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PROTECTIVE EFFECTS OF CACTUS AND/OR PAPAYA JUICES AGAINST HEPATIC AND TESTICULAR TOXICITY INDUCED BY CHLORPYRIFOS IN ALBINO RATS

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ABSTRACT

This study aimed to evaluate the effect of cactus and/or papaya juices on chlorpyrifos induced toxicity in rat's liver and testes. A total of sixty albino rats weighing (180g -200g) were used for this study. Rats were divided into 5 groups each of 12 rats. First group was left as control group. Second group was given chlorpyrifos 6mg/kg b wt. The other three groups were given cactus, papaya juices and both of them as 20ml/kg b wt, subsequently along with chlorpyrifos dose. All doses were orally administered daily for 28 successive days. At the end of the experimental period, blood samples, liver and testes were collected from each rat for biochemical analysis. Oral administration of chlorpyrifos for 28 days in a dose of 6 mg/kg bwt, significantly increased the percentage of DNA fragmentation, caspase-3 level in liver and testes tissues. A significant increase in serum alanine aminotransferase, aspartate aminotransferase, catalase, cytochrome P450, paraxonase-1 activity as well as tumor necrosis factor- α, malondialdehyde and follicle stimulating hormones levels and decrease in testosterone and luteinizing hormone levels was observed. On the other hand the administration of tested chlorpyrifos with juices, reduce the percentage of DNA fragmentation, caspase-3 level and directed enzymes activity and hormones levels toward normal. The effect on the liver is one of the main toxic effects of this product. In conclusion cactus and papaya juices have beneficial effects as it tends to dampen chlorpyrifos toxicity in rats.

KEYWORDS: cactus, papaya, chlorpyrifos, testosterone, DNA fragmentation, paraxonase-1.

1. INTRODUCTION

Pesticides poisoning is a major clinical problem worldwide, especially in developing countries. Workers of pesticide manufactories, agriculture workers and their families, people living in proximity to farms, and those exposed heavily to home application of pesticides or eat food rich in pesticides residue are highly vulnerable to pesticides intoxication.[1]

Pesticides fall into several chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms. Organo phosphorus (OP) insecticides are the largest and the most diverse group of insecticides. The broad application of OP insecticides in public health and agricultural programs are accompanied by potentially hazardous impacts on humans, animals, plants and environment (water, air, soil and food) and cause severe acute and chronic poisoning.[2]

Chlorpyrifos (CPF) (O, O-diethyl O- 3, 5, 6- trichloro 2- pyridinyl phosphorothioate) is a widely OP insecticide used for agricultural and domestic applications in the whole world. Similar to other phosphorothioate pesticides, chlorpyrifos acts primarily by inhibiting neuronal acetylcholine esterase (AChE) activity, thereby interfering with normal cholinergic nerve transmissions. Studies have indicated that chlorpyrifos affects several biochemical pathways that are independent of the modulation of the Ach E enzyme. One such mechanism associated with both acute and chronic poisoning is oxidative stress. Several studies point to the generation of reactive oxygen species as a secondary molecular mechanism of some pesticides toxicity.[3]

Cactus (*Opuntia ssp.*) is used as a common medicinal plant. There are about 200 recognized species of *Opuntia*. Cactus pears are sweet edible fruits from the cactus that belong to the *Cactaceae* family. These fruits contain a wide variety of trace elements, sugars and other bioactive compounds, such as betalains, carotenoids, ascorbic acid, flavonoids and other phenolic compounds. Cactus pear fruits are now recognized as a rich source of nutritional compounds with health-promoting activities, including antioxidant, neuroprotective, cardioprotective, anti-inflammatory, anti-diabetic, anti-clastogenic and anti-genotoxic actions. In addition, they have protective effects on erythrocyte membranes and on acute gastric lesions, and they improve platelet function and cancer chemoprevention.[4,5]

Papaya *(Carica papaya)* belongs to *Caricaceae* family is known by many other names such as papaya, papaw, pawpaw, mamao and melon tree. It is cultivated for its young leaves and

fruits which are cooked as a vegetable or for its ripe fruit which is consumed as a beverage. Papaya had positive effect against bacterial infections. Treatment with *carica papaya* improved efficiency of phagocytic cells that destroy bacteria. Papain which is the enzyme found in *Carica papaya* is effective natural medicine in controlling both inflammation and edema associated with surgical procedures. It also produced therapeutic beneficial effects in patients with inflammatory disorders of liver, intestine and eye.[6]

Papaya is a rich source of three powerful antioxidant vitamins C, E and precursor of vitamin A; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer.[7]

2. MATERIALS AND METHODS

2.1 Plant materials

Ripe cactus and papaya fruits were obtained from the Ministry of Agriculture and Land Reclamation, Cairo, Egypt in August and December 2017, respectively.

