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# Effect of Different Doses of Pumpkin Seed Oil on Liver Function in Adult Male Rats Treated with Chlorpyrifos.

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## Abstract:

They present study aimed to investigate the effect of effect of different doses of pumpkin seed oil on liver function in adult male rats treated with chlorpyrifos . Fifty adult male rats were used and randomly divided into five equal groups of 10 rats each group.**Group 1**(control) were given corn oil (1ml /kg.bw) , whereas animals of **Group 2** were given CPF 6.7 mg/kg bw in corn oil. **Group 3, 4** and **5** were given CPF (6.7mg /kg.bw) plus PSO (20, 40, and 80) mg/kg.bw respectively. The treatment were given once daily by oral gavages for 8 weeks. At the end of the experiment, animals were sacrificed and serum isolated from blood samples were used for measurement of liver enzymes and liver samples were taken for histopathological study. Chlorpyrifos treated group recorded significant increase($p<0.05$)in aspartate aminotransferase(AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and histopathological examination of liver revealed marked congestion in the centrilobular area with hepatocellular necrosis .On other hand groups treated with 20,40and 80 mg pumpkin seed oil induced improved of these parameters

**Keywords***:* Chlorpyrifos, Pumpkin, Liver Function, Adult Male Rats.

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## Introduction

The liver is one of the largest organs in the body and the main site of metabolism and intensive secretion (1). The liver plays a major role in the removal of toxins and excretion of many internal and external compounds and any damage or impairment of liver function may lead to many effects on health (2).

Pesticides are toxic chemical compounds designed to control pests, causal organisms of plant disease, weeds and other living organisms that reduce the quantity and quality of crop yields (3).They are one of the most potentially harmful toxic chemicals introduced into the environment (4).

Chlorpyrifos was introduced in 1965 by Dow Chemical Company and widely used indoors and outdoors to control fleas, insects, termites, pests and mosquitoes (5).Chlorpyrifos [0, 0-diethyl-o(3,5,6-trichloro-2-pyridil) phosphor -dhioate]is member of organophosphate class of pesticides that elicits broad spectrum insecticidal activity against a number of important arthropod pests (6).CPF is commercially used to control foliar insects that affect agricultural crops (7) and subterranean termites(8). CPF with a trade name of Dursban (9). CPF was eliminated slowly from fat and relatively rapidly from liver, heart, and kidney (10).In fact, the toxicity of CPF results in negative effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system (11).

Treatment options for liver disease are limited and modern medicine may be ineffective. In addition, some treatments such as corticosteroids and interferons are inappropriate and may cause adverse events, in addition to being expensive (12). Here the need arose to find effective, low-cost, and side-effects, alternative therapeutic agents. Plants are of special importance used in herbal medicine which have the ability to protect liver activity as in the Indian, Chinese and Korean medicine system (13). Herbal medicines are used individually or in combination with standard

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medicines in the treatment of many different diseases. Pumpkin is one of the plants that are used to eat as well as have many medical properties as used in the treatment of diabetes, anti-inflammatory, anti-oxidant and anti-cancer and many chemicals are extracted (14). Pumpkin seed oil is rich in unsaturated fatty acids, which account for 84% of total fatty acids. It is also rich in minerals and antioxidants such as tocopherol and carotenoids (15). It also contains a high percentage of essential amino acids and a number of sugars and a large amount of other essential elements such as potassium, copper, sodium, magnesium, chromium and selenium (16). Pumpkin oil also contains traditional antioxidants such as zinc, manganese and phenolic antioxidants that include phenolic acid hydroxybenzoic, caffeic, coumaric, ferulic, sinapic, vanillic, protocatechuics. Pumpkin seeds also contain antioxidant plant nutrients which include Lignans, pinoresinol, medioresinol and lariciresinol (17; 18). The aim of this study was to determine the protective role of different doses of pumpkin seed oil (PSO) on liver functions in chlorpyrifos treated male rats.

