Protective Role of Wheat Germ Oil in Clozapine-Induced Oxidative Stress and Biochemical Alterations in Liver of male albino rats

Abeer M. Abd El- Hameed[1] ; Hanan A. Soliman[1]; Eman S. Abd El- Reheem[2]

[1] Chemistry Department Biochemistry branch, Beni Suef University, Beni Suef, Egypt.
[2]Zoology Department Physiology branch , Beni Suef University, Beni Suef, Egypt.
Afnan_abrar@yahoo.com

Abstract: The purpose of the present study was to assess the antioxidant role of wheat germ oil (WGO) in clozapine-induced oxidative stress and biochemical changes in liver of male albino rats. Clozapine (CLZ) was given orally and daily for 6 weeks at a dose of 27.0 mg/kg b. wt rat. WGO was given orally 3 times/week for 4 weeks at the dose level of 900 mg/kg b. wt rat . In all groups ,CLZ and WGO was given directly into the stomach using a gastric tube , At the end of the experiment , the liver was extirpated in all of the animals . Tissue homogenates prepared from the tissue specimens were analyzed for malondialdehyde (MDA) levels ,superoxide dismutase (SOD) activities and liver glutathione content (GSH). **The results:** showed that the enzyme activities such as alanine aminotransferase (ALT), aspartate aminotransferas (AST), alkaline phosphatase (ALP) and were significantly increased in rats administrated only by CLZ . In addition, CLZ caused a significant increase in the activities of superoxide dismutase (SOD), liver glutathione content (GSH) and malondialdhyde level in liver tissue. **In conclusion:** It was determined that CLZ led to adverse alterations in the majority of the oxidative stress markers and biochemical parameters. Wheat germ oil supplementation caused significant improvement in different biochemical parameters of all rat groups.

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Introduction

Antipsychotic drug medication is an important therapeutic option for the treatment of patients suffering from schizophrenia and other psychoses. Based on their mechanism of action, antipsychotics are classified as either typical or atypical drugs. (Covell et al., 2004) . Clozapine [8-chloro-11-(4-methyl-1-piperazinyl)-5H dibenzo(b,e) (1,4) diazepine] is superior over other anti-psychotic medications in treatment of refractory or treatment-resistant schizophrenia (Taylor and Duncan-McConnell, 2000). Clozapine is predominantly metabolised in the liver by cytochrome P450 1A2, in addition by iso enzymes 2D6 and 3A3/4 to polar metabolites suitable for elimination. The major metabolite, norclozapine (desmethyl-clozapine), is pharmacologically active (Buur-Rasmussen and Brosen, 1999).

Atypical antipsychotics represent a new class in the treatment of schizophrenia. Due to the presence of risk factors like polymedication, alcoholism, or drug abuse, hepatic tolerance represents a significant issue in short, mid, and long term in schizophrenia treatment. (Stamatiadis *et al.,* 2002). A lot of medications may induce a clinical or/and Biological hepatic toxicity and need to be carefully supervised Sixteen percent of those medications belong to the neuropsychiatric class (Biour *et al.,* 1996). The first signs appear generally within 2–8 weeks after the introduction (Stamatiadis *et al.,* 2002).

A lot of atypical antipsycotics namely clozapine and others induce a clinical and/or biological hepatic toxicity (elevation of ALT, AST, ALP and GGT activities) (Gaertner *et al.,* 2001).

Oxidative stress increases when the level of reactive oxygen species exceeds the cellular anti-oxidant defence capacity(Zhang *et al.,* 2003). Indirect biochemical alterations of ROS formation have been shown for patients treated with antipsychotics as well as for untreated patients in the cases of haloperidol and clozapine the two higher concentrations induced a significantly enhanced formation of ROS (Heiser *et al.,* 2010). Cells have several ways to alleviate the effects of oxidative stress. They can either repair the damage or directly reduce the pro-oxidative state via enzymatic and non-enzymatic antioxidants. Non enzymatic(vitamins E and C, flavonoids, etc.) and enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)) antioxidants have been shown to scavenge free radicals and ROS (Durak *et al.,* 2010) .