2.2 Chemicals and kits

Chlorpyrifos was purchased from The National Company for Fertilizers and Chemicals. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), catalase (CAT) and lipid peroxidation malondialdehyde (MDA) kits were obtained from Biodiagnostic Co. (Giza, Egypt). testosterone, luteinizing hormone (LH), follicle stimulating hormones (FSH), cytochrome P450 Isozyme, paraxonase-1 (PON 1) and tumor necrosis factor- α (TNF-α) Kits were purchased from Cusabio Co. USA. Kit for caspase-3 level was purchased from MyBiosource Co. (USA). All other chemicals were of the highest purity commercially available.

2.3 Preparation of plant juices

Fruit juices were prepared from peeled cactus and papaya fruits according to ***Madrigal-Santillán et al.***[8] ***and Gopinathan and Naveenraj***[9]**,** respectively. The juices were extracted with a juicer machine subsequently then the juices were strained and filtered through muslin cloth. Finally, they were stored frozen at −4 °C until used.

2.4 Analysis of bioactive components in juices by GC analysis

Juices of cactus and papaya fruits juices were prepared according to the methods previously described. The main phytocomponents of cactus and papaya juices were identified using GC–MS detection system.

GC–MS analysis was accomplished using an agilent 6890 series II gas chromatography system set up with an agilent 5973 mass spectrometer with electron ionization. The MS system was performed in electron ionization mode with Selected Ion Monitoring generated at 70eV. The ion source temperature and quadruple temperature were set at 230°C and 280°C, respectively. The GC was performed using HP5-MS capillary column (30 m x 0.25 mm x 0.25 µm). Operating conditions were as follows: carrier gas, helium with a flow rate of (1.5ml/ min). The temperature program was set as follows: The initial temperature was programmed from 60 °C to 90°C ramp 10°C per min then 90°C to 280°C ramp 8 °C per min held 2 min, and the total run time was 30 min.

Identification of phytocomponents was performed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST08.L and Wiley7n.l libraries.[10]

2.5 Experimental animals

Sixty healthy adult male albino rats Spargue-Dawley strain, weighing 180-200g, were obtained from the Breading Unit of the Egyptian Organization for Biological Products and Vaccines, Helwan, Egypt. All rats were maintained on standard lab diet prepared according to American Institute of Nutrition (AIN -93) and adjusted by ***Reeves et al.***[11] with some modifications[12]; all the amount of starch was used in the form of corn starch and corn oil was used instead of soybean oil, in 12/12h light/dark cycle and temperature controlled room.

2.6 Experimental design

After an acclimatization period of 1 week, toxicity with chlorpyrifos was induced in 48 rats intragastrically at a dose of 6 mg/kg bwt daily for 28 days dissolved in corn oil.[3] While 36 intoxicated rats co-administered (20ml/kg bwt) cactus and/or papaya juices daily for 28 days.[8,9]

- Group 1 (healthy rats): received corn oil.
- Group 2 (CPF intoxicated rats): received CPF dose dissolved in corn oil.
- Group 3: received CPF dose + cactus juice dose.

- Group 4: received CPF dose + papaya juice dose.
- Group 5: received CPF dose + mixture of cactus and papaya juices dose.

During the experimental period (28 days) food intake was measured daily by subtracting the residual and refusal diet from the served diet. The animals were weighed weekly to monitor the body weight changes.

Food intake (g) = Served diet – (Residual diet + Refusal diet).

2.7 Collection of blood and tissue samples

After the end of the experimental period, rats were fasted overnight, then all rats were scarified under ether anesthesia. Blood samples were collected from the hepatic portal vein for the separation of serum, then it was stored at -20◦C until used for biochemical analyses.

Liver and testes were immediately removed and cleaned, rinsed with cold physiological saline solution (0.9%) then blotted on filter paper and weighed to calculate the relative weight (g%) as described by ***Guo et al.***[13]

$$\text{Relative organ weight} = \frac{\text{Absolute weight}}{\text{Final body weight}} \times 100$$

2.8 DNA fragmentation in liver and testes tissues

The percentage of DNA fragmentation was assessed by the method proposed by ***Boraschi and Maurizi.***[14] This method is based on the notion that extensively fragmented double stranded DNA can be separated from chromosomic DNA upon centrifugal sedimentation. Includes the lysis of cells and the release of nuclear DNA centrifugation step with the generation of two fractions (corresponding to intact and fragmented DNA respectively), precipitation of DNA, hydrolysis and colorimetrical quantitation upon staining with diphenylamine (DPA), which binds to deoxyribose.