**Martials and methods:**

Pumpkin oil extraction: Pumpkin (*cucurbita pepo L.* ) seeds were purchased from a local market . After cleaning and removal of the sand and foreign materials, the dried pumpkin seeds were grounded to a fine powder using a grinder. The oil was extracted with n-hexane (1:4 w/v) by agitation in a shaker at room temperature in the dark for 36 h. The solvent was evaporated in vacuo at 40 °C to dryness. The extracted oil was stored in sealed and dark bottles under nitrogen gas until analysis (19).

## Experimental design:

Fifty adult male rats weighting (280- 290 gm) were used in this study. The animals were kept in the animal house for acclimatization fifteen days before the beginning of the experiments. The animals were maintained

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under optimum conditions (25±2°C) and (12/12 hours light/dark) cycle throughout the study, with standard pellets and tap water *ad libitum*.

After the period of acclimation, 50 male rats were divided into 5 equal groups with 10 animals in each as the following:

1- **Group 1 (Control group):** Animals of this group administered corn oil (1 ml /kg bw)/day orally by gavages.
2- **Group 2 (CPF treated group):** Animals of this group administered orally CPF 1/20th LD50 (6.7 mg/kg bw),/day dissolved in 1ml corn oil by gavages (20 ).
3- **Group 3 (CPF + 20 PSO):** Animals of this group administered CPF (6.7 mg) + pumpkin seed oil (20 mg)/kg bw/ day orally by gavages.
4- **Group 4 (CPF + 40 PSO):** Animals of this group administered CPF (6.7 mg) + pumpkin seed oil (40 mg)/kg bw/ day orally by gavages.
5- **Group 5 ( CPF + 80 PSO):** Animals of this group administered CPF (6.7 mg) + pumpkin seed oil (80 mg)/kg bw/ day orally by gavages.

The experimental was continued for 8 weeks.

At the end of the experiment, animals of each group were anaesthetized by chloroform and sacrificed. Blood sample were collected from the heart via the cardiac puncture by using 5cc sterile syringe and dropped in plain without anticoagulant tubes and serum samples were isolated from blood by centrifugation at 3000rpm for 15 min, and separated in eppendroff tubes and used for measurement of AST, ALT and ALP enzymes concentration. Liver of treated groups were removed and kept in 10% formal saline for histopathological study.

## Studied parameters:

## Measurements of Aspartate Aminotransferase (AST) (U/I):

Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (21).

**Measurements Alanine Aminotransferase (ALT) (U/I):**

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine(21).

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## Measurements Alkaline Phosphatase (ALP) (U/I):

This measurement was done by using the colorimetric determination of alkaline phosphatase activity (Biolabo-France) (22).

## The Histological Study:

Liver samples were dissected and fixed in formalin, dehydrated and imbedded in paraffin wax. Then, the sections were stained by haematoxylin and eosin (H and E) stain. Each slide was examined for histopathological changes under light microscope. Based on (23).

## Statistical Analysis:

The data were subjected to analysis of variance and the significance differences at ($p \leq 0.05$) which were determined by analysis of variance (ANOVA), one-way by using the statistical soft ware's sigma statistical (24).

## Result

The results represented in Table (1) revealed a significant increase ($P \leq 0.05$) in serum concentration of ALT, AST and ALP enzymes in CPF treated group for 8 weeks compared with control group. A significant decrease ($P \leq 0.05$) in AST,ALT and ALP concentrations were recorded in CPF group treated with 20 mg / k. bw PSO compared with CPF group but remain significantly higher ($P \leq 0.05$) compared with control group, except in ALP enzyme concentration where no significant difference was observed compared with control group. On the other hand no significant differences were showed in AST, ALT and ALP concentrations in CPF groups treated with 40 and 80 mg / kg. bw PSO respectively compared with control group.

