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Ameliorations of *Carrisa carandus* fruit pulp extract on hepato-histopathologies of carbofuran exposure in mice

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ABSTRACT

Purpose: The purpose of this study was to explore the ameliorative effects of *Carissa carandus* fruit pulp extract against the hepato histopathological changes of Carbofuran treated mice.

Methods: There were 40 animals divided into four groups (10 animals in each group) and study consists of 8 days. 1:Vehicle Control group (VC) (0.1mL 5% DMSO in corn oil was provided daily for first three days and for remaining days (4 to 8) of study pure water was given) , 2: *Carrisa carandus* group (CC) (0.1mL of 5% DMSO in corn oil was given for first three days, followed by 4th day rest and from 5th-8th days of study CC was provided), 3: Carbofuran group (CF) (treated with 0.1mL CF in 5% DMSO in corn oil for first 3 days and for remaining days (4 to 8) of study pure water was given), 4: Carbofuran+ *Carrisa carandus* group (CF+CC) (provided with 0.1mL CF in 5% DMSO in corn oil for first three days, followed by one day rest and for 5-8 days ,0.1mL of CC fruit extract in 5% DMSO in corn oil was given). All the animals were dissected on 9th day to remove liver lobes for further processing.

Results: Carbofuran exposure induced several hepato-histopathological changes that included damaged hepatic vein, apoptotic hepatocytes, irregular hepatic cords with disrupted sinusoids, macrophages infestation and micronuclear formation. Most of these pathologies were ameliorated in *Carrisa carandus* treated group. In CF group, significant decrease in mean number of CSA of hepatocytes, CSA of hepatic nuclei, number of hepatocytes and oval cells/per unit area (2,495.45µ²m), and animal body weight was significantly ($p<0.05$) lower than CF+CC (29.556±0.293) and CF (24.111±0.85) groups as compared to VC (30.667±0.5773) and CC (31.22±0.8570) groups respectively.

Conclusions: Findings of this study concluded that CF exposure damage the histopathology of liver and CC (*Carrisa carandus*) extract can be used as hepato-protective agent against exposure of Carbofuran.

Key-words: *Carbofuran; Carissa carandus; Koranda; Furadan; Liver*

INTRODUCTION

Carbamate ($C_{12}H_{15}NO_3$) family is commonly used in agriculture as pesticide.[1,2] Carbamates are used more commonly than organochlorine and organophosphates due to its low toxicity and relatively degraded easily in the environment. Choline esterase enzyme activity inhibition by carbamate is reversible.[3]

Histopathological alterations had been reported in all vital organs like liver, stomach, kidney, pancreas and intestine in mice and rats treated with Carbofuran[4] as well as in reproductive organs.[5] Liver is the principal organ for the metabolism of pesticides and excretion of the harmful substances occur through the kidney therefore alterations in histopathological and biochemical parameters of these organs serve as a tool to evaluate the toxicity of insecticides.[6]

In african catfish, histopathological alterations like sinusoidal congestion, cellular infiltration, hepatic necrosis was observed in CF treated group along with increased level of glucose,cortisol and aspartate amino transferase.[7] Hepatic cell necrosis was also observed in exposed wister rat liver.[8] CF exposure was considered as the leading cause of oxidative stress.[9] Histological alterations like hepatic necrosis,proliferation of kupffer cells, hepatic cellular changes, disorganized nucleus and endoplasmic reticulum was observed against fluoride exposure in the liver of albino rat. [10] Moreover, negative effects on the fetal growth of pregnant mice was also observed against CF exposure.[11]

In mice LD50 value of CF is 0.5mg/Kg body weight.[11] *Carrisa carandus* fruit extract is the rich source of sugar, flavonois, protein, terpenoids, steroids, phenolic compounds, saponins, carbohydrates, glycosides, ascorbic acid,[12] calcium, phosphorus[13] and iron as well different vitamins (C & A).[14] Therefore, was decided to check the rescuing potential of CC against CF induced liver damage.

MATERIAL AND METHODS

The present study was conducted on 40 male albino mice (*Mus musculus*) reared in animal house of The Department of Zoology, University of Sargodha. Mice were divided into four groups in separate cages. Room temperature was regularly monitored and kept at 20-23°C. Water and feed with appropriate amount of essential nutrients was provided daily. For the experiment, 40 healthy mice weighing between 28-30g were used.

Preparation of required dose of insecticide

The standard solution was 5mg/kg, it was prepared by

For 1000g animal desired dose is = 5mg

For each 1g animal desired dose is= 5mg/1000g

For 30g animal desired dose is= 5mg/1000g*30g

= 0.15mg/30g

The dose of 0.1mL of carbofuran solution was given to the animals. So the dose of each 0.1mL of 5mg/kg of carbofuran solution must contain 0.15mg of carbofuran.