2.9 Statistical analysis

The data were statistically analyzed by SPSS version 18.0 statistical packages. Data were presented as mean ± S.D. statistical differences between groups were performed using student t-test, differences considered significant when $P< 0.05$.[15]

3. RESULTS

3.1 Phytocomponents analysis in cactus and papaya juices

The analysis of phenolic compounds of cactus was performed by GC-MS allowing to identify compounds kaempferol, isorhamnetin, quercetin, rutin and caffeic acid. In terms of phenolic compounds identifed, the most abundant was cafeic acid with respectively 9.8% of plant material. In terms of flavonoid composition, isorhamnetin derivatives was the major component, it accounted for 20.6%, quercetin derivatives for 20.0% kaempferol derivatives for 13.1% and rutin derivatives accounted for 6.7% (figure 1).

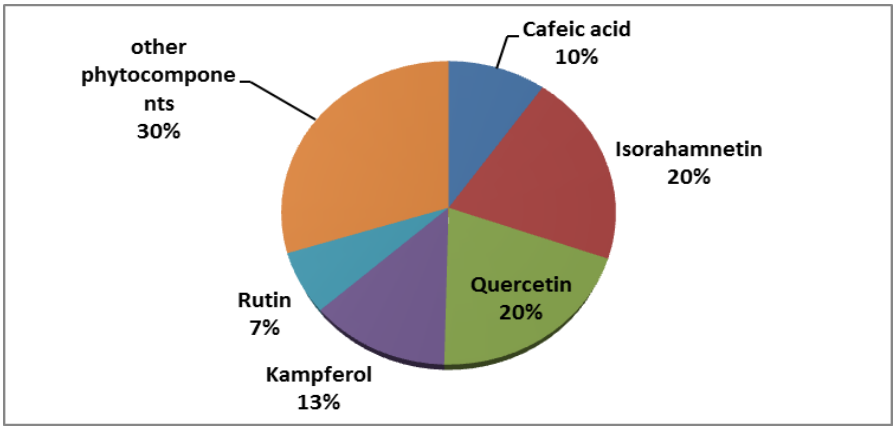

Figure 1: Bioactive components in cactus juice.

Phenolic compounds were identified in papaya juice. caffeic acid 16.0%, ferulic acid 1.6% and rutin 5.0%, flavonoid quercetin-3-O-rutinoside 13.0 were the most abundant phenolics and only traces of gallic 0.8% and protocatechuic acids conjugates 0.6% were found. The other phytocomponents present in papaya in terms of their relative abundance were tetradecanoic acid, octadecanoic acid and hexadecanoic acid, methyl ester (figure 2).

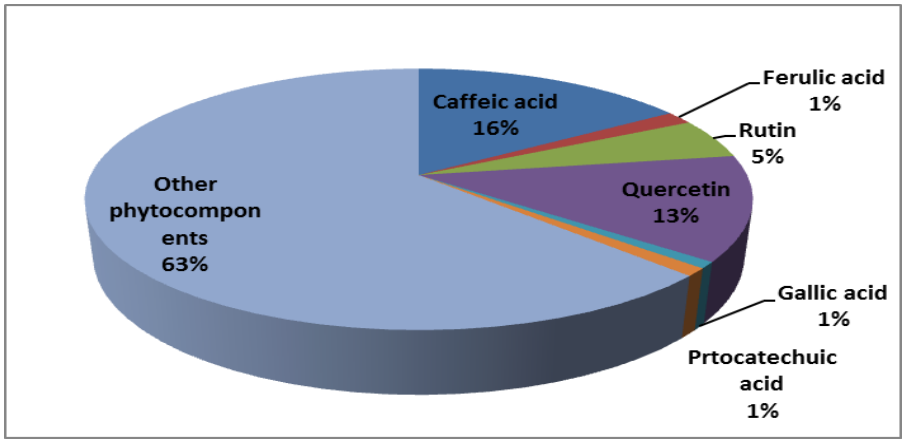

Figure 2: Bioactive components in papaya juice.