**Table (1): Effect of different doses of PSO on ALT, AST and ALP enzymes concentration in adult male rats exposed to CPF.**

**(Mean ±SD) (n=8)**

| Parameters / Groups | ALT (U/l) | AST (U/l) | ALP (U/l) |
|---|---|---|---|

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| | | | |
|---|---|---|---|
| **Control** | **26.85** ± 3.87 **c** | **56.00** ± 11.26 **c** | **24.28** ± 3.32 **b** |
| **G1**<br>**CPF (6.7mg/kg.bw/day)** | **41.71** ± 6.15 **a** | **96.71** ± 11.78 **a** | **33.28** ± 5.94 **a** |
| **G2**<br>**CPF+20 mg / kg PSO** | **31.42**± 4.13 **b** | **66.71** ± 10.08 **b** | **28.28** ± 4.80 **b** |
| **G3**<br>**CPF+40 mg / kg PSO** | **29.00**± 3.89**bc** | **60.28** ± 4.94**bc** | **26.00** ± 4.34 **b** |
| **G4**<br>**CPF+80 mg / kg PSO** | **27.00**± 3.62**bc** | **56.14** ± 7.62 **c** | **25.00** ± 3.33 **b** |
| **LSD** | **4.57** | **10.57** | **5.00** |

**Values expressed in the small letters mean significant differences at the (P≤0.05) level.**

Histopathological examination of liver in control group revealed normal histological appearance of liver Figure (1). While in figure (2) liver of CPF group showed marked congestion in the centri-lobular area with hepatocellular necrosis.

Figure (3) liver of CPF+20mg PSO group showed moderate congestion in the centri-lobular area with hepatocellular swelling. In figure (4) liver of CPF+40mg PSO group showed the moderate congestion in the portal area with normal appearance of hepatocytes while in figure (5) liver of CPF+80mg PSO group reveal the normal appearance of hepatocytes in the centri-lobular area.

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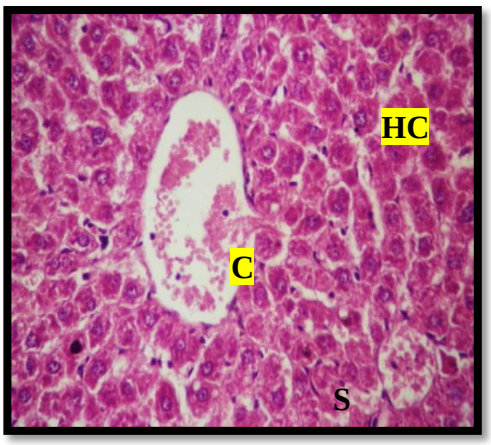


**Fig1: Liver of control rat. Showing normal architecture of centri-lobular area(C) hepatocyte (HC) and Sinusoid(S).( H&E) stain. 500X**

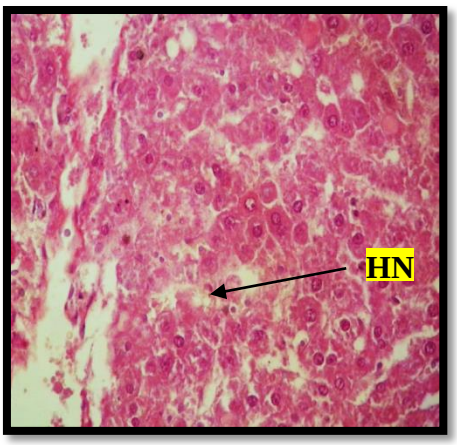


**Fig. 2: Liver of rat treated with CPF. Showing hepatocellular necrosis (HN), disarrangement of hepatic cells.(H&E) stain. 500X**

