Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/6048
DOI URL: http://dx.doi.org/10.21474/IJAR01/6048
ISSN NO. 2320-5407

RESEARCH ARTICLE

EVALUATION OF PROTECTIVE EFFECT OF WHEAT GERM ON CHLORPYRIFOS INDUCED TOXICITY IN RATS.

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Manuscript Info

Manuscript History

Received: 13 October 2017
Final Accepted: 15 November 2017
Published: December 2017

Key words:-
Chlorpyrifos, Wheat Germ, Hematology, Antioxidants, Comet, Histopathology.

Abstract

Chlorpyrifos (CPF) is a widely used insecticide causing harmful effects in human beings and animals. Determining effective and economic antioxidants is an ultimate goal for all toxicologists. In this regard, the current study focused on the protective effect of wheat germ (WG) administered as 20% of diet for 45 days against the toxic effects of CPF given at 50 mg/kg/day for the last 3 days of experiment by gastric intubation. CPF induced hematological changes in the form of reduction of red cell count (RBC), hemoglobin (Hb) level and packed cell volume (PCV) with leukocytosis, neutrophilia and lymphocytopenia. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities and serum concentrations of iron, total protein, globulin, ceruloplasmin and haptoglobulin were elevated. Malondialdehyde (MDA) level increased with concomitant reduction of reduced glutathione (GSH) level and catalase (CAT) activityin liver, brain and spleen tissues. This was associated with histopathological alterations in these organs. Moreover, CPF produced genotoxic effects in liver and brain as demonstrated by comet assay. Administration of WG-enriched diet ameliorated most of the toxic effects produced by CPF. In conclusion, it is recommended to use WG to protect animals and human being from harmful effects of CPF and other organophosphates.

Introduction:-

Chlorpyrifos (CPF) is an organophosphorus insecticide with contact, stomach, and respiratory action and nonsystemic anticholinesterase activity. It is widely used in agriculture on a wide range of crops and in applications used to control pests in animal houses **(Testai et al., 2010)**.

The extensive use of CPF in developing countries was associated with hundreds of thousands of deaths per year due to acute intoxication episodes resulting from use of large quantities of CPF in agriculture without use of adequate protection devices, e.g., gloves and protective clothes **(Eddleston et al., 2007)**.

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CPF produces its action, like other organophosphates, by inhibition of AChE. It induces its effects after being converted via oxidative desulphuration to the potent active metabolite CPFO **(Huff et al., 1994)**.CPF has been demonstrated to induce many harmful effects such as hyperglycemia and hyperlipidemia **(Acker and Nogueira, 2012)**, hepatotoxicity **(Raina et al., 2015)**, genotoxicity**(Zeljezic et al., 2016; Kopjar et al., 2017)** and reproductive and developmental toxicity **(Testai et al., 2010)**.

As oxidative stress is reported to be the main mechanism of CPF-induced toxicity **(Lahouel et al., 2016; Savithri et al., 2016; Yazdinezhad et al., 2017)**, it is necessary to search for safe, reliable, effective and economic natural herbal medicines to ameliorate the toxic effects of CPF.

Among the natural products, wheat germ (WG) is of beneficial nutritional and medicinal values. It constitutes 2-3% of the wheat grain and it contains 8-14% oil content **(Pomeranz, 1988)**. It is a byproduct of industrial wheat milling, being separated from the endosperm during this process, and used mainly as forage and as a source of wheat germ oil. Most of the nutrients in wheat grain, except starch, are concentrated in WG. It contains oil with favorable polyunsaturated fatty acid pattern and high nutritional value protein. Besides, it is one of the richest known sources of tocopherols and vitamin B complex **(Rizzello et al., 2013)**.

WG and WG oil induced anti-inflammatory, antioxidant, radioprotective and hepatoprotective effects due to their contents of essential fatty acids and vitamin E **(Hussein et al., 2014; Sliai, 2015; Soliman et al., 2015)**. Also, they were effective in protecting against various diseases such as cancers, hypercholesteremia and diabetes **(Cara et al., 1991; Boros et al., 2005, Merghani et al., 2015)**.

Therefore, the aim of the current study is to elucidate whether and to what extent WG enriched diet may protect rats from CPF- induced hematotoxicity, biochemical alterations in blood, oxidative stress, genotoxicity and histopathological alterations.