3.2 Effects of oral doses of cactus and/or papaya juices on biological parameters

The results in table (1) show the mean values± SD of body weight changes (g), food intake (g), and the relative weight of liver and testes (g%) in case of CPF intoxicated rats treated with juices as compared with CPF intoxicated control and healthy control groups. The current study showed that CPF causes significant ($P < 0.05$) decrease in body weight gain by 30.52% and significant increase in food intake by 4.72% in intoxicated rats as compared with healthy rats. While, there was no significant difference in body weight gain for groups administered cactus and/or papaya juices as compared with CPF intoxicated group. Moreover, treatment with CPF significantly affect relative liver and testes weights that recorded 4.37 g%, 1.19 g%, respectively when compared with corresponding means in healthy rats that recorded 2.99 g%, 0.99 g%, respectively. While in treatment with juices improvement was observed.

Table 1: The effects of cactus and/or papaya juices on some biological parameters in chlorpyrifos intoxicated rats compared with healthy rats.

Parameters / Groups	Body weight gain (g)	Food intake (g/day)	Relative liver wt (g%)	Relative testes wt (g%)
Healthy rats	78.92±16.94^{a}	24.34±1.35^{b}	2.99±0.22^{a}	0.99±0.1^{a}
CPF intoxicated rats % of change*	54.83±6.04^{b} -30.52	25.49±1.54^{a} 4.72	4.37±0.22^{c} 46.15	1.19±0.1^{b} 20.20
Intoxicated rats treated with cactus juice % of change* % of change**	57.00±9.98^{b} - 27.77 3.96	24.38±1.25^{b} 0.21 -4.35	3.73±0.5^{b} 24.75 -14.65	1.23±0.1^{b,c} 24.24 3.36
Intoxicated rats treated with papaya juice % of change* % of change**	54.92±6.05^{b} -30.41 6.16	24.77±1.55^{a,b} 1.81 -2.82	3.7±0.5^{b} 23.75 -15.33	1.28±0.11^{c} 29.29 7.56
Intoxicated rats treated with cactus and papaya juices % of change* % of change**	55.58±11.52^{b} -29.57 1.37	24.13±0.91^{b} -0.81 -5.34	3.26±0.27^{a} 9.03 -25.4	1.19±0.1^{b} 20.20 0
L.S.D.	8.91	1.09	0.299	0.08

Values expressed as mean ± S.D.; 12 rats in each group; There was no significant difference between means have the same superscript in the same column at P<0.05.% of change from healthy rats, % of change** from CPF intoxicated rats*

3.3 The effects of cactus and/or papaya juices on some liver functions parameters

The results in table (2) show the mean value ±SD of ALT and AST CytochromeP450 Isozyme and PON1 activities. There was a significant increase in ALT and AST activity by

150.34 % and 152.2 %, respectively, in rats treated with CPF as compared with healthy group and this confirms the hepatotoxic effect of CPF. However, co-administration of cactus and/or papaya juices with CPF ameliorates the toxic effect and cause significant improvement in enzymes activity for all treated groups.

Exposure to CPF causes a significant increase in CytochromeP450 and PON1 activity by 101.7 % and 15.17 % as compared with healthy group. While cactus and/or papaya juices co-administration for rats exposed to CPF caused a significant decrease for cytochromeP450 by 6.6 %, 14.47 % and 1.43 % and increase for PON1 activity 28.41 %, 3.77 % and 2.28 %, respectively as compared with CPF intoxicated rats.

Table 2: The effects of cactus and/or papaya juices on some liver function parameters in chlorpyrifos intoxicated rats compared with healthy rats.

Parameters / Groups	ALT activity (U/L)	AST activity (U/L)	CytochromeP450 Isozyme (pg/ml)	Paraoxonase 1 activity (U/ml)
Healthy rats	8.82±0.64^{c}	14.78±0.25^{d}	122.79±3.11^{e}	174.38±6.92^{d}
CPF intoxicated rats % of change*	22.08±0.72^{a} 150.34	37.27±0.60^{a} 152.2	247.72±3.4^{a} 101.7	200. 85±6.10^{b} 15.17
Intoxicated rats treated with cactus juice % of change* % of change**	10.61±0.93^{b} 20.29 -51.95	15.52±0.67^{c} 5.0 -58.36	231.37±5.51^{c} 88.43 -6.60	257.91±9.24^{a} 47.90 28.41
Intoxicated rats treated with papaya juice % of change* % of change**	10.94±1.45^{b} 24.04 -50.45	16.74±0.83^{b} 13.26 -55.08	211.88±5.39^{d} 72.55 -14.47	193.27±4.38^{c} 10.83 -3.77
Intoxicated rats treated with cactus and papaya juices % of change* % of change**	11.19±1.53^{b} 26.87 -49.32	16.77±0.67^{b} 13.46 -55.0	244.18±2.46^{b} 98.86 -1.43	205.42±7.54^{b} 17.80 2.28
L.S.D.	0.914	0.52	3.41	5.75