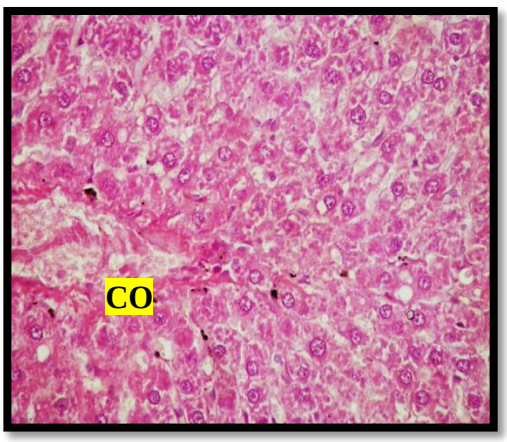


**Fig. 3: Liver of rat treated with CPF+20 mg PSO. Showing moderate congestion in the centri-lobular area (CO).(H&E)stain. 500X**

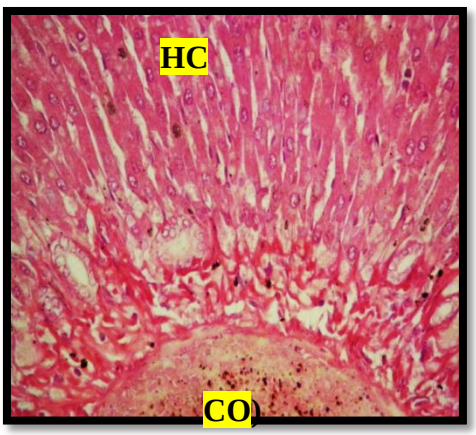


**Fig. 4: Liver of rat treated with CPF+40 mg PSO. Showing congestion in the portal area (CO) with normal appearance of hepatocytes (HC) (H&E) stain. 500X**

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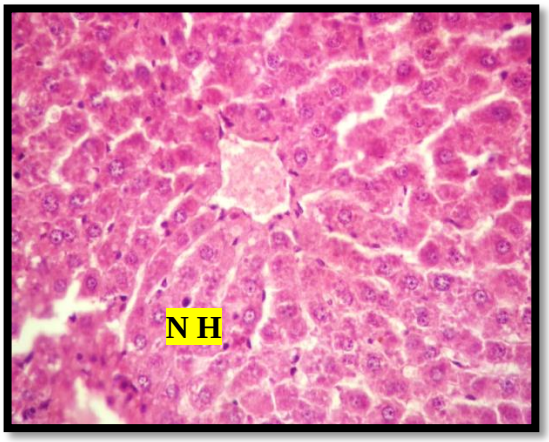


**Fig. 5: Liver of rat treated with CPF+80 mg PSO. Showing normal appearance of hepatocytes in the centri-lobular area.( H&E) stain. 500X.**

## Discussion

The results revealed a significant increase in serum ALT, AST and ALP concentration in CPF treated group compared with the control group. The elevation in serum ALT, AST and ALP of CPF treated group in the present study may be resulted from the histopathological changes in the liver cells represented by cellular degeneration as seen in figure (2). This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation and indicating a necrosis and inflammatory reactions (25). Ambali *et al.,* (26) showed that administration of CPF caused a significant increase in the activity of serum AST, an enzyme found not only in the liver but also in the skeletal muscle and myocardial cells.

Data in the present study revealed that serum liver enzymes (AST, ALT and ALP) improved and renormalized when animal treated with different doses of pumpkin oil . (27, 28, 29) they all observed that the administration

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of pumpkin seeds after intoxication resulted in significant reduction in activity levels of ALT, AST and ALP. The recovery of ALT and AST as a result of pumpkin oil administration along with CPF may be occurred due to the presence of polyphenols. The various extracts (petroleum ether, ethyl acetate and alcohol) of Pumpkin caused significant reduction in the activities of ALP, AST and ALT to normal levels which might be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by streptozotocin (30 ; 25). Vitamins C and E protected mice from subchronic CPF-induced alteration in liver enzymes (31). Pre-treatment with vitamin C caused a decrease in the activity of AST, ALT and ALP suggesting amelioration of hepatic or muscular damage. This shows that vitamin C protect the organs from the damage caused by administration of CPF (31).

