.c, PBH, CH and Sp.T (Fig 2-CrM-p).

The Jambul improve Leydig's cell, basement membrane along with smooth muscles fill up the vacuolated regions (Fig 2-CrJ-r), bulbous spermeogenesis (Fig 2-CrJ-s), reorganization of primary and secondary spermatocytes (Fig 2-CrJ-t,u). The co-treatment with synchronized effects of Morus and Jambul remove vacuolated cell and reduce the debris by re-organizing cellular texture and symmetrical whirls of ST (Fig 2-CrJM-w,x,y,z).

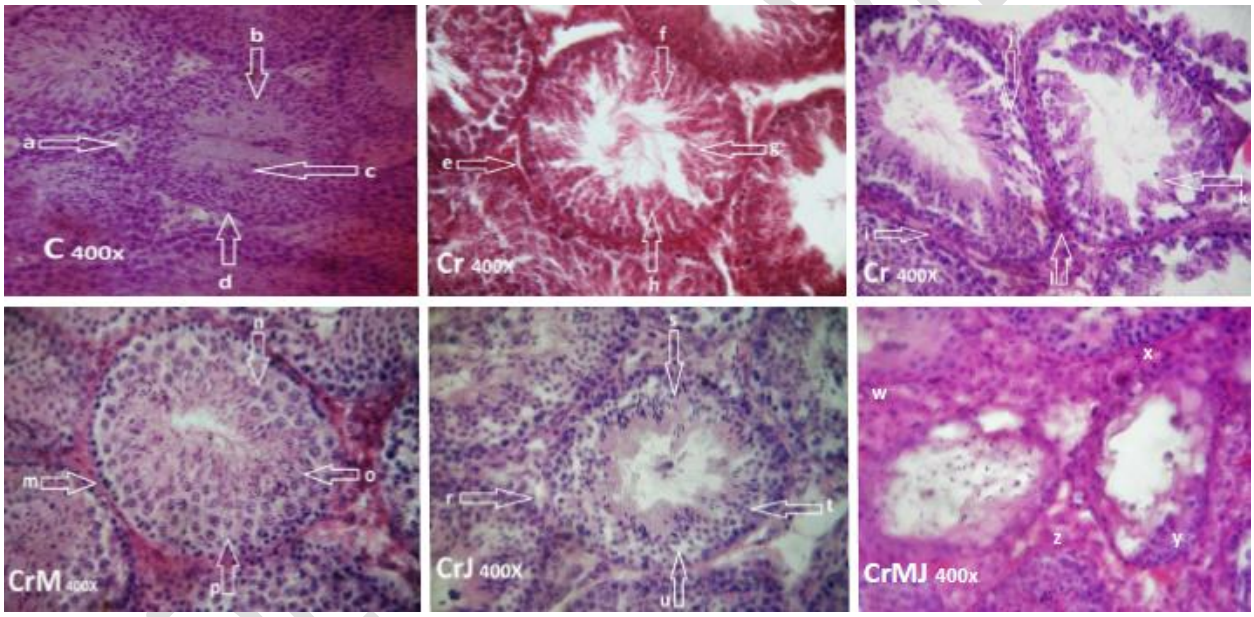

Fig: 2; Histological sections of mice testis in control (C), Cr treated (Cr), Morus (CrM), Jambul (CrJ) and combine co-postreatment of Morus and Jambul (CrMJ) groups.

The testicular and seminal vesicle smear of normal animals as control group have PBH, with long tail without any kink (Fig 3-1,5) inverse to Cr treated in which sperm ha,ve kink and weak connection b/w sperm head and tail (Fig 3-2,7) with halted spermatocytes and sperms without tail (Fig 3-8,9). The Morus and Jambul rehabilitate the anomalies (Fig 3-3, 4, 6) the Cr induced anomalies.