Legend as table 1

3.4 The effects of cactus and/or papaya juices on some fertility parameters

The levels of serum fertility hormones testosterone (ng/ml), LH (mlU/ml) and FSH (mlU/ml) were measured and results mean value ± SD are shown in table (3). A significant decrease in testosterone and LH and increase in FSH levels 0.74±0.14 ng/ml, 1.16±0.04 mlU/ml and 0.83±0.06 mlU/ml, respectively in CPF intoxicated group in comparison with levels in

healthy group which are 4.75±0.11 ng/ml, 4.7±0.16 mlU/ml and 0.3±0.02 mlU/ml, respectively. The percentage improvements after treatment with cactus and/or papaya juices were 54.05%, 458.11%, 516.21% & 76.73%, 102.59%, 80.17% and 50.6%, 32.53 %, 39.76% for testosterone, LH and FSH, respectively.

Table 3: The effects of cactus and/or papaya juices on some fertility parameters in chlorpyrifos intoxicated rats compared with healthy rats.

Parameters / Groups	Testosterone (ng/ml)	LH (mlU/ml)	FSH (mlU/ml)
Healthy rats	4.75±0.11[a]	4.7±0.16[a]	0.3±0.02[e]
CPF intoxicated rats % of change*	0.74±0.14[e] -84.4	1.16±0.04[e] -75.3	0.83±0.06[a] 176.7
Intoxicated rats treated with cactus juice % of change* % of change**	1.14±0.06[d] -76 54.05	2.04±0.09[c] -56.38 76.73	0.41±0.04[d] 36.67 -50.60
Intoxicated rats treated with papaya juice % of change* % of change**	4.14±0.07[c] -12.84 458.11	2.35±0.06[b] -50.0 102.59	0.56±0.07[b] 86.67 -32.53
Intoxicated rats treated with cactus and papaya juices % of change* % of change**	4.56±0.12[b] -4.0 516.21	2.09±0.04[c] -55.53 80.17	0.6±0.04[c] 66.67 -39.76
L.S.D.	0.086	0.073	0.045

Legend as table (1)

3.5 The effects of cactus and/or papaya juices on some inflammatory and oxidative stress markers

The data on the influence of cactus and/or papaya juices and CPF co-exposure on biomarkers of inflammation and oxidative stress are shown in table (4). Exposure to CPF significantly increased the level of TNF-α reach 169.79± 6.08 pg/ml compared with healthy group 22.54±0.87 pg/ml and also serum MDA level was increased to 81.98± 1.05nmol/ml in CPF intoxicated group as compared with healthy group 35.42±1.37nmol/ml and serum catalase activity was increased in CPF treated group by 196.4 % as compared with healthy group. On the other hand, the increase in these indices of inflammation was significantly diminished in the rats co-treated with cactus and/or papaya juices by 59.46 %, 83.53 % and 75.53 %, respectively for TNF-α and decrease MDA level to 42.15±0.68, 56.04±0.33 and 43.93±0.32 nmol/ml, respectively when compared with intoxicated rats. while the percentage change was -4.02%, -42.65% and -20.77% for cactus and/or papaya juices co-administered rats, respectively as compared with CPF group.

Table 4: The effects of cactus and/or papaya juices on some inflammatory and oxidative stress markers in chlorpyrifos intoxicated rats compared with healthy rats.

Parameters / Groups	TNF-α level (pg/ml)	MDA level (nmol/ml)	CAT activity (U/L)
Healthy rats	22.54 ±0.87 ^{e}	35.42±1.37^{e}	116.27±8.63^{e}
CPF intoxicated rats % of change* % of change**	169.79±6.08^{a} 653.3 196.4	81.98± 1.05^{a} 131.5	344.60±22.08^{a}
Intoxicated rats treated with cactus juice % of change* % of change**	68.83±6.26^{b} 205.37 -59.46	42.15±0.68^{d} 19.0 -48.59	330.75±5.35^{b} 184.47 -4.02
Intoxicated rats treated with papaya juice % of change* % of change**	27.97±0.92^{d} 24.09 -83.53	56.04±0.33^{b} 58.22 -31.64	197.63±7.84^{d} 69.98 -42.65
Intoxicated rats treated with cactus and papaya juices % of change* % of change**	41.55±3.5^{c} 84.34 -75.53	43.93±0.32^{c} 24.03 -46.41	273.02±4.59^{c} 134.82 -20.77
L.S.D.	3.47	0.699	9.49

Legend as table 1

3.6 The effects of cactus and/or papaya juices on apoptosis markers (DNA damage and caspase-3)

The results of the present study in table (5) illustrate the effect of CPF on DNA of examined tissues. Treatment with CPF causes 15.37 % and 15.67 % DNA fragmentation in liver and testes respectively, as compared with control group 1.11 % and 1.04%.