Preparation of Carrisa carandua pulp extract

Carrisa carandus fruit was collected from University of Sargodha, Sargodha. The fruit was washed with tap water and grinded by juicer blender after removing its seeds. Finally juice was centrifuged to obtain the supernatant from pulp.[15]

Corn oil and DMSO

5% DMSO was mixed with corn oil in proportion of 0.5mL DMSO and 9.5mL corn oil.

Dose Groups

Forty animals were divided into four groups and each group contains 10 animals.

- **(i)** **Vehicle Control (VC) group:** 0.1mL 5% DMSO in corn oil was given for 1-3 days and pure water for 4-8 days.
- **(ii)** ***Carrisa carandus* (CC) group:** 0.1mL of 5% DMSO in corn oil for 1-3 days and 5-8 days CC fruit extract.
- **(iii)** **Carbofuran (CF) group:** was treated with 0.1mL CF of 5% DMSO in corn oil for 1-3 days and pure water for 4-8 days.
- **(iv)** **Carbofuran + *Carrisa carandus* (CF+ CC) group:** was provided with 0.1mL

CF in 5% DMSO in corn oil for 1-3 days followed by CC fruit extract for 5-8 days.

Excision of liver lobes and histological observation

All the animals were recovered on day 9 and liver of each animal was excised. The excised liver lobes were fixed in formyl ethanol, followed by dehydration process by passing in 50%, 70%, 90%, 100% alcoholic grades, cleared in xylene and wax embedded. Thin sections of 2-3µ were obtained by microtome (ERMA TOKYO 422) and stained by H & E.

Photomicrography

After staining, photography of liver section was done at 100X and 400X magnifications by digital camera (Sony DSC-W35) of 7.2 mega pixel with trinocular research microscope (Labomed CXR2). Selected photographs of liver section were further improved by using the CorelDRAW11.

Micrometry

The measurement of mean Cross Sectional area (CSA) of hepatocytes, CSA of hepatic nuclei, number of hepatocytes and oval cells/per unit area (2,495.45µ²m) were measured according to the following formula.

CSA = (Length x Width/4) x 3.14

Analysis of data and statistical application

The micrometric data was analyzed by applying to ANOVA and Tukey multiple Range test (TMRT) in SPSS20.

RESULTS

Histological Results

Healthy histological signs were observed in vehicle control and *Carrisa carandus* liver including lobular structure of central vein with normal size surrounded by organized hepatic cords, sinusoids arranged regularly with narrow spaces. Hepatocytes have circular to polygonal shape with normal sized nuclei and uniform distribution of cytoplasm. Moreover,components of hepatic triad were clearly distinguishable (Fig 1 and 2).

In *Carrisa carandus* liver, some stem cells and rehabilitatory cells including progenitor cells and biliary epithelial cells found abundantly which were very rarely found in vehicle control group (Fig 1 and 2). Carbofuran treated group showed hepato-histopathological damage. Degenerative signs includes enlarged and punctured central vein, distorted hepatic cords with irregularly spaced sinusoids, apoptotic hepatocytes, perinuclear vacoulation and disrupted shaped hepatic nuclei and micro-nuclear formation. The hepatic lobule also showing infestation of macrophages and some regenerative cells which shows natural rehabilitation tendency of liver (liver regeneration).There is no distinguish between components of triad (Fig 1 and 2).

The Carbofuran+*Carrisa carandus* group showed rehabilitatory signs included the rehabilitation of central vein with nascent and rehabilitated hepatic cords. Triad components of this group were comaparatively distinguishable. Presence of white blood cells, regenerative cells,cholangeocytes around triad and hepatoblast in hepatic lobule clearly indicate ameliorative features of karonda against carbofuran. (Fig 1 and 2).

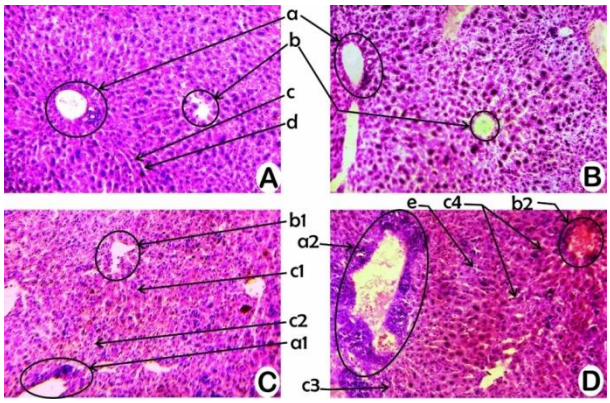

Figure 1: Hematoxylin and Eosin stained histological section (100X) of mice liver; A:(Vehicle Control), **B**:(*Carrisa carandus*), **C**:(Carbofuran), **D**:(Carbofuran+ *Carrisa carandus*)a:normal triad, a1: damaged triad, a2:rehabilitation of triad, b:normal central vein, b1:damaged central vein, b2: rehabilitated central vein c:normal hepatic cord, c1:distorted hepatic cord, c2:micronuclear formation, c3: nascent hepatic cord, c4:rehabilitated hepatic cords, d:normal sinusoids, e:hepatoblast.