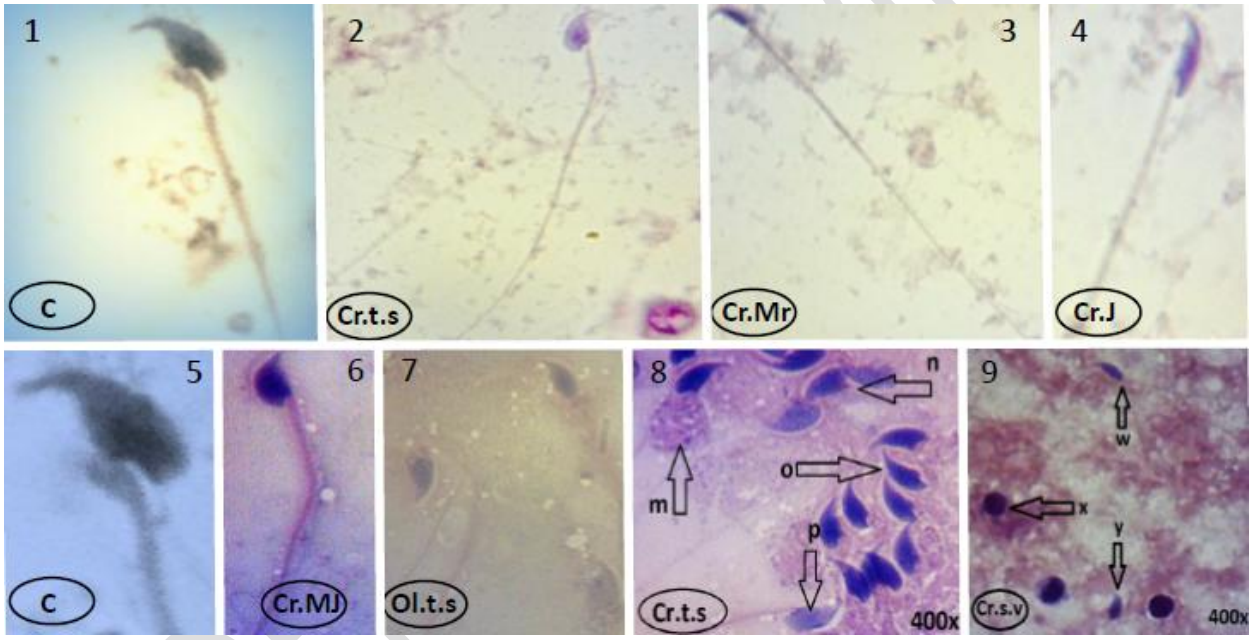

Fig: 3; Testicular smear with sperm morphology in control, Cr-treated and fruit extracts groups.

C; control, Cr; chromium, t.s; testicular smear, Mr; Morus, J; Jambul, ol; oil emulsion, s.v; seminal vesicle fluid.

3.1 MICROMETRIC HISTOLOGICAL STUDY OF MICE TESTES

3.1.1 SPERM MICROMETRIC STUDY: The mean CSA of head, length of tail and diameter of mid piece was measured from testicular smear as;

Table:2. Sperm micrometry, ameliorative effects of fruit extracts on Cr induced anomalies.

PARAMETERS	GROUPS				
	C	Cr	Cr-M	C-J	Cr-MJ
PBH CSA(μ^2) of head ***	† 13.91±0.64^{a}	7.86±0.65^{b}	15.39±0.74^{a}	12.89±1.25^{a}	15.78±1.59^{a}
PBH tail length (µ)*	85.57±12.11^{a}	75.92±12.05^{a}	102.10±5.45^{b}	85.99±3.25^{a}	86.84±1.70^{a}
PBH Middle piece. DM (µ)**	0.08±0.06^{a}	0.58±0.09^{b}	0.75±0.05^{c}	0.95±0.14^{a}	1.05±0.05^{d}
CH CSA(μ^2) of head*	11.37±0.98^{a}	9.16±1.85^{a}	14.05±4.05^{a}	15.7±7.04^{a}	11.19±0.73^{a}
CH taillength(µ)*	81.07±5.55^{a}	56.27±14.43^{b}	86.28±16.89^{a}	87.46±5.55^{a}	67.51±10.90^{ab}
CH Middle piece. DM (µ)*	0.96±0.12^{a}	0.39±0.15^{c}	0.78±0.61^{ac}	0.67±0.08^{bc}	1.25±0.35^{d}