Wheat germ oil, which makes up only 7-12% of the seed. Wheat germ oil ,known to be the natural source richest in tocopherol ,is extracted from wheat germ .Of tocopherol derivatives, this oi l contains all three alpha ,beta-and gamma- tocopherols. Wheat germ oil also contains alpha-and gamma- tocotrienols

(Leenhardt *et al.,* 2008; Hassanein and Abedel-Razek, 2009). Wheat germ oil induces the tocopherol-mediated redox system and inhibits the synthesis of eicosanoid ,which activates the lipid peroxidation process (Paranich *et al.,* 2000; Chang *et al.,* 2010). Although less than its tocopherol content, wheat germ oil also contains fat-soluble carotenoids ,which have antioxidant effect ,such as lutein, zeaxanthin and beta-carotene (Panfili *et al.,* 2003; Leenhardt *et al.,* 2008). The fatty acid composition of wheat germ oil is made of 42–59% linoleic acid, 12–28% oleic acid,11–19% palmitic acid,2–11% a-linolenic acid and 1% stearic acid. These fatty acids have a major role in the metabolism of the organism and cannot be synthesized in the body. Wheat germ oil contains unsaturated and multiple saturated fatty acids at rates of 81% and 64 , respectively (Zacchi *et al.,* 2006; Eisenmenger and Dunford,2008). Of these fatty acids, alfa-linolenic acid ,in relation to its anti-inflammatory effect, decreases O2- production and NADPH oxidase activity, and thereby, has antioxidant activity (Alessandri *et al.,* 2011). It is known that the phenolic compounds found in this oil also have antioxidant effect (Niu *et al.,* 2011). Animal studies show that intake of wheat germ oil results in a rapid increase in the content of vitamin E in the brain, liver, heart, lungs, kidneys, and spleen and gives powerful antioxidant protection to these organs and tissues (Mehranjani *et al.,* 2007; Field *et al.,* 2008). Wheat germ oil has been attributed to improved physical endurance, delayed aging, and compensated the imbalance of the serum biochemical factors in the rats to make it as the control level (Megahed, 2011).

Materials and Methods

Materials :

Clozapine was purchased from Multi-Apex Pharma-Badr city Cairo. The commercial name is clozapex®. IT was given orally and daily for 6 weeks at a dose of 27.0 mg/kg b. wt rat . WGO was purchased from SEDico pharmaceutical 6 October city-Egypt. The commercial name is Extra-1000 SEDIco WGO. The oil was given orally 3 times/week for 4 weeks at the dose level of 900 mg/kg b. wt rat.

Experimental animals:

The health experimental animals used throughout the present work were 40 adult male albino rats, mean weight varied between 90g to 170g. They were obtained from El-Salam-Farm, Giza, Egypt. The animals were allotted to 4 homogenous groups and housed individually in plastic cages and fronts in a room maintained at 25-30 °C with about 50% relative humidity. The room was lighted on a daily photo period of 12hr light and dark. They were given normal diet.

Experimental Design:

All rats offered a balanced diet for 7 days for adaptation period on the environmental conditions before starting the experiment. The animals were divided into four groups of rats (ten for each group). The first group was maintained as the control group. The second group served as positive control of clozapine . It was orally administered clozapine dissolved in distal water at the dose level of 27.0 mg/Kg BW 3 times/week for 6 weeks. The third group it was orally administered the equivalent volume of distal water 3 times/week for the first two weeks and was orally administered wheat germ oil (WGO) as emulsion with distal water at the dose level of 900 mg/Kg BW rat for another 4 weeks (from the third week to the sixth week). The fourth group it was orally administered clozapine dissolved in distal water at the dose level of 27.0 mg/Kg BW rat 3times/week for 6 weeks. After the second week it was orally administered WGO daily at dose level of 900 mg/Kg BW rat for 4 weeks(from the third week to the sixth group) beside clozapine treatment .Clozapine was administered in the morning hours, whilst wheat germ oil was administered 2 hrs after the first treatment performed that day.

Methods:

1- Collection and preparation of samples for analysis

At the end of the experimental period, the animals were fasted for 12hrs, and then anesthetized under diethyl ether anesthesia and whole blood samples were taken from hepatic portal vein in three centrifuge tubes. The second tube contained heparin then centrifuged for 10 minutes at 4000 rpm and plasma kept in plastic vials at -20 °C till used for the biochemical analysis. The second tube were left for 15 minutes at 37°C then centrifuged at 4000 rpm for 20 minutes for separating serum, and then serum were removed and kept in plastic vials at – 20 °C until analysis. The liver was extirpated. The extirpated organs were washed in deionised water to cleanse remaining blood, and connective tissue was removed. The tissue specimens were homogenized at certain rates in phosphate buffer (pH 7.4) and were centrifuged at 20,000 rpm for 1 h. Following centrifugation, the supernatants were collected into separate Eppendorf tubes for analysis.