Materials and Methods:-

Animals:-

Forty adult male Wistar albino rats (120-150g) were obtained from Al-Zyade Experimental Animals Production Center (Giza, Egypt). Animals were housed in plastic cages on wood-chip bedding in well ventilated laboratory animal room (25±3°C, 45-60% relative humidity, natural daily dark/light cycle). Rats were provided with balanced diet and tap water *ad libitum* for 2 weeks prior to start of the experiment. The experimental protocol was ethically approved by the International Animal Care and Use Committee IACUC, Faculty of Veterinary Medicine, University of Sadat City.

Chemicals:-

Chlorpyrifos (CPF) was obtained as the commercial product Clorzane 48% EC from International Kormandle Company, India. Wheat germ (WG) was purchased from Trigo, Good Food Company, Egypt. Diagnostic kit for ceruloplasmin was purchased from sigma Aldrich, while rat haptoglobulin ELISA kit was obtained from Life Diagnostics. Test kits for assaying other serum and tissue biochemical parameters were purchased from Biodiagnostic Company (Dokki, Giza, Egypt). Other utilized chemicals and reagents were of analytical grade and commercially available.

Experimental design:-

Control group was fed basal diet for 45 days. CPF group was fed basal diet for 45 days and administrated 3 doses of CPF by gastric intubation at a dose level of 50 mg/kg/day, 1/3*rd* LD_{50}**(Bebe and Panemanglore, 2003)** on the last three days of the experiment. WG group was fed on WG enriched basal diet (containing 20% WG) **(Abdel-Rahim and Mahmoud, 2011)** for 45 days. WG+CPF group was fed WG enriched basal diet (20%) for 45 days and orally administered CPF like the second group.

Samples collection:-

Five animals from each group were anesthetized by inhalation of diethyl ether, and blood samples were obtained by cardiac puncture. Blood samples were divided into two portions. The first portion was collected on EDTA for hematological assays, while the other portion was used for collection of serum which was stored at -20 ºC until used for biochemical analysis. Liver, brain and spleen were excised immediately and stored at −20°C for further tissue

biochemical analysis.

The remaining animals were euthanized under diethyl ether anesthesia. Parts of Liver and brain were immediately removed and placed in cold BPS for comet assay. Additional parts of liver, brain and spleen were fixed in 10% neutral buffered formalin for histopathological investigations.

Hematological parameters:-
Estimation of red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV)%, and total (TLC) and differential leukocytic counts were performed according to the routine hematological procedures adopted by **Weiss and Wardrop (2010)**. Mean corpuscular volume (MCV) was calculated as PCV divided by red cell count and multiplied by 10. Mean corpuscular hemoglobin (MCH) was calculated as hemoglobin divided by red cell count and multiplied by 10, while mean corpuscular hemoglobin concentration (MCHC) was calculated as hemoglobin divided by PCV multiplied by 100.

Serum biochemical parameters:-
Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) **(Reitman and Frankel, 1957)**,lactate dehydrogenase (LDH) **(Bais and Philcox, 1994)** and concentration of iron **(Dreux, 1977)**were measured using commercial kits following the manufacturer's instructions. Serum total protein **(Gornall et al., 1949)**, albumin **(Doumas et al., 1971)** and ceruloplasmin (Cp) **(Samygina et al., 2013)** and haptoglobin (Hp) **(Giffen et al., 2003)** were measured according to manufacture instructions.

Oxidant- antioxidant biomarkers:-
Malondialdehyde (MDA) and reduced glutathione (GSH) levels and catalase (CAT) activity were estimated in liver, brain and spleen according to**Ohkawa et al. (1979), Beutler et al. (1963) and Aebi (1984),** respectively, using commercial kits following their instructions.

Comet assay:-
DNA damage in liver and brain cells was determined by comet assay (single cell gel electrophoresis) according to **Singh et al. (1988)**. Out of 100 randomly selected nuclei were photographed and scanned using software program for detection of tail length and the percentage of DNA in the tail. The tail moment was expressed as tail length × percentage of migrated DNA / 100.

Histopathological examination:-
Liver, brain and spleen specimens were fixed in 10% neutral buffered formalin solution, processed and stained by hematoxylin and eosin (H&E) for light microscopical examination according to **Bancroft and Gamble (2002)**.

Statistical analysis:-
Statistical analysis was determined by one-way ANOVA followed by Duncan's Multiple Range test for post hoc analysis. All statistical analyses were performed using SPSS (Statistical package for Social Sciences) Version 16 released on 2007.Values were presented as mean ± SE. Differences were considered statistically significant at $P \leq 0.05$.