In addition, cactus and papaya juices have a protective effect against DNA damage caused by CPF; for cactus juice % protection from liver and testes DNA damage was 87.25 % and 84.94%, respectively, for papaya juice 84.84 % and 85.39 %, respectively and for mix of juices 86.21 % and 87.68 % as compared with CPF group. The results in table (5) show that CPF can induce apoptosis in liver and testes cells, and this effect is partially mediated by activation of intracellular caspase-3. CPF can increase caspase-3 activity by 327.1 % and 201.69 % for liver and testes respectively as compared with healthy group, moreover the treatment with cactus and/or papaya juices cause a significant decrement in caspase-3 activity in all examined tissues when compared with CPF intoxicated group. However papaya juice was the most effective.

Table 5: The effects of cactus and/or papaya juices on DNA damage and caspase 3 activity in chlorpyrifos intoxicated rats compared with healthy rats.

Parameters / Groups	DNA fragmentation in liver (%)	DNA fragmentation in testes (%)	Caspase 3 activity in liver(ng/ml)	Caspase 3 activity in testes(ng/ml)
Healthy rats	1.11±0.06[d]	1.04±.016[d]	2.25±0.36[d]	2.96±0.11[d]
CPF intoxicated rats	15.37±0.61[a]	15.67±0.83[a]	9.61±0.64[a]	8.93±1.12[a]
% of change*	1284.6	1406.7	327.1	201.69
Intoxicated rats treated with cactus juice	1.96±0.14[c]	2.36±0.20[b]	4.36±0.20[b]	5.10±0.21[b]
% of change*	76.58	126.92	93.78	72.3
% of change**	-87.25	-84.94	-54.63	-42.89
Intoxicated rats treated with papaya juice	2.33±0.19[b]	2.29±0.15[b]	3.79±0.19[c]	4.19±0.07[c]
% of change*	109.9	120.19	68.44	41.55
% of change**	-84.84	-85.39	-60.56	-53.08
Intoxicated rats treated with cactus and papaya juices	2.12±0.21[b,c]	1.930±0.14[c]	4.37±0.21[b]	4.61±0.23[c]
% of change*	90.99	85.58	94.22	55.74
% of change**	-86.21	-87.68	-54.53	-48.38
L.S.D.	0.25	0.32	0.298	0.44

Legend as table 1

4. DISCUSSION

The analysis of phenolic compounds of cactus was performed by GC-MS allowing to identify compounds belonging to two families in favonoids (kaempferol, isorhamnetin, quercetin, rutin and phenolic acids (quinic acid, caffeic acid, acetylcafeic acid, caffeic acid derivative, chlorogenic acid, cafeoylquinic acid, dicafeoylquinic acid, rosmarinic acid, p-coumaric acid, ferulic acid and syringic acid.

Fernandez-Lopez et al.[16] reported a more phenolic content but it referred to a whole cactus fruit (skin and pulp). For Aissa cultivar quercetin derivatives accounted for 21.6%, rutin derivatives for 18.5% and kaempferol derivatives accounted for 16.4% of total favonoids. These values were lower than those reported by ***El Mostapha et al.***[17] While ***Kuti***[18] analyzed different types of cactus and concluded that purple skinned fruits contained the highest amounts of favonoids. Almost half of flavonoid compounds are composed of isorhamnetin.

Moreover, many terpenes derivatives have been found in the analyzed samples like camphene. The current results showed that it represented 0.6% of the aroma profile. The most

abundant compounds were (2E)-nonenal, tetradecanoic, hexadecanoic acid and E) -3-butyldiene phthalide and n-hexanol with a percentage of 18.7%.

Phenolic compounds were identified in papaya juice. caffeic acid, ferulic acid and rutin flavonoid (quercetin-3-O-rutinoside) were the most abundant phenolics and only traces of gallic and protocatechuic acids conjugates were found. The other phytocomponents present in papaya in terms of their relative abundance were tetradecanoic acid, octadecanoic acid and hexadecanoic acid, methyl ester.