2- Biochemical Measurements:

Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum were performed the colorimetric method as described by Reitman and Frankel (1957). Alkaline phosphatase activity in serum was measured by colorimetric method as described by Belfield and Goldberg (1971). Determination of lipid peroxides as malondialdehyde concentration in serum was determined by the colorimetric procedure as described by Ohkawa *et al.* (1979). Superoxide dismutase (SOD) activity was measured as described by Sun *et al.* (1988). Liver glutathione content (GSH) was determined

according to the procedure of Beutler *et al.* (1963).

3- Statistical Analysis:

The present data were analyzed by using statistical analysis t- student distribution test to evaluate the significant differences between the studied variables at confidence level 95%. The statistical package for the social science (version 18 SPSS inc) was used.

3.Results:

The results in table (1) show the effect of clozapine and WGO on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities. The results revealed that CLZ administration in untreated group (G2) induced liver injury which is reflected by the significant increase in serum level of ALT, AST, and ALP activities than control group (G1)and treated group (G4). From the results we observed that there is a noticeable improvement in all studied enzyme activities according to G4 (CLZ+WGO) with respect to G2 (CLZ). While there is no significant differences between G3 (WGO only) and the normal control group (G1) in both case of (ALT) and (AST) which indicate to the safety effect of WGO. It was found that serum activities of (ALT), (AST)and (ALP) was reduced significantly in treated group(G4) than untreated group(G2).

Table 1: Effect of WGO on activities of some serum enzymes related to liver function in clozapine-administered rats.

Parameters / Groups	AST (U/L)	Change%	ALT (U/L)	Change%	Alkaline Phosphatase (U/L)	Change%
Group 1(normal control)	83.00 ±1.460	-	21.16 ±0.307	-	18.05±0.593	-
Group 2(CLZ)	108.16±7.091 ***	30.31	36.17±1.447***	70.94	44.03 ±2.245***	143.93
Group 3(WGO)	82.66±4.730	-0.41	25.00±1.366	18.15	23.48±0.914*	30.08
Group 4(CLZ+WGO)	91.66±5.840+	-15.26	27.50±1.910+++	-23.97	23.18±1.477+++	-47.35

Significantly differ in comparison with the corresponding normal control group (group 1) at α=0.05 and 0.001, respectively. Percentage of changes was calculated by comparing clozapine-administered group (group 2) and WGO-administered group (group 3) with normal control group (group 1). and comparing clozapine group treated with WGO (group 4) with (group 2). Values were expressed as mean± standard error.

The results in table (2) demonstrates the effect of CLZ administration alone and with treatment of WGO on malondialdhyde (MDA) level, superoxide dismutase (SOD) level and liver glutathione content (GSH) in liver tissue . From the results, the level of MDA, SOD and GSH were statistically significant differences in group that administered CLZ alone (G2) when compared with normal control group (G1). The findings of the present experimental study demonstrate the improvement effect of WGO in all enzymes exhibited in treated group(G4) when compared with group administered only CLZ(G2).

Table 2: Effect of WGO on antioxidant and some oxidative stress activities in liver tissue of clozapine-administered rats.

Parameters / Groups	Glutathion (n mol/100 mg)	Change %	Lipid peroxidation (n mol/g)	Change%	SOD (U/ml)	Change%
Group1(normal control)	19.91 ± 0.943	-	16.72 ± 1.066	-	3.44 ± 0.117	-
Group 2(CLZ)	76.84 ± 1.685 ***	285.94	41.12 ± 1.763***	145.93	4.24 ± 0.117***	23.26
Group 3(WGO)	15.60 ± 1.216*	-21.65	11.94 ± 1.265*	-28.59	3.08 ± 0.058*	-10.47
Group 4(CLZ+WGO)	30.92 ± 0.700+++	-59.76	25.07 ± 2.859+++	-39.03	3.40 ± 0.055+++	-19.81

*, ***: Significantly differ in comparison with the corresponding normal control group (group 1) at α=0.05and 0.001, respectively. Percentage of changes was calculated by comparing clozapine-administered group (group 2) and WGO-administered group (group 3) with normal control group (group 1). and comparing clozapine group treated with WGO (group 4) with (group 2). Values were expressed as mean± standard error

4.Discussion:

Oxidative stress caused by various agents (toxins, metals, dioxin and pesticides) is considered as an imminent threat for many organisms since it can lead to death. However, the imbalance between production of oxygen free radicals (OFRs) and antioxidant defenses in the body is called oxidative stress which has important health implications reported by Ranjbar et al.