C: control. **Cr:** chromium treated, **Cr-M:** chromium+MFE, **Cr-J:** chromium+JFE,**Cr-MJ:** chromium+MFE+JFE.CSA: mean cross- sectional area of (PBH) sperm, DM: diameter (thickness) of parrot beak headed sperm and club headed (CH) sperm mid piece at 400X.* : $p \leq 0.05$-0.01, * * : $p \leq 0.001$ * * * : $p \leq .0001$ {Statistical analysis (ANOVA: two factors without replication)} , †group means ±SEM, µ = µm, $^{a\ b\ c}$: Anyone two groups not sharing a lower case letters differ significantly from each other (Duncan's Multiple Range comparison- post hoc analysis), n=10.

3.1.2 MICROMETRIC HISTOLOGICAL STUDY OF MICE LIVER:

The cross sectional of liver indicate destruction of hepatocytes, with significant ($p \leq 0.001$) elevation of hepatocytes nuclei, central vein CSA and mean width (µm) of Sinusoidal Spaces (S.S) at (400×) while mean number of hepatocytes and mean relative area occupied by hepatocytes per unit area were significantly ($p \leq .0001$) reduced in Cr treatment as compared to control.

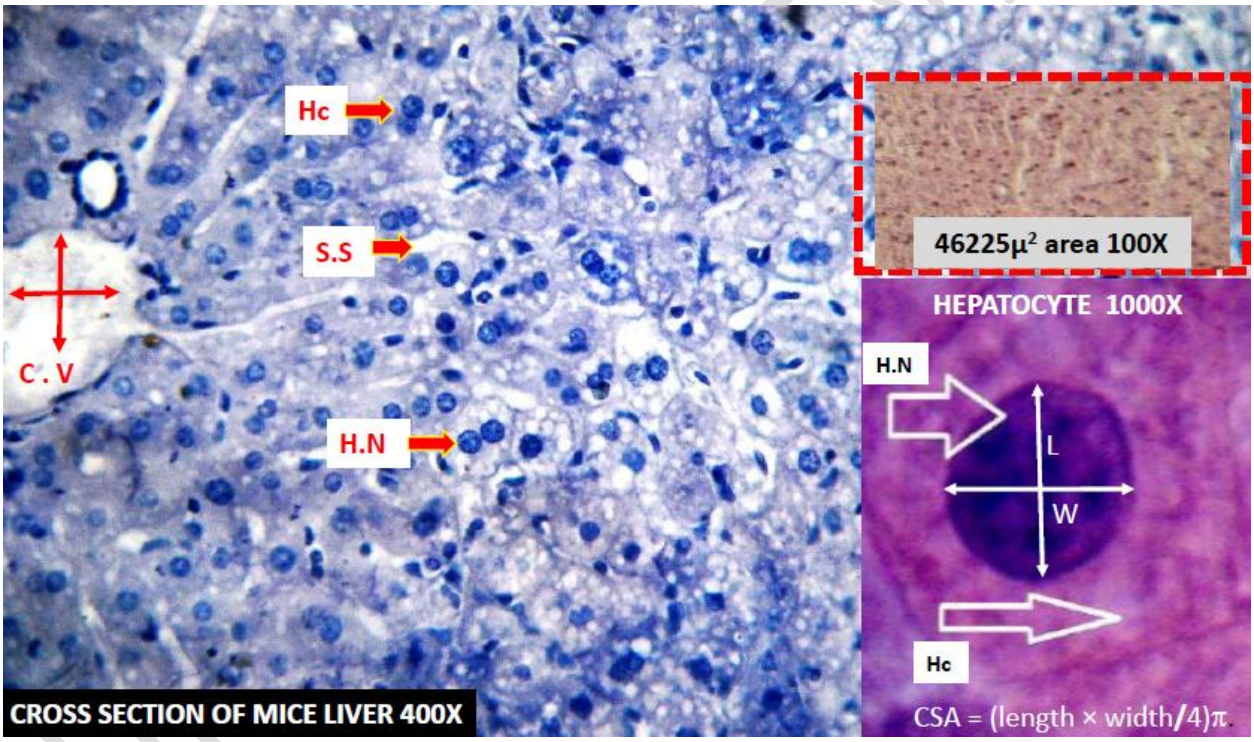

Fig: 4; Histology of Liver. The cross sectional of liver indicate hepatocytes, their nuclei and central vein CSA, Mean width (µm) of Sinusoidal Spaces (S.S) at (400×), Mean number of hepatocytes(N. Hepat) in $46225\mu^2$ area (100×), Mean relative area occupied by hepatocytes (R.A.Hepat) in $46225\mu^2$ area (100×).

Table3: Micrometry of mice liver, ameliorative effects of fruit extracts on Cr induced anomalies.