Results:-

Effects on animals' general condition:-
Both control and WG groups did not exhibit any clinical manifestations. However, dullness, depression, rough hair coat, lacrimation, conjunctivitis, mild tremor, diarrhea, reduced feed intake and dyspnea were the apparent toxic signs observed in CPF intoxicated group. Animals of the WG+CPF group showed nearly normal appearance.

Effects on hematological parameters:-
Rats of the CPF group revealed significant decreases ($P \leq 0.05$) in RBC count, PCV% and Hb level compared to control group. However, no significant changes were observed in values of MCV, MCH and MCHC. Rats of the WG+CPF showed partial improvement in RBC count, PCV% and Hb level compared to CPF group (Table 1).

CPF intoxicated rats showed significant ($P \leq 0.05$) elevations in TLC and neutrophils percentage with reduction in the percentage of lymphocytes compared to control group. These changes were neutralized in WG + CPF group. The group received WG alone showed significant increase in lymphocyte percentage (Table 1).

Table 1:-Hematological parameters in control and experimental groups.

	Control	CPF	WG	WG + CPF
RBCs ($x10^6$/µl)	6.19±0.07[a]	5.31±0.01[b]	6.32 ± 0.07[a]	5.43±0.08[b]
PCV (%)	37.12±0.40[a]	31.73±0.18[b]	37.00±0.66[a]	32.58± 0.52[b]
Hb (g/dl)	12.37±0.14[a]	10.47±0.06[b]	12.34±0.22[a]	10.86±0.18[b]
MCV (fl)	59.91±0.81[a]	59.75±0.62[a]	58.54±0.91[a]	60.00±1.01[a]
MCH (pg)	19.88±0.83[a]	19.71±0.99[a]	19.44±0.92[a]	19.90±0.18[a]
MCHC (%)	33.31±1.1[a]	32.99±0.89[a]	33.33±0.79[a]	32.30±1.00[a]
TLC ($x10^3$/ µl)	7.08±0.36[c]	10.71±0.45[a]	7.12±0.61[c]	8.83±0.46[b]
Neutrophils (%)	18.60±0.51[bc]	22.40 ±0.40[a]	17.40± 0.68[c]	19.20±0.37[b]
Lymphocytes (%)	75.60 ± 0.51[b]	72.60 ± 0.81[c]	78.4 ± 0.68[a]	76.2±0.37[b]
Eosinophils (%)	1.40 ±0.24[a]	1.0 ± 0.31[a]	1.40 ± 0.24[a]	1.2±0.20[a]
Basophils (%)	0.40 ± 0.02[a]	0.20 ± 0.02 [a]	0.20± 0.02 [a]	0.40± 0.02[a]
Monocytes (%)	3.40 ± 0.23[a]	3.40 ± 0.24 [a]	2.60 ± 0.24 [a]	3.00±0.32[a]

Values are mean ±SE. n=5

Means in the same raw followed by different superscripts (a,b,c) are significantly different at (P≤0.05).

Effects on serum biochemical parameters:-

Table 2 reveals that CPF significantly increased (P≤ 0.05) the activities of ALT, AST and LDH compared to control group. In WG+CPF group, WG restored the normal control values of ALT and AST, while partially improved LDH activity.

Significant elevations (P≤ 0.05) in serum iron, total protein, globulin, haptoglobin and ceruloplasmin levels were recorded in CPF group compared to control, while serum albumin level showed no changes.

In WG+CPF group all parameters returned to normal range and levels of iron, total protein and globulin were significantly different (P≤ 0.05) from to CPF intoxicated group (Table 2).

Table 2:-Serum biochemical parameters in rats of all groups.

	Control	CPF	WG	WG+CPF
ALT (U/L)	72±2.61[bc]	118.2±6.92[a]	65.6±4.08[c]	82.4±3.20[b]
AST (U/L)	178.2±2.63[b]	238±4.22[a]	168.4±6.34[b]	179.2 ±1.2[b]
LDH (U/L)	964±27.7[b]	1338±26.8[a]	946±23.4[b]	1280±29.3[a]
Iron (µg/dl)	138.7±1.1[b]	150.5±1.9[a]	142±0.7[b]	143±1.4[b]
TP (g/dl)	6.66±0.08[b]	7.41±0.20[a]	7.52±0.03[a]	6.95±0.3[b]
Alb (g/dl)	2.56±0.04[a]	2.60±0.10[a]	2.52±0.06[a]	2.70±0.07[a]
Globulin (g/dl)	4.10±0.15[b]	4.81±0.14[a]	5.01 ±0.03[a]	4.25±0.30[b]
Ceruloplasmin (mg/dl)	7±0.44[b]	10±0.57[a]	8±0.57[b]	9±0.57[ab]
Haptoglobin (g/dl)	0.09±0.01[b]	0.24±0.06[a]	0.14±0.01[ab]	0.17±0.02[ab]

Values are mean ±SE. n=5

Means in the same raw followed by different superscripts (a,b,c) are significantly different at (P≤0.05).