(2005). If there are too many OFRs or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic damage (Abdollahi et al., 2004). Indirect biochemical alterations of reactive oxygen species (ROS) formation have been shown for patients treated with antipsychotics as well as for untreated patients. Clozapine induced a significantly enhanced formation of ROS and antipsychotics induce the formation of ROS in the whole blood of rats, which can be reduced by the application of vitamin C (Heiser et al., 2010) . ROS can cause cellular damage such as peroxidation of membrane lipids, oxidation of proteins and damage to DNA, if not removed by antioxidant defenses .Oxidative stress is described as the imbalance between the generation defence system to detoxify reactive products(Auten and Davis, 2009; Mena et al.,2009) . One of the results of the level of free radicals exceeding the limit that can be compensated by cells is the peroxidation of lipid membranes .Cell membranes predominantly contain unsaturated fatty acids and due to this property are a target for free radicals. The peroxidation of lipid membranes alters the permeability of the cell membrane and damages the cell transport system ,and thereby ,triggers the development of intracellular adverse effects(Srivastava et al.,1989; Van der Vliet and Bast,1992; Evans and Halliwell, 2001; Halliwell, 2007; Ogino and Wang,2007; Niki, 2009; Negre-Salvayre et al.,2010) . The conjugation of reactive drug metabolites to GSH is considered an important detoxification mechanism. Dragovic et al., 2010 .

In the present study, activities of antioxidant enzymes such as superoxide dismutase (SOD) in liver tissue was examined in addition, glutathione (GSH) content in liver tissue was determined.

in the present study , as compared to normal control, the administration of clozapine in rats produced a significant increase in the activities of (SOD) and a significant increase in liver (GSH) content .these results are supported by Gama et al. (2006) who examined the effects of long-term clozapine treatment on blood anti-oxidant defence system enzymes in schizophrenia and reported that activity of serum SOD was higher in chronic medicated schizophrenic patients under clozapine compared with control values. Also Cedo et al 2010 found an increased SOD1 activity in RBCs isolated from schizophrenic patients which in agreement with our results. Vinay Parikha et al. 2003 reported that for the first time, none of the atypical antipsychotics studied , CLZ or olanzapine were found to cause oxidative stress or oxidative injury. Zhang et al. (2006) noted reduced levels of plasma SOD in chronic medicated schizophrenic patients under clozapine compared with control values. These disparate findings could be due to differences in exposure to and duration of neuroleptic treatment and the mean daily doses of medication, which have all been known to influence such indices Ranjekar et al., 2003. In the present study we determined the level of lipid peroxidation in the liver tissue of animals and we found that clozapine-administered rats show a significant increase of lipid peroxidation level as compared to normal control rats .and our results are supported by the finding of Dr. Clare Beasley 2010, who suggest that antipsychotic drugs may result in oxidative stress, leading to cell damage in the brain and other organs and ultimately to adverse effects. And found increased levels of lipid peroxidation in the liver of animals given clozapine. increase in lipid peroxidation probably due to enhanced superoxide production (Polydoro et al., 2004). Lipid peroxidation has been implicated in the toxic effect of many chemicals and in many tissue injuries and disease processes(Dal-Pizzol et al., 2000) .

Wheat germ is a rich source of antioxidants that include carotenoids, tocopherols, flavonoids and phenolic acids. (Vaher et al., 2010). Most of the essential amino acids from wheat germ proteins are present at concentrations higher than in the reference egg protein pattern (Ge et al., 2001). Since the rapid increase of the global demand for protein consumption, wheat germ may represent one of the most attractive and alternative source of proteins from cheap vegetable sources (Zhu et al., 2006).