PARAMETERS	GROUPS				
	C	Cr	Cr-M	Cr-J	Cr-MJ
CSA (μ^2) Hc***	†237.95±12.56^{a}	187.39±19.19^{b}	143.14±5.58^{c}	171.95±8.59^{b}	228.76±10.78^{a}
CSA (μ^2) H.N***	32.59±1.29^{a}	43.98±1.17^{b}	29.18±1.25^{c}	39.88±1.87^{d}	32.89±0.75^{a}
CSA (μ^2) C.V ***	2849.14 ±255.09^{a}	4213.55 ±773.89^{b}	2090.25 ±329.59^{a}	2250.24 ±201.58^{a}	6748.31 ±1466.29^{c}
Width(µm)S.S***	6.17±0.63^{a}	7.25±0.83^{b}	5.18±0.66^{c}	7.09±0.67^{b}	5.05±0.26^{c}
N. Hc /area ***	112.06±6.07^{a}	80.64±3.47^{b}	56.75±5.49^{c}	95.13±4.45^{d}	85.89±0.09^{b}
R.A.Hc /area***	26504.77 ±1457.93^{a}	12995.14 ±555.12^{b}	7593.72 ±722.14^{c}	16380.55 ±699.12^{d}	19639.12 ±762.14^{e}

C: control. **Cr:** chromium treated, **Cr-M:** chromium+MFE, **Cr-J:** chromium+JFE,**Cr-MJ:** chromium+MFE+JFE.Mean cross sectional area (μ^2) of hepatocytes (Hc) at (400×), Mean CSA (μ^2) of hepatocytic nucleus (H.N) at (400×), Mean CSA (μ^2) of central vein (C.V) at (40×), Mean width (µm) of Sinusoidal Spaces (S.S) at (400×), Mean number of hepatocytes(N. Hepat) in 46225μ^2 area (100×), Mean relative area occupied by hepatocytes (R.A.Hepat) in 46225μ^2 area (100×). n=10. Statistical analysis (ANOVA: two factors without replication).***:** $p \leq 0.05$-0.01; ****:** $p \leq 0.001$; *****:** $p \leq .0001$, † group means ±SEM, $^{a\ b\ c}$ **:** Anyone two groups not sharing a lower case letters differ significantly from each other (Duncan's Multiple Range comparison- post hoc analysis).