Oxidant/antioxidant biomarkers:-

Administration of CPF to rats significantly increased (P≤ 0.05) the level of MDA in liver, brain and spleen concomitantly with reduction in GSH content and CAT activity compared to control group. Coadministration of WG with CPF ameliorated the effect of CPF and restored the values of all parameters to normal range except that of CAT in spleen (Fig. 1).

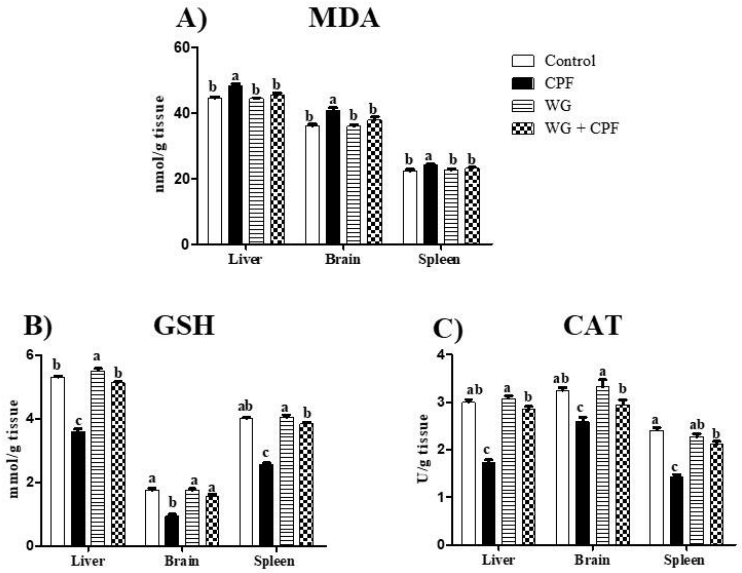

Fig. 1:-Oxidant-antioxidant biomarkers in liver, brain and spleen of all groups of rats.
Values are mean ±SE, n=5.
Different superscripts (a,b,c) are significantly different at (P≤0.05).

Comet assay:-

Oral administration of CPF induced significant increases in tail length, DNA% in the tail and tail moment of hepatic and brain cells compared to control group (Table 3, Fig.2). Despite of the considerable ameliorative effects recorded in WG+CPF group compared to CPF group, the changed comet parameters were still significantly higher than control values.

Table 3:-Comet assay of liver and brain cells of control and experimental groups.

		Control	CPF	WG	CPF+WG
Liver	Tail Length(µm)	$3.2±o.13^{c}$	$4.9±0.12^{a}$	$2.8±0.11^{d}$	$3.8±0.12^{b}$
	DNA% in Tail	$8.8±0.4^{c}$	$11.4±0.3^{a}$	$8.6±0.2^{c}$	$9.9±0.4^{b}$
	Tail Moment	$0.28±0.015^{c}$	$0.56±0.02^{a}$	$0.24±0.01^{c}$	$0.37±0.02^{b}$
Brain	Tail Length(µm)	$2.95±0.21^{c}$	$4.61±0.31^{a}$	$2.62±0.15^{c}$	$3.63±0.20^{b}$
	DNA% in Tail	$7.3±0.3^{c}$	$11.3±0.5^{a}$	$8.2±0.3^{c}$	$9.5±0.3^{b}$
	Tail Moment	$0.18±0.02^{c}$	$0.51±0.04^{a}$	$0.21±0.02^{c}$	$0.34±0.02^{b}$

Values are mean ±SE. n=5
Means in the same raw followed by different superscripts (a,b,c) are significantly different at (P ≤0.05).