Tetradecanoic acid (myristic acid) is the major antibacterial and antioxidant principles isolated from *Myristica fragrans.* Furthermore, tetradecanoic acid impedes cell proliferation and exhibits nematocidal and hypocholesterolemic activities. activities. In addition to the antioxidant activity of the n-hexadecanoic acid, like its ester derivative also serve as anti-cancer, anti-microbial, anti-haemolytic, anti-diabetic agents in addition to causing pesticidal inhibitory action to 5-α reductase activity.[19]

The effect of CPF on biological parameters was investigated in many previous works. The present results agreed with the results of ***Akhtar et al.***[20] who observed a significant decrease in body weight gain in CPF treated rats. In addition, their result showed an increase in liver relative weight. In addition, the current results matched the results of ***Tanvir et al.***[21] who showed that body weight gain of CPF-intoxicated rats was markedly reduced when compared with the control group; however, a significant change in food consumption following CPF exposure was observed. In addition, CPF caused a slight but non-significant increase in relative liver weight. A possible explanation for the reduction in body weight gain may be the overall increased degradation of lipids and proteins directly induced by CPF.[22] Also could be due to the toxicity of CPF on the gastrointestinal tract leading to a relative impairment of nutrients digestion or to inhibition of protein synthesis.[23] Moreover, the slight increase in relative liver weight may relate to the reduction in body weight gain in experimental animals.[24]

In the current study, cactus and/ or papaya juices significantly mitigates the effects of CPF on food intake and on body weight, relative liver and testes weights. Cactus provides a wide range of natural antioxidants including polyphenols, flavonoids, ascorbic acid, carotenoids, and betalains compounds.[25] This kind of antioxidants could protect body from toxic damage.[26] Also, the tissue protective properties of papaya have been traced to its phenolic

composition**.** Papaya fruits rich in antioxidant nutrients like carotene, vitamin C, vitamin B, flavonoids and phenolic compound which scavenge free radicals generated by CPF and alleviating toxic effects.[27]

Akhtar et al.[20] discus in their study the elevation of serum ALT and AST as indicators of liver damage, when the liver cell membrane damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. This agreed with the low ALT and AST activities in the liver of rats exposed to CPF in the previous findings of ***Zama et al.***[28] Therefore, the increase in these enzymes in blood may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place. Moreover, ***Zeashan et al.***[29] reported that the oral administration of the cactus extract promoted liver recovery at histochemical and biochemical levels. It has been proposed that the mechanism of hepatoprotection was due to its antioxidant activity**.** Also**,** ***Ncibi et al.***[30] showed that cactus protect against liver damage induced by CPF. A study of ***Sadeque and Begum***[31] was performed to evaluate the hepatoprotective effects of papaya against CCl_4 induced hepatotoxicity and compared it with that of vitamin E and results confirmed that papaya and vitamin E showed significant hepatoprotection against CCl_4 induced hepatotoxicity, but papaya showed more significant protection than vitamin E. Chlorpyrifos is activated *in vivo* to their oxygen analogs chlorpyrifos oxon mainly by enzymes of cytochrome P450 family. These oxidized metabolites are highly toxic but can be detoxified by esterases such as PON1. Thus, higher activity of paraoxonase enzyme in serum originates increased dialkyl phosphate levels in urine and decreased levels of the substrates (oxon forms), which in turn results in reduced toxicity of these oxon-metabolites.[32] Therefore the present results concerning that the increased level of PON1 activity towards diazoxon elicited a reduced effect of CPF metabolites on the organs functions, are consistent with the higher metabolicability to detoxify some organophosphates when higher enzyme activity occurs.

The previous study of ***Sai et al.***[33] demonstrated that non significant changes in the levels of LH were observed, but the levels of serum testosterone showed a decreasing tendency and the levels of FSH showed an increasing tendency. The reduction of testosterone content may be due to the hypothalamus-pituitary-testicular axis, which was damaged by CPF. In males, FSH is produced by the anterior pituitary gland. It acts on the Sertoli cell of the testes, stimulating them to synthesize and secrete the male sex hormone. Meanwhile, FSH was regulated by the

negative feedback of testosterone.[34] Therefore, the increased levels of serum FSH may be the negative feedback to hypothalamus-pituitary-testicular axis of testosterone. While the present results showed that juices of papaya and cactus fruits have the ability to improve fertility markers in rats affected by CPF intoxication. This improvement may be due to the effect of flavonoids, polyphenols and antioxidant components in fruit juices. On the other hand, ***Boeira et al.***[35] showed that lycopene (pigment from papaya fruit) prevented the reduction in the number and motility of spermatozoa and the testicular tissue damage induced by Zearalenone (ZEA). Furthermore, other studies reported that lycopene has been suggested as an alternative treatment for sperm toxicity after chemotherapy.[36,37,38] Moreover, ***Sharma et al.***[39] showed that quercetin (found in papaya and cactus fruits) protected against cypermethrin and deltamethrin induced reproductive system toxicity and oxidative damage in rats.