4. DISCUSSION: Cr as environmental toxicant produce ROS and induce andro-hepatic anomalies evident by presence of necrosis and steatosis in testes and liver due to β-oxidation which divert metabolic pathway towards lipogenesis(Damek-Poprawa, 2003). That process effect hepatocytes and kuffer cells to induce cirrhosis, dehydration and fibrosis. The lipid peroxidation distract the steroid level and elevate testosterone. The ketonic bodies from fatty acids also drop pH which induce atrophy and causes the fluctuations of biochemical hematological and lipid profiles (Fig; 2, 4) as Cd induce atrophy, testicular architecture

disorganization and germinal epithelium disruption (Sharma*et al.*, 2013).These process increases the acidity which ruptured the endothelial lining of testes indicate the sloughing and degeneration by the exposure of Cr as debris and dislodge spermatid in the lumen of ST, which altered their diameter which exactly co-related with Fabricia *et al.,* (2010) study about Cd toxicity. The primary hypertrophy with Crexposures seems to cause accumulation of undifferentiated cells due to the lack of meiosis and spermatogenesis to produce spermatogenic arrest (Boekelheide *et al.,* 2000). The necrosis of sertoli cells leading to the dislodgment of dead spermatic cells in ST due to the deficiency of nutrition, evident by significant elevation of CSA of ST may cause testicular hypoplasia anddestruction of Leydig's cell in long run cause infertility (Giagulli and Vermeulen, 1988). ROS by Cr exposure affect the mitochondrial membrane permeability with deficiency/insufficiency of sugar metabolism which causes the depletion of germinal epithelium in the ST and induce desquamation; shedding of the outermost ST basement membrane (Fig; 2). Sertoli cells are interconnected by tight junctions; for blood-testis barrier, which temporarily open to permit the passage of spermatogenic cells and the loss of interstitial cells affects the process of spermatogenesis and spermiogenesis. Cr exposure distort the sperm heads with significant elevation of tail-less spermatozoa also indicate the possible deformities at the androgen receptor during terminal differentiation of spermatids (Fig; 3, Tab; 2). The rodent sperm have one or more apical regular symmetrical hooks (Firman *et al.*, 2011) and the spermatozoa show the prominent anomalies in head of sperm with irregular and wavy intermediate shape and tail less appearance in Crgroup (Fig; 3). Micrometric analysis (Tab; 2) of spermatozoa specified the alterations in CSA of sperm head, thickness of mid-piece, and length of the tail, that can be extrapolated with Crrelated DNA damage and loss of polymerization of the micro tubular array into microtubules of the sperm tail (Vijaya *et al.*,

2013). The anabolic androgenic steroids can be ameliorated by drugs and the pharmacological products of plants like anthocyanin, β-sitosterol and α-Tocopherol (vitamin E). Phytochemicals are the most important lipid-soluble antioxidants and interrupts the chain reactions of lipid peroxidation to enhance the activity of natural antioxidants that scavenge the free radicalsevident by blue green algae (Won *et al.,* 2012). The synchronized effect of MFE and JFE significantly higher spermatogonia and primary spermatocyte and have more ameliorative effect to improve the histological and micrometric cellular level of testis and liver, so the phytochemicals are the best natural sugar and cholesterol regulators as compared to synthetic drugs(Rahmani *et al.,* 2013).

Apart from hepato-lipogenicity, atrophy, dehydration, fibrosis and hepatocytic necrosis reported in this study has been considered oxidative stress attribute by Cr exposure and the defense against free radicals is associated with activities of SGOT, SGPT and bio-chelation of heavy metals from body.The environmental chemical exposure in the induction and progression of various diseases is significant (Kaiser *et al.,* 2013). The hyperlipidemia may indicate anomalies of anterior pituitary, liver-biliary dysfunction, and lipoprotein lipase cofactor deficiency corticosteroids (McCarty, 2004). LDL cholesterol and excess lipid with rise in HDL and VLDL damage liver and cause hepatic steatosis anddisplace the nucleus towards periphery. Histological studies of liver at micrometric level indicate the alteration central vein CSA in Crtreated group, due to accumulation of debris by necrosis. Micrometric data (Tab; 3) show significant decline in the CSA of central veins (CV), number of hepatocytes/area and hepatocytes along with their nuclei CSA increase the sinusoidal spaces (SS) clearly indicate hepatocytes necrosis liver parenchyma and hepatocytes (Muthukumaravel and Rajaraman, 2013), also indicate hypothyroidism, glucocorticoid excess and the adrenal gland overproducing cortisol