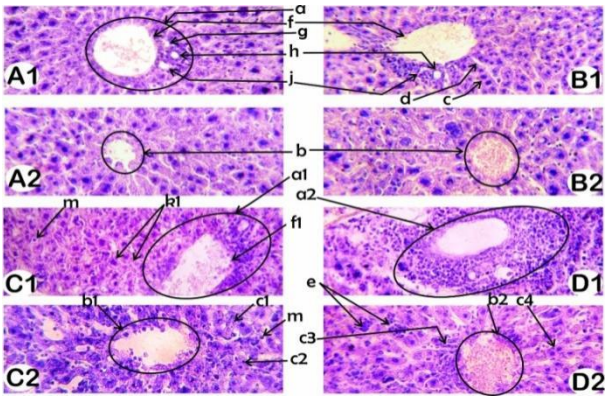

Figure 2: Hematoxylin and Eosin stained histological sections (400X) of mice liver. A1+A2(VehicleControl); **B1+B2**:(*Carrisacarandus*);**C1+C2**:(Carbofuran);**D1+D2**(Carbofuran+*Carrisa carandus*) a:normal triad, a1:damaged triad a2:rehabilitated triad b: normal central vein,b1: damaged central vein, b2:rehabiliated central vein ,c: normal hepatic cord c1: distorted hepatic cord, c2: formation of micronuclei,c3: nascent hepatic cord, c4: rehabilitated hepatic cords,d: normal sinusoid, e:hepatoblast, f:hepatic portal vein,f1:enlaerged hepatic portal veing: lacteal of triad, h:bile ductule, j:hepatic artery, k1:apoptotic hepatocytes, m:macrophages showing phagocytic activity.

Micrometric Results

Statistical analysis have shown significant ($p<0.01$) difference among the groups for all parameters. Post hoc analysis showed lowest mean value of CSA of hepatocytes in Carbofuran treated group (72.48±3.91) than CF+CC(125.49±8.09), VC(168.65±5.31) and (169.69±4.47) groups. Significant lower value of CSA of hepatic nuclei was observed in CF (18.58±2.80) than CF + CC (24.19±1.73), CC(39.16±1.73) and VC (39.58±2.12) group (Table. 1).

Mean value of number of hepatocytes/unit area (2495.45μ²m) were significant higher in CC (3.92±0.13) and VC group (3.64±0.15) than CF+CC(3.00±0.15) and CF (2.21±0.14) groups. Mean value of number of oval cells/unit area(2495.45μ²m) were significant higher in CF+CC (3.71±0.29) group than CF (2.67 ± 0.27), CC(2.03± 0.23) and VC (1.6±0.32) group. Significant lower mean animal body weight was observed in CF (24.111±0.857) than CF+CC(29.556±0.293), VC(30.667±0.5773) and CC(31.22±0.8570) group (Table. 1).

Biochemical Results

Statistical analysis(ANOVA) have shown significant ($p<0.01$) difference among the groups for all parameters. Post hoc analysis showed slightly higher mean value of Bilirubin in CF treated group (0.667±0.033) than CF+CC(0.613 ± 0.03), VC(0.55 ± 0.057) and CC group (0.55 ± 0.057). Significant higher value of AST were observed in CF+CC(106 ± 15.8) than CF (54.00 ± 23.43), VC (52.66 ± 12.11) and CC (52.33 ± 13.81) groups (Table. 2).

The mean value of Alkaline phosphate were lower in CF+CC(58.18 ± 33.01) than CF(173.6 ± 3.17), CC (237.61 ± 18.9) and VC (323.3 ± 33.01) groups. Mean value of total protein were observed slightly higher in CF (6.26 ± 0.145) than CF+CC(5.56 ± 0.218), VC(5.46 ± 0.145)and CC (4.93 ± 0.29) groups. Significant lower mean value of albumin were observed in CC(1.93 ± 0.145) than VC(2.53 ± 0.88), CF+CC(3.00 ± 0.404) and CF(3.066 ± 0.176) (Table.2).