Microscopical examination of the liver of CPF-treated group reveal the marked congestion in the centri-lobular area with hepatocellular necrosis Fig (2). These results are in line with those of Newairy and Abdou (32) who reported that administration of CPF resulted in degenerative changes in liver ,comprise fatty change, individual cell necrosis, cellular infiltration and areas of necrosis that are suggestive of acute hepatitis. The damage of the liver tissues in CPF-treated rats could be due to injured mitochondria (33). Heikal *et al.*,(34)showed degeneration and coagulative necrosis in the hepatocytes, inflammatory cells infiltration, and Kupffer cells proliferation in CPF-treated groups which sustained the leakage of liver enzymes. Barakat *et al.*,(35) showed an increased vacuolization of hepatocytes and focal necrosis ,congestion of the portal area and inflammatory infiltration increased in comparison to untreated normal controls. These observations could be due generation of reactive oxygen species causing damage to the various membranous components of the cell.

Pumpkin seed oil contains a high percentage of phenols, beta-carotene, vitamin C and alpha-tocopherol, which are effective antioxidants protect against peroxidation of cell membranes, maintain a high level of antioxidant enzymes and prevent the formation of free radicals that cause damage to cell

membranes and thus maintain the concentration of hepatic enzymes within normal limits as demonstrated in the two CPF groups treated with 40 and 80 mg / kg of pumpkin seed oil compared with control group.

Antioxidant supplementation may protect the protein structures, prevent the reactive oxygen species induced enzyme inactivation, and stabilize cell membranes, which may be responsible for this protective and favorable effect of ascorbic acid and α- tocopherol (36; 37; 38). The natural plant components found in pumpkin could improve the liver against alcohol-induced liver toxicity and oxidative stress (28). Abdel Aal *et al,* (39) Showed that treated of male rats with pumpkin reduced the toxic effect of azathioprine on liver and this might be due to high content of β-carotene. The antioxidant and hepatoprotective effect of pumpkin may be due to properties of polyphenol and β-carotene content which had been proved to be a powerful antioxidant and protective actions against cell injury (40).

## Conclusion:

We concluded that oil extracted from pumpkin seed has an ability in protection of liver function in dose dependent manner from the deleterious effect of chlorpyrifos.

## References:

1- **Ahsan, R.; Islam, M.; Bulbul,J.I .; Musaddik, A.; Haque, E** . Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats Eur J Sci Res, 37 (2), pp. 302-310 **(2009).**

2- **Subramaniam, S.; Khan, H.B.H.; Elumalai, N.; Lakshmi S.Y.S.** Hepatoprotective effect of ethanolic extract of whole plant of Andrographis paniculata against CCl4-induced hepatotoxicity in rats Comp Clin Path, 24, :. 1-7**. (2015).**

3- **El-Deeb, A.E.A.; Abd El-Aleem I.M. and SherinS.Ghaleb .** Harmful effect of some insecticides on vital parameters of albino rats. *J Egypt. Soc. Toxicol,*. 36: 53-60**. (2007)**.

*University of Thi-Qar Journal*

ISSN (print): 2706- 6908, ISSN (online): 2706-6894

*Vol.16 No.1 Mar 2021*

4- **Sunanda, M.J.;Rao C.C.;Neelima K.; Rao G. and Simhachalam G .** Effects of Chlorpyrifos (an Organophosphate Pesticide) in Fish. *Int.* J. Pharm. Sci. Rev. Res., 39(1):299-305.(2016).

5- **Oyewole. O.I. and Olabiyi B.F**. Ameliorating Effect of Moringaoleifera Leaf Extract on Chlorpyrifos-Induced Toxicity in the Brain of Wistar Rats. American Journal of Biochmistry ,4(5): 93-97. **.(2014).**

6- **Racke, K.D.** Environmental fate Of Chlorpyrifos. Reviews of Environmental Contamination and Toxicology; 131: 1-150.(1993).