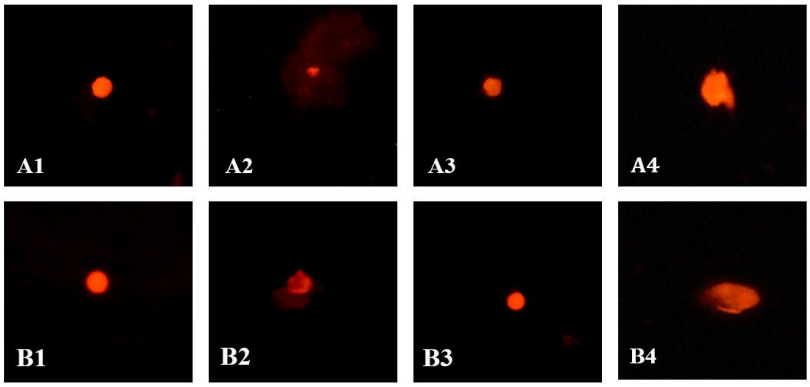

Fig.2:- Comet assay for evaluation of genotoxicity of CPF in liver (A) and brain (B) cells. 1: Control group, 2: CPF group, 3: WG group, and 4: WG+CPF group.

Histopathological changes:-

Liver of rats treated with CPF revealed congestion of central vein, focal necrosis of hepatocytes, dissociation of hepatic cords and inflammatory cells infiltration (Fig. 3A1). Coadministration of WG with CPF protected the liver and no histopathological alterations were seen (Fig. 3A3). Brain of CPF treated rats showed degeneration, loss and necrosis of Purkinjean cells (Fig. 3B1), while brain of rats treated with both WG and CPF showed apparently normal structure (Fig. 3B3). Spleen of CPF treated group revealed lymphoid depletion in white pulp (Fig. 3C1) but no apparent histopathological alterations were seen in the WG+CPF group (Fig. 3C3). Administration of WG alone did not produce any pathological changes in liver, brain or spleen (Figs. 3A2, B2, C2).

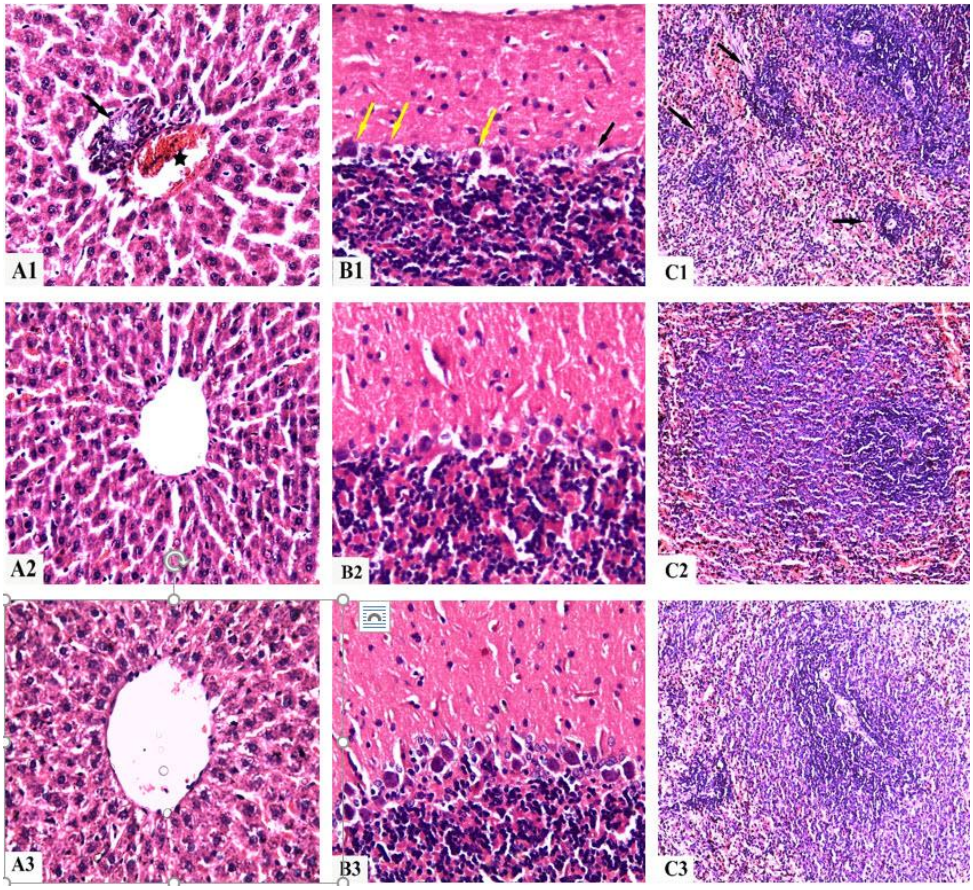

Fig.3:- Histopathological alterations in liver, brain and spleen of different groups.