behavior by heavy metalsexposure (Agnes *et al*., 2012). MFE and JFE analogous to citrus fruits protect the membrane integrity resulting in a reduction of RBC hemolysis and increased the amount of platelets due to their flavonoids (Heroor*et al*., 2013).In humans the stomach acts as a site for anthocyanin absorption from food, cyanidin-3-glucoside and cyanidin-3, 5-diglucoside, penetrate into human's blood through liver cells (Milbury *et al.*, 2010). The anthocyanin-rich extract decreases the lipid peroxidation; to normalize cholesterol by up regulating the peroxisome proliferator-activated receptors and influence the testosterone metabolism to ameliorate hyperglycemia and reproductive anomalies.

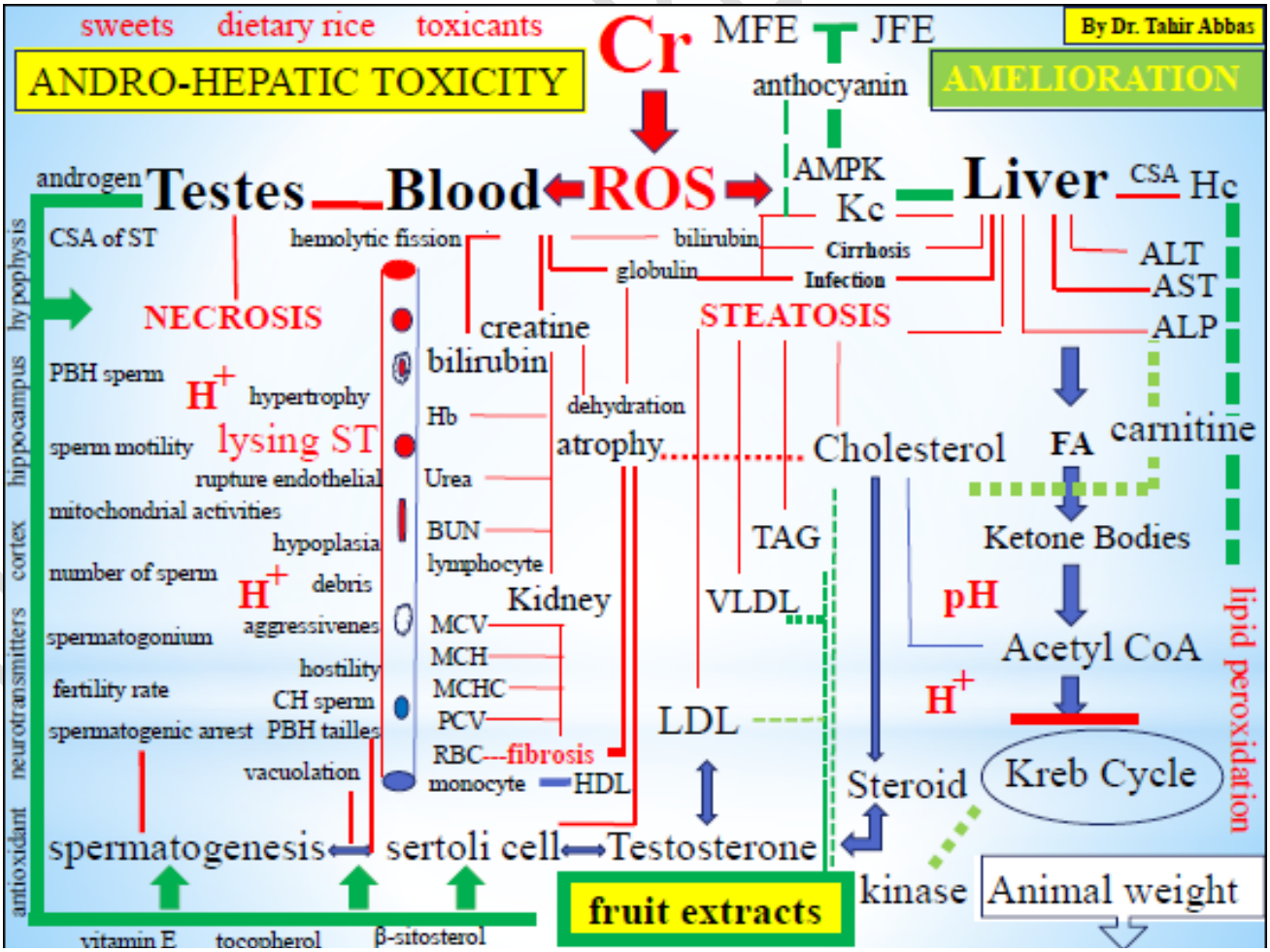

Fig: 5; Comprehensive summary of Ameliorative role of phytochemicals against Cr induced

Andro-hepatic anomalies in mice.

The ameliorative effect of melatonin also affects the spermatogenic proliferation in ST and metalloid exposure like vitamin-E reduces the oxidative damage induced by Pb (Penna-Videau *et al.,* 2012). Protective activities of plants (deferiprone) act as an aluminum chelator and nullify the Al-induced toxicity (Albina *et al.,* 2000).Chemical compounds isolated from *Morus nigra*like anthocyanin, betulinic acid, flavonoid, β-sitosterol and germanicol (Naowaboot *et al.,* 2009), and from *Syzygium cumini*areoleanolic acid, isoquercetin, quercetin, myricetin,tannins, delphinidin, petunidin, malvidin-diglucosides, friedelin, ellagic acid, gallotannin, ellagitannin betulinic acid, kaempferol, gallic acid, myricetine, pinocarveol, cineole, gerany-l-acetone, α-cadinol, eucarvone, muurolol and α-myrtenal have medicinal abilities and can be used in drugs (Kaneria, 2009).