7- **Rusyniak, D .E. and Nanagas K.A.** Organophosphate poisoning . *Semen. Neurol.* 24: 197-204. **(2004).**

8- **Rao, J.V.** Biochemical alterations in euryhaline fish, Oreochromis mossambicus exposed to sub-lethal concentrations of an organophosphorus insecticide, monocrotophos. Chemosphere, 65:1814- 1820**.(2006)**

9- **Riutta, A.** Occupational determinants of chlorpyrifos adducts to plasma cholinesterase in chlorpyrifos exposed agricultural workers in Washington State. Master's thesis. University of Washington, Department of Environmental and Occupational Health Sciences Available at: http:// gradworks .umi .com /15 /15 /1515977.html. **(2012).**

10- **Agency of Toxic Substances and Disease Registry (ATSDR)** .Interaction profile for: chlorpyrifos, lead, mercury, and methylmercury Atlanta, GA: Agency for Toxic Substances and Disease Registry(2006).

11- **Mansour, S.A and Mossa A.H.** Oxidative damage, biochemical and histopatholo-gical alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pest BiochemPhysiol , 96: 14-23. **(2011)**.

12- **Stickel, F. and Schuppan, D .** Herbal medicine in the treatment of liver diseases Dig Liver Dis, 39: 293-304. (2007).

13- **Thyagarajan, S.P.; Jayaram, S.; Gopalakrishnan, V.; Hari, R.; Jeyakumar, P.; Sripathi**, **M.S**. Herbal medicines for liver diseases in India. *J Gastroenterol Hepatol*, 17, :. S370-6. **. (2002)**

14- **Mukesh, Y.** Medical and biological potential of pumpkin'an updated review, National Research Reviews. 23: 184-190. **(2010).**

15- **Procida, G; Stancher, B; Cateni, f.; Zacchigna M.** Chemical composition and functional characterization of commercial pumpkin seed oil. Journal of the Science of Food Agriculture. 93(5): 1035-1041. **(2013).**

16- **Glew, RH; Glew RS.; Chuang LT.; Huang YS.; Millson M.; Constans D.; Vanderjagt D.J** .Amino acid, mineral and fatty acid content of pumpkin seeds (Cucurbita spp) and Cyprus esculents nuts in the Republic of Niger. *Plant Food Hum Nutr* .61:51-56. **.(2006).**

17- **Butinar, B; Bucar-Miklavcic M; Mariani C et al. (2011).** New vitamin E isomers (gamma-tocomonoenol and alpha- tocomonoenol) in seeds, roasted seeds and roasted seed oil from the Slovenian pumpkin variety 'Slovenska golica'. *Food Chemistry.* 128: 505-512**.(2011).**

18- **Xanthopoulou, M.N; Nomikos, T; Fragopoulou, E *et al.*,** Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. *Food Research International*. 42: 641-646. **(2009).**

19- **Ardabilih, G.; Farhoosh, R. and Khodaparast, M.H**. Chemical Composition and Physicochemical Properties of Pumpkin Seeds (Cucurbita pepo Subsp. pepo Var. Styriaka) Grown in Iran. J. Agr. Sci. Tech, 13: 1053-1063. **(2011).**

20- **Lamfon, H.A**. Effect of selenium on chlorpyrifos-induced thyroid toxicity in albino rats. *Research in Endocrinology*, DOI: 10.5171/2014.751500. **(2014).**

21- **Schumann, G. and Klauke R.** New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: Preliminary upper reference limits obtained in hospitalized subjects. *Clin. Chim. Acta*; 327(1-2): 69-79. **(2003).**

22- **Klin, Chem. Z.,** Klin Biochem., 8,658. **(1980).**

23- **Luna, L.G.** Manual of; histologic staining methods of the armed forces institute of pathology. 3 rd. ed. McGraw-Hill, New York, NY. **(1968).**

24- **Statistical Packages for the Social Sciences (SPSS**). Statistical soft ware for windows version 19.0 Micrisoft. SPSS, Chicago, Illinois, USA. **) ,. (2010).**