In the present study the administration of WGO with clozapine for 4 weeks minimizing the elevation of SOD, liver GSH content and lipid peroxidation level as compared to clozapine-administered group.these results are supported by M¨ ursel Karabacak et al., 2011 who found that SOD activity in the group which received coumphos alone had significantly increased in lung tisses . Values pertaining to the group, which was given both coumaphos and wheat germ oil displayed positive alterations, in other words, drew closer to the values of the control group. And suggested that positive alterations in SOD activity occurred more rapidly in liver and heart tissues. Also M¨ ursel Karabacak et al., 2011 found that In the group, which received coumaphos alone, it was ascertained that MDA levels had increased significantly in all the tissues analysed. On the other hand, in the group, which was administered with both coumaphos and wheat germ oil, values improved and drew closer to the values of the control group. This suggested that lipid peroxidation was eliminated in the tissues more rapidly. The improvements observed in both tissue MDA levels and tissue antioxidant enzyme activities can be explained by the influence of mechanisms targeting the reduction or inhibition of free radicals that cause the lipid peroxidation of cell membranes. Several mechanisms may be involved in the aforementioned improvement,

which is considered to have arisen directly from the composition of wheat germ oil. Of the structural components of wheat germ oil, one is the abundantly found tocopherols. As is known, owing to it radical scavenging activity, tocopherol is considered as a strong antioxidant (Chow, 1991; Liu et al., 2008) . Although to a less extent than tocopherols, it is considered that carotenoids found in the composition of wheat germ oil may also contribute to antioxidant activity. Furthermore, it is possible that the phenolic compounds found in wheat germ oil/extract also have free radical scavenging activity (Niu et al., 2011; Zhu et al., 2011),

Similar to vitamins, and thus, prevent the peroxidation of lipid membranes.

Liver enzymes activities were used as important biomarkers for detection of hepatotoxicity. Four serum hepatic marker enzymes (ALT, AST, ALP and γGT) were evaluated for hepatotoxicity. The liver is the most sensitive organ to preoxidative damage because it is rich in oxidizable substances. The increment of the oxidative stress on the cells of the liver and the consequent decrease in the antioxidant ability of the cells result in the occurrence of aggressive cellular damage to the liver cells with destruction of their membranes and the release of the enzymes into the blood stream .The more severe the liver damage the higher the release of the liver enzymes (El-Khayat et al., 2009). Increase in serum level of ALT, AST as observed in groups dosed with CLZ may reflect damage of liver cells and cellular degeneration or destruction occurs in this organ and the increase in the activities of ALP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis, and this showed the stress condition of the treated animals with CLZ. Also, the results of the present study indicate that wheat germ oil (WGO) significantly reduce the toxic effects of CLZ by altered the hepatic enzyme activities and thus can be considered a potential hepato protective agent in conditions of CLZ poisoning. Alia et al. (2003) explained that the liver is the main detoxifying organ in the body, and as such it possesses a high metabolic rate and it is subjected to many insults potentially causative of oxidative stress. Consequently, a correct status of the hepatic antioxidant defense system is of major importance for the maintenance of health. When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Elevation of AST and ALT indicates the utilization of amino acids for the oxidation or for gluconeogenesis and is used to determine liver damage (Etim et al., 2006). The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage or necrosis of hepatocytes (Pari and Arumugam, 2008). The membrane bound enzymes like ALP and γGT are released unequally into bloodstream depending on the pathological phenomenon. (Li and Zhong,2004). These results are supported by the observations of Laurence et al (2002) who reported that in a small series of 23 patients, we confirm that treatment with various antipsychotic drugs (risperidone, amisulpride, olanzapine, and clozapine) may frequently cause Asymptomatic increase in liver function tests. A lot of atypical antipsycotics namly clozapine and others induce a clinical and/or biological hepatic toxicity (elevation of ALT, AST, ALP and GGT activities) (Gaertner et al., 2001) .

These elevation were improved after treatment with WGO for 4 weeks of treatment. These data are supported with the finding of OKita et al. (2001) in obese patient s and Du et al. (2004) in female rats who found decrease s in serum ALP after omega-3 fatty acids and linolenic acid treatment. . In many studies it was found that Vitamin E has a protective role against liver toxicity caused by carbon tetrachloride, nitrosamines, nitrites etc. Vitamin E is capable of inhibiting peroxidation and nitrosation. Vitamin E had a protective effect on the hepatic cellular structure in CC14 hepatotoxity (Egilmez -1994) . Ammar (2009) and this agreed with the result of the present work that declared significant elevation in liver enzymes as a result of γ-radiation exposure where as this elevation were alleviated when treated with wheat germ oil. Also{Fares et al., 2011 stated that the results of the his study indicate that wheat germ oil (WGO) significantly reduce the toxic effects of CPF by altered the hepatic enzyme activities and thus can be considered a potential hepatoprotective agent in conditions of organophosphate poisoning.

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