The heavy metals induced testicular and hepatic histoarchitecture grievances which can be attenuated and chelated by using specific plants extracts as supplements (Singh *et al.,* 2012).Traditional medicinal plants are used in amelioration of diseases because plants components like antioxidants have ability to chelate the heavy metals and organs can be recovered after chelation. Antioxidant play significant role in the treatment of Cr induced oxidative stress as efficient chelators and have radical scavengingand apoptosis preventing abilities (Atef and Al-Attar, 2011; Lim *et al.,* 2013).Natural fruit extract ~~have~~has no side effect and do not alter the normal cecal microbial composition and intestinal microbiota; essential for carnitine palmitoyl transferase-1 pathway during rehabilitation(Koeth *et al.,* 2013).The

traditional Chinese herbal medicines are preferred as compare to modern synthetic drugs (Jieyu Zuo *et al.,* 2017) and auxiliary modern chimeric antigen receptor T-cell therapy against cancer (Van Schandevyl *et al.,* 2018) along with phytochemicals supplementations will ensure amelioration without side effects in future should be encouraged and sponsored.

5. CONCLUSION: There is no literature available for the co-treatment of Morus and Jambul, but these findings open the door and results authorized findings about bio-chelation and amelioration of heavy metals in the body. The modern allopathic medicines have limited therapeutic options due to their huge serious side effects while herbal drugs must be used as an alternative way to cure diseases.As this study is being conducted on a mammalian model the results obtained will be helpful in order to make clinical recommendation of its safe and beneficial use for the human populations and that approach should be encouraged and sponsored.

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