Tumor necrosis factor- α is a "master-regulator" of inflammatory response because it recruits immune cells at the sites of injured tissues. Hence, the increase in the concentrations of TNF-α in CPF-exposed rats evidently connotes induction of inflammation in the treated rats. Moreover, elevated TNF-α level has been shown to activate inducible nitric oxide synthase to produce NO and induce cytotoxicity through varying mechanisms including the overproduction of ROS, which in turn damages the cellular components such as protein, lipids and DNA.[40,41]

A number of the phytochemicals found in papaya bioactive secondary metabolites (e.g. alkaloids, phenolics, flavonoids, carotenoids tannins, saponins, etc.) and proteolytic enzymes (papain and chymopapain), have been shown to reduce chronic inflammatory conditions and associated side effects by modifying the levels of inflammatory markers.***[42]*** One explanation of anti-inflammatory effect of papaya is the proteolytic enzymes present in papaya (papain and chymopapain) have also shown immune modulatory and anti-inflammatory activities.[43,44] In the same line ***Pietrzkowski et al.***[45] reported that betalain-rich extract reduces osteoarthritis-associated inflammation. A growing body of evidence illustrates that betalains show anti-inflammatory functions through interfering with pro-inflammatory signaling cascades.[46] ***Vidal et al.***[47] observed that betalains have also been shown to suppress *in vitro* expression of cyclooxygenase-2, an important enzyme converting arachidonic acid to leukotrienes and prostaglandins, which are chemical mediators of inflammation. Additionally, ***Tan***[48] has been indicated that betanin may have

nephroprotective effects against paraquat-induced acute renal injuries in rats via inflammatory reactions inhibition.

Malondialdehyde is the major oxidation product of polyunsaturated fatty acids, and increased MDA levels are a crucial indicator of lipid peroxidation. ***Heikal et al.***[49] confirmed the significant accumulation of CPF in the liver, which induce severe oxidative stress in hepatocytes as evidenced by the significantly elevated liver MDA levels in experimental rats when compared with the healthy group. The antioxidant activity of cactus fruit extract was attributed to the constituents (mainly flavonoids and betalains) which were shown to be potent antioxidants *in vitro*.[8] This was in agreement with previous studies reported that, papaya contains antioxidant phytochemicals, such as vitamin C, beta-carotene, lycopene and vitamin E all of which acts as antioxidant and subsequently decrease the consumption of these antioxidant enzymes to combat oxidative stress.[27]

Several studies have suggested that chronic exposure to OP can cause DNA damage leading to adverse long-term health effects. The results of a study by ***Ojha et al.***[50] showed that both acute and chronic exposures to chlorpyrifos were equally damaging to DNA and that even a single dose of CPF causes significantly high levels of DNA single strand breaks as measured by the Comet assay in all examined rat tissues. They concluded that CPF has the potential to cause mutagenic effects by inducing oxidative DNA damage. The study of ***Owumi and Dim***[41] confirmed the apoptotic activity of CPF which significantly increased hepatic and renal caspase-3 activity in the treated rats when compared with control.

On the other hand, ***Siriwardhana et al.***[51] demonstrated that extracts from cactus, had a powerful antioxidant that reduced up to 60% the damaged lymphocytes DNA that was altered via oxidation by H_2O_2. This study suggest that consumption of cactus or its extracts can reduce the action of free radicals, inhibit the oxidation of molecules as lipids and DNA, thus lowering the risk of developing chronic degenerative diseases associated with high oxidative stress levels. In addition, ***El-Nekeety et al.***[52] showed that ochratoxin-contaminated diet induced a significant increase in DNA fragmentation percentage. However, animals treated with the papaya extracts alone did not show any significant differences in DNA fragmentation. Administration of the papaya extracts to the animals fed ochratoxin - contaminated diet succeeded to induce a significant reduction in the elevation level of DNA fragmentation.

5. CONCLUSION

It was concluded that the toxicity study of CPF (6mg/kg bwt) administered orally to *Sprague Dawley* rats caused adverse effect on the liver and testes biochemical markers of the treated rats. However, from the biochemical analysis, it showed that cactus and/or papaya juices consumption may cause protective effect against CPF toxicity.

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