A1) Liver of CPF group showing congestion and inflammatory cells infiltration in central vein (star), newly formed bile duct (arrow), focal necrosis of hepatocytes with pyknotic nuclei and dissociation of hepatic cords. **A2**) Liver of WG group and **A3)** Liver of WG+CPF group showing apparently normal histology (H&EX20).

B1) Brain of CPF group showing loss (black arrow), necrosis and degeneration of Purkinje cells (yellow arrows).B2) Brain of WG group and B3) Brain of WG+CPF group showing apparently nearly normal architectures (H&EX20).

C1: Spleen of CPF group showing lymphoid depletion in white pulp (arrows). **C2)** Spleen of WG group and**C3)** spleen of WG+CPF group showing normal histology (H&EX10).

Discussion:-

Chlorpyrifos (CPF) is utilized extensively as a broad-spectrum insecticide in agriculture and veterinary medicine. This compound has spurred interest as it elicits many additional toxic effects in human and animals **(Testai et al., 2010)**. Thus, the possible protective effect of wheat germ (WG) as a natural antioxidant against the toxic effects of CPF in rats was investigated.

Evaluation of blood cells and serum variables has higher predictive value in assessing status of the body because it tells us changes occurring internally with toxicity or disease. In this regard, our hemogram findings revealed

significant decreases in RBC count, Hb level and PCV in CPF intoxicated rats. In agreement with our results, **Savithri et al. (2010)** reported that acute exposure of rats to CPF reduced RBC count, Hb and PCV. This effect could be attributed to the adverse effects on blood forming organs or disruption of erythropoiesis **(Hall and Everds, 2014)**. In addition, direct destructive effect on RBCs is suggested, as CPF was found to induce oxidative stress inside the RBCs *in vitro***(Saxena et al., 2011)**.

Our findings demonstrated that intoxication of rats with CPF was associated with leucocytosis, neutrophilia and lymphopenia. These changes may be attributed to the stress associated with CPF intoxication. The stressful conditions increase production of endogenous glucocorticoids causing leucocytosis characterized primarily by increased neutrophil count and decreased lymphocyte and eosinophil counts. Increase of monocyte count may or may not be present **(Everds et al., 2013)**. In this respect, **Acker and Nogueira (2012**) reported that acute exposure of rats to CPF caused increases in corticosterone level in plasma after 12 and 24 hours.

Neutrophilia is usually caused by increased production or reduced margination. The elevated production is usually caused by signals emerging from inflammatory conditions due to infectious and non-infectious agents. This signal may be inherent property of the toxicant (e.g., hematopoietic growth factors or cytokines) or may be secondary to the toxic effects such as tissue necrosis and intravascular hemolysis. Lymphopenia is most frequently observed as part of the stress- or glucocorticoid-induced leukocyte response **(Hall and Everds, 2014)**.

In the present study, CPF administrated to rats at a dose of 50 mg/kg/day for three consecutive days provoked significant elevations in serum ALT, AST and LDH activities. Our results agree with previous studies reporting elevations of serum transaminases and LDH activities after CPF intoxication **(Raina et al., 2015; Tanvir et al., 2015)**.

ALT is the most sensitive biomarker for the detection of hepatocellular damage. Because ALT is mainly cytosolic, and its level intracellular is much higher than that in serum, ALT may leakage to the serum due to degeneration or necrosis which affect hepatocellular membrane integrity. The rise in serum activity is usually proportional to the number of affected liver cells. After acute hepatotoxic insult, ALT activity in serum increases rapidly to reach peak within 1 or 2 days **(Hall and Everds, 2014; Plaa et al., 2014)**.

AST is equally found in liver and heart muscle, but minimally present in skeletal muscle, RBCs, lung, kidney, spleen and pancreas. It is usually measured with ALT. It is not specific to the liver like ALT. In liver injury, AST activity in blood increases with ALT where leaky cell membranes release it into blood with peak levels at 24-36 hrs. However, in cardiac infarction, AST activity elevates while ALT activity increases only slightly **(Whalan, 2015)**.