Table 1. Shows micrometric parameters related to liver

Micrometric Parameters	Groups			
	VC	CC	CF	CF+CC
CSA of hepatocytes	168.65±5.31 [c]	169.69 ±4.47 [c]	72.48± 3.91[a]	125.49± 8.09[b]
CSA of hepatic nuclei	39.58±2.12 [b]	39.16± 1.73 [b]	18.58± 2.80 [a]	24.19±1 .73[a]
Number of hepatocytes per unit area (2495.45μ²m)	3.64±0.15 [c]	3.92±0 .13 [c]	2.21±0 .14 [a]	3.00±0. 15[b]
Number of oval cells per unit area (2495.45μ²m)	1.6±0.32 [a]	2.03± 0.23 [a]	2.67 ± 0.27 [a]	3.71±0. 29 [b]
Mean of animal body weight	30.667±0. 577[b]	31.22± 0.85 [b]	24.111 ±0.85 [a]	29.556± 0.29[b]

ł: analyzed by ANOVA, [abc] the mean values in a row not sharing a common superscript differ significantly (p≤0.05) with each other.

Table 2. Shows biochemical parameters related to mice liver

Biochemical Parameters	Mean + SEM			
	VC	CC	CF	CF+CC
łBilirubin	0.55 ± 0.057 [a]	0.55 ± 0.057 [a]	0.667± 0.03[a]	0.613 ± 0.03 [a]
ł AST	52.66± 12.11 [a]	52.33± 13.81 [a]	54.00± 23.4 [a]	106± 15.8 [a]
ł Alkaline phosphate	323.3± 33.01 [b]	237.61 ± 18.90 [a]	173.6± 3.17 [a]	58.18± 33.00[b]
ł Total protein	5.46± 0.145 [a]	4.93± 0.29 [a]	6.26± 0.145 [b]	5.56± 0.218 [b]
ł Albumin	2.53± 0.88 [ab]	1.93± 0.145 [a]	3.066± 0.17 [b]	3.00± 0.40[ab]

ł:analyzed by ANOVA, [abc] the mean values in a row not sharing a common superscript differ significantly (p≤0.05) with each other.

DISCUSSION

Liver is a vital organ which is involved in synthesis of useful products, detoxification and elimination of harmful agents.[16] During its detoxification process,different harmful chemicals induce oxidative stress in liver cells.[17] Carbofuran, belongs to carbamate family used to control harmful agricultural insects. Carbofuran exposure inflicts the toxicological effects on living organism by damaging its organs including liver, kidney reproductive organs.[18,19]

In present study different histopathological alterations were observed in mice liver exposed with carbofuran including necrosis, enlarged and punctured central vein,distorted hepatic cords with irregularly spaced sinusoid, apoptotic hepatocytes, micronuclear formation, perinuclearvacoulation and dispersion of nuclear material in some cells. The infestation of macrophages and regeneration of cells (liver regeneration); these signs were also reported in previous studies.[20] Biochemical analysis including higher mean value of billirubin, AST and total protein in carbofuran treated group than alkaline phosphate value that was lower in carbofuran treated group.

Carrisa carandus has been used as an ameliorative and soothing agent in hepatic ailment[18,19] but here is no previous study that showed hepatoprotectant effects of *Carrisa carandus* against Carbofuran. Certainly all above mentioned changes in liver histology of mice may be due to oxidative stress caused by carbofuran exposure. It has been reported that vitamin C has ameliorative effects against the carbofuran treated liver in rat[20] and *Carrisa carandus* is a rich source of vitamin C[21] and according to literature *Carrisa carandus* is also a rich source of these components[22] .The leaf extract of Justicia gendarussa (a medicinal herb) has been used against Carbofuran hepatohistopathologies and leaf extract have significant amount of flavinoids and phenols[23].

Additionally our study also support ameliorative effects of *Carrisa carandus* which is clearly observed in Carbofuran+*Carrisa carandus* group like the rehabilitation of central vein with nascent hepatic cords, transformation of micronuclei to normal size. Components of triad were comparatively distinguishable. Presence of white blood cells, regenerative cells, cholangeocytes around triad and hepatoblast in hepatic lobule clearly indicate amelioration of karonda against carbofuran.

CONCLUSIONS

Exposure of carbofuran to non-target organism cause severe hepatohistopathologies. It is concluded that unnecessary use of insecticides should be avoided. If its use is extremely needed, should be administered properly. People who are occupationally exposed with carbofuran may be recommended with use of *Carrisa carandus* fruit extract. But still need a thorough study about the use of *Carrisa carandus* fruit extract for human.

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Not Applicable.

CONFLICT OF INTERESTS

None

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