25- **Sharma, A.;Sodhi,S.; BrarA.S. and Ghai J.K.** Chlorpyrifosinduced hematological, biochemical and histoarchitectural alterations in albino mice. *International Journal of Advanced Research*, 1(10): 825-835. **(2013).**

26- **Ambali, S.F.; Angani, M.; Adole, A.O.; Kawu, M.U.; Shittu, M.; Akande, M.G. and Oladipo, O.O.** Protective effect of vitamin C on biochemical alterations induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats. Environmental & Analytical toxicology, 1:3**.(2011).**

27- **Nkosi, C.Z.; Opoku A.R. and Terblanche S.E.** Antioxidative Effects of Pumpkin Seed (*Cucurbitapepo*) Protein Isolate in Ccl -Induced Liver Injury in Low-Protein Fed Rats. *Phytotherp. Res.*, 20(11): 935-940.**(2006).**

28- **Oboh, G.** Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (Telfairiaoccidentalis) leaves against garlic-induced oxidative stress. *J Med Food ;*8(4):560- 563.**(2005).**

29- **AbouSeif, H.S.** Ameliorative effect of pumpkin oil (Cucurbitapepo L.) against alcohol-induced hepatotoxicity and oxidative stress in albino rats . *Benisuef University Journal of Basic and Applied Sciences*, 3:178 - 185**.(2014).**

30- **Baldi, A.; Choudhary N.; MaruJ.and Joshi R**. Effect of pumpkin concentrate on alloxan induced diabetic rats. *Journal of Global Pharma Technology*,2(10): 24-27. **(2010).**

31- **Ambali, S.; Akanbi, D; Shittu, M.; Giwa, A.G.; Oladipo ,O.O. and Ayo ,J.O.** Chlorpyrifos-Induced Clinical, Hematological and Biochemical Changes in Swiss Albino Mice- Mitigating effect by co-administration of vitamins C and E. Life *Science Journal*, 7(3):37-44**.(2010).**

32- **Newairy, A.A. and Abdou H.M**. Effect of propolis consumption on hepatotoxicity and brain damage in male rats exposed to chlorpyrifos . *African Journal of Biotechnology*, 12(33): 5232-5243. **.(2013).**

**33- Goel, A.; Dani, V. and Dhawan D.K.** Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos induced toxicity. *Chem. Biol. Interact*. 156: 131-140. **(2005).**

**34- Heikal, T.M, Mossa, A.H, AbdelRasoul, MA, Marei , GKh.** The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. *Asian J Pharm Clin Res;* 6:48-55 .**(2013).**

**35- Barakat, H; Khalil, F.A.;Khalifa, F.K. and Hessin, M.M**. Biochemical role of fermented wheat germ on liver and kidney functions alteration induced by chlorpyrifos in rats. *African J. B iol. Sci.,*7(1): 45-60. **.(2011).**

**36- Rayman, M.P.** The importance of selenium to human health. *Lancet.* 15:356 (9225) :233 -41. **(2000).**

**37- Schroder, H.; Navarro, E.; Mora, J.; Galiano, D. and Tramullas, A .** Effects of alpha-tocopherol, beta-carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players. *Eur J Nutr* , 40:178–84. **(2001).**

**38- Lodhi, G.M.; Hameed, W.; Ahmed T. Latif R. and AslamM.** Effects of ascorbic acid and alpha tocopherol supplementation on basal testosterone cortisol ratio in male spraguedawley rats**.** *Pak J Physiol*, 7(2):7-10. **(2011).**

**39- AbdelAal, F.S.** The Protective Effect of Pumpkin Seed Oil on Azathioprine – Induced Hepatic Toxicity in Adult Male Albino Rats: Histological and Immunohistochemical Study. *Basic Sciences of Medicine* , 3(4): 85-100. **(2014).**

**40- Oboh, G.** Antioxidant and hepatoprotective properties of polyphenol extracts from Telfairia occidentalis (Fluted Pumpkin) leaves on acetaminophen induced liver damage. Pak. J. Biol. Sci .10: 2682-2687 . **(2007).**