LDH is not tissue-specific, being found in almost all tissues and thus it is considered as a marker of no-specific cellular injury. Thus, the elevated activity of LDH in CPF intoxicated rats indicates cellular damage and cytotoxicity resulting in cell lysis**(Bagchi et al., 1995)**.

The recorded increment of ALT, AST and LDH activities in CPF intoxicated rats is compatible with our findings demonstrating occurrence of lipid peroxidation and histopathological alterations in the liver of rats.

Liver is the main organ controlling iron homeostasis by secreting hepcidin which regulates iron flux through the hepcidin-ferroportin regulatory axis, resulting in decrease of iron egress from macrophages and absorption of iron from the small intestine **(Ganz, 2011)**. Our results demonstrated that CPF caused increase of serum iron level in intoxicated rats which may be attributed to inhibition of iron gene (hepcidin) expression in hepatocytes and elevated expression of ferroportin in macrophages**.** This in turn promotes absorption of iron from duodenal enterocytes and release of iron from macrophages **(Sun et al., 2014)**.

The serum iron level may also be elevated due to the release of stored iron from necrotizing hepatic cells, as the liver is the main storage site for iron **(Saito, 2014)**. The increase in serum iron could also be attributed to oxidation of ferrous ions to the ferric form. Free radicals and a subset of iron oxidases contribute to this oxidation process **(Xie et al., 2016)**.

Our findings demonstrated that CFP intoxication caused elevations in serum total protein, globulin, ceruloplasmin and haptoglobin. The increases in total protein are due to elevation of globulin level which in turn can be explained

by increases in haptoglobin and ceruloplasmin levels. **Walter et al. (2013)** reported that globulin levels in blood may increase due to dehydration and/or as a general response to inflammation causing elevated release of the acute-phase proteins such as ceruloplasmin and haptoglobin.

Ceruloplasmin is a copper dependent acute phase reactant protein which belongs to alpha globulins and possesses antioxidant property **(Gursel et al., 2010)**. Haptoglobin, haemoglobin binding protein synthesized and secreted by liver cells, plays role in providing protection against heme driven oxidative stress **(Murata et al., 2004)**. Inflammatory conditions and tissue damage stimulate pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α which in turn stimulate acute phase protein release from hepatocytes **(Gruys et al., 2005)**. The recorded increase in serum ceruloplasminlevel in this study may contribute to the recorded elevation in serum iron level. This is supported by the observation that ceruloplasmin enhances the efflux of iron out of hepatocytes **(Richardson, 1999)**.

Oxidative stress is a disturbance in the prooxidant - antioxidant balance in favor of the prooxidants causing tissue injury **(Sies, 1991)**. It usually occurs due to increased production of reactive free radicals, suppression of enzymatic and non-enzymatic antioxidants and/or impaired repair of oxidative injury. The damage induced by free radicals includes important cellular components such as membrane lipids, protein and DNA. Finally, cell function changes with disruption of cellular homeostasis followed by cell death **(Kehrer et al., 1990)**.

The present investigation demonstrated that exposure of rats to CPF caused oxidative stress in liver, brain and spleen evidenced by increase of MDA level, reduction of GSH level and suppression of CAT activity. The elevation of tissue MDA indicates increased rate of lipid peroxidation leading to tissue injury and subsequent failure of antioxidant system in the body to detoxicate the elevated release of ROS. The reduction of cellular GSH, the main intracellular antioxidant, results from consumption of such protein in the process of free radical detoxication**(Nordberg and Arnér, 2001)**. Also, the inhibition of CAT activity is suggested to result from increased utilization to detoxify the H_2O_2 resulting from the process of lipid peroxidation**.** Depletion of CAT causes H_2O_2 and ROS accumulation which triggers protein and DNA oxidation causing cell death **(Halliwell and Gutteridge, 1999)**.

Our results agree with previous literature reporting that single or repeated exposure to CPF increased levels of MDA, reduced GSH level and inhibited enzymatic antioxidants in liver **(Raina et al., 2015; Savithri et al., 2016;Yazdinezhad et al., 2017)** and brain **(Malhotra et al., 2011**; **Lahouel et al., 2016; Abolaji et al., 2017)** of intoxicated rats. Moreover, **Bebe and Panemangalore (2003)**reported similar effect in spleen of rats exposed to CPF.
The recorded oxidative stress in this study is compatible with the elevation observed in serum enzyme activities and the histopathological alterations recorded in the liver, brain and spleen of intoxicated animals. Peroxidation of membrane lipids has been considered as a main molecular mechanism involved in toxicity induced by organophosphorus insecticides **(Kehrer, 1993)**. Moreover, the observed oxidative stress is consistent with the recorded neutrophilia in this study. **Ambali et al. (2010)** reported that neutrophils play an essential role in free-radical mediated injury by inducing extracellular release of superoxide and other free radicals.

In this study, comet parameters of liver and brain cells revealed significant increases in tail length, DNA% in tail and calculated tail moments in CPF intoxicated rats in relation to control group. This indicates that CPF caused DNA single and/or double strand breaks **(Nandhakumar et al., 2011)**. The observed DNA damage could be attributed to the potential of CPF to cause oxidative stress, DNA adduct formation, and failure of DNA repair system which leads to apoptosis and cell death **(Ojha and Gupta, 2017)**.

The genotoxicity of CPF was previously reported in liver and brain of intoxicated rats **(Mehta et al., 2008; Kopjar et al., 2017)** and whole blood of mice **(Rahman et al., 2002)**.Similar findings were reported *in vitro* in human lymphocytes and HepG2 cells **(Želježić al., 2016)** and human HeLa and HEK293 cells **(Li et al., 2015).**

Natural antioxidants are required as food additives to oppose the oxidative damage and lower risk caused by free radicals. In this study, we tested the potential of WG enriched diet (20% WG) to combat the oxidative stress evoked by CPF.

WG protected against the toxic effects of CPF as evidenced by partial improvement of the values of RBC count, PCV, Hb level and TLC with complete improvement of the values of neutrophils and lymphocytes. WG restored the normal values of serum ALT, AST and LDH activities, ameliorated histopathological alterations in liver, brain and

spleen. In addition, WG reduced lipid peroxidation and restored values of GSH and CAT to be around the normal values in most of these organs. Furthermore, WG ameliorated the DNA damage produced by CPF.

Our results agree with those of **Barakat et al. (2011a)** who demonstrated the protective effect of fermented WG against CPF in rats. However, these authors used WG fermented by *Saccharomyces cerevisiae* as a commercial product called avemar, but in our study, we used crude WG. The protective effect of WG against other toxic agents was previously reported. Animals fed on WG enriched diet showed marked protection against profenofos induced disturbance in lipid profile and related enzymes (G6PD and 6PGD) in liver, kidney and brain of rats **(Abdel-Rahim and Mahmoud, 2011)**.In addition, WG inhibited colon carcinogenesis induced in rats by azoxy-methane **(Zalatnai et al., 2001)**.

Wheat germ oil exerted similar protective potential against radiation induced oxidative damage **(Barakat et al., 2011b)** and cyclophosphamide induced testicular toxicity **(Mahmoud et al., 2016**) in rats, and against doxorubicin-induced hepatotoxicity in mice **(Sliai, 2015)**.

The protective effect of WG is attributed to its high content of antioxidants which can detoxicate ROS such as hydroxyl radical and ameliorate oxidative stress **(Park et al., 2015)**. WG contains the highest tocopherol content of all vegetable oil, and also the highest content of α-tocopherol, which represents around 60% of the total content **(Piras et al., 2009)**. The oil present in WG rapidly increases vitamin E content in different tissues and gives powerful antioxidant protection to all tissues of rats **(Mehranjani et al., 2007)**. This oil is also rich in unsaturated fatty acids, mainly oleic, linoleic, and α-linolenic acids that may exert inhibition of oxidative stress **(Alessandri et al., 2006)**.Moreover, WG oil contains high levels of vitamin B complex, pigments, minerals and functional phytochemicals**,** such as glutathione, flavonoids, sterols and octacosanols**(Zhu et al., 2006)**.

Based on the present observations, we can conclude that exposure of rats to high doses of CPF caused hematotoxic and hepatotoxic effects in addition to oxidative stress in liver, brain and spleen. Genotoxic effects were also demonstrated in liver and brain.

Supplementation of WG in the diet protected rats against the toxic effects of CPF as evidenced by the noticeable improvement of almost all evaluated parameters and histoarchitectural pictures. Therefore, WG can be considered as one of the powerful protectives against hepatotoxicity, oxidative stress and genotoxicity. It is recommended to enrich diet with a daily dose of WG to protect animals and human from toxic effects of organophosphates like chlorpyrifos.

Acknowledgment:-

The authors are grateful to all workers and lab staff for their cooperation and efforts to complete this study.

Funding:-

The authors declare that there are no sources of funding to be acknowledged.

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