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Research Article

EVALUATION OF POSSIBLE AMELIORATIVE ROLE OF WHEAT GERM OIL ON HEPATOTOXICITY INDUCED BY THE COMBINATION OF TWO ANTI-TUBERCULAR DRUGS (ISONIAZID AND RIFAMPICIN) IN RATS

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ABSTRACT

The study was designed to evaluate the protective effect of wheat germ oil (WGO) against hepatotoxicity induced during treatment with the combination of two anti-tubercular (anti-TB) drugs, Isoniazid (INH) and Rifampicin (RIF) in rats. Fatty acid and Vit E. contents of WGO were assayed by GC-MS and HPLC respectively. Rats received WGO at two different doses (250 and 500 mg/kg. body weight, p. o, for 30 days) 30 minutes before Anti-TB drugs. Liver functions test, inflammatory mediator marker (IL-10 and NF-κB), oxidative stress (GSH, MDA) and NO were determined with different techniques. Furthermore, histological findings, immunohistochemical and ultra-structure were carried out. *In-vitro* analysis of the WGO revealed that linoleic and oleic acids were the major compounds, and WGO was rich in vitamin E. Significant elevation in liver enzymes, NF- κB, and depletion in IL-10, along with a disturbance in the antioxidant defense systems. Meanwhile, WGO improves all these changes in a dose- dependent manner as compared with the anti-TB intoxicated group. The hepatoprotective effect of WGO was confirmed with immune histochemical and histopathological findings. WGO has a hepatoprotective effect against hepatotoxicity induced by combined therapy of anti- TB drugs (INH+RIF).

Keywords: Wheat germ oil; Isoniazid; Rifampicin; inflammatory mediator; immunohistochemical examination.

INTRODUCTION

Tuberculosis (TB) is a highly infectious disease in developing countries. Isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol, and streptomycin, are the most commonly used drugs for the treatment of TB.[1] Isoniazid (INH), and Rifampicin (RIF) are the most effective drugs that were commonly used for the treatment of TB. The incidence of hepatotoxicity is increased, when INH, and RIF are used as a mixture.[2] It was confirmed the combination produces sliver injury by damaging the liver membrane that leads to the leakage of bilirubin (T-Bil) and other biochemical marker enzymes such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).[3,4] The mechanism of liver damage induced by toxic intermediaries and reactive oxygen species (ROS) derived from INH biotransformation has been concerned in the progression of hepatotoxicity.[5] INH is metabolized to Hydrazine (HYZ) and Acetyl hydrazine. Oxidation of these metabolites by cytochrome P450 (CYP 450), generates an electrophilic intermediate, and free radical, which covalently adduct the hepatic macromolecules resulting in acute liver failure.[6] RIF is a strong inducer of CYP2E1[7] and activation of CYP2E1 leads to oxidative stress.[8] Moreover, hydrazine depletes the reserve sources of reduced glutathione (GSH) in hepatocytes, which results in altered mitochondrial permeability and induce apoptosis. Oxidative stress in the hepatocytes results in apoptosis is one of the attributing mechanisms of liver dysfunction caused by INH and RIF.[9,10] Also, the transforming growth factor -β (TGF-β) and matrix metalloproteinase (MMP2) activate the hepatic stellate cell (HSCs) to produced fibers due to elevating the amount of glycoprotein which transforms stellate cells to fibrogenic cells causing liver fibrosis.[11]

Wheat germ oil (WGO) is an excellent source of natural vitamin E (Vit. E), the most powerful natural antioxidant.[12,13] WGO is also rich in unsaturated fatty acids, mainly oleic, linoleic, and α-linolenic acids (precursors of omega 6 and omega 3 fatty acids) that may inhibit the inflammatory response and oxidative stress.[14,15] Furthermore, WGO is rich in functional phytochemicals, mainly flavonoids, sterols, octacosanols, and glutathione.[13] Recently, it has been shown that WGO intake results in a rapid increase in the level of Vit. E in different rat tissues, and exerts high protection against oxidative damage.[16,13] Thus, it was interesting to investigate the potential protective role of WGO against anti-TB intoxicated drug-induced hepatotoxicity in rats.

MATERIAL AND METHODS

All chemicals, solvents (HPLC grade) and reagents were purchased from Sigma Aldrich.

Anti-tuberculosis drug capsule is purchased from the local pharmacy. It consists of a combination of Isoniazid (150 mg/cap) and Rifampicin (300 mg/Cap). The dose of this drug was calculated based upon the human dose after conversion to that of rat according to[17] conversion tables.

Extraction of the wheat germ oil

Wheat germ oil is prepared by a hydraulic press machine (Cold press). Fatty acids in WGO were measured by gas chromatography according to.[18] While, investigation of Vit. E was carried out on an Agilent Technologies 1100 liquid chromatography according to.[19]

Experimental animals

Adult female rats (180 ± 20 g) obtained from the National Organization for Drug Control and Research (NODCAR), Giza, Egypt, was used in this study. The rats were housed in wire mesh fence cages for one week to become acclimated to the environment. During the entire research, the rats were kept under standard conditions (temperature 25 ± 2ºC, 12 hours. light and, 12 hours. darkness cycles). Animals were fed a pellet standard rat diet and water *ad libitum.* The study was conducted by following the recommendations from the declaration of Helsinki on guiding principles in the care and use of animals.

Experimental design

The rats were randomly divided into four groups (6 rats each) and treated as follow:

Group 1: Normal control, animals received distilled water 0.5 ml /kg. b. w. p. o. daily
Group 2: Anti-TB intoxicated drug, animals received INH + RIF (27 + 54 mg/kg. b. w. p. o. daily, respectively).
Group 3: Treated group, animals received WGO (500 mg/kg. b. w. p. o, daily) and then received (INH + RIF with previous concentration) after half an hour.
Group 4: Treated group, animals received WGO (250 mg/kg. b. w. p. o. daily) and then received INH + RIF (with above concentration) after half an hour. The experiment maintained for 30 days.

Blood sample and tissue collection

At the end of the experimental period, all animals fasted overnight. Blood samples were withdrawn from the retro-orbital plexus vein. Serum was separated by centrifugation at 3000 rpm at 4ºC and stored at -20 °C until further biochemical analysis. Serum levels of ALT, AST, ALP, T. Bil, Total protein (TP), and Albumin (ALB) were assayed using biochemical kits supplied by Spin react, Barcelona, Spain. Necrosis factor Kappa- B (NF-κB) and interleukin-10 (IL-10) were measured by ELISA kits according to the manufacturer`s instruction. The excised liver in each group was fixed with 10% formaldehyde for histopathological examination, and the remnants of the liver tissues were stored at -80°C for later analysis.

Tissue collection

Liver tissues were crushed into small pieces, homogenized in ice-cold 0.05 M potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were centrifuged at 4000 rpm. for 15 minutes at 4°C and used to determine of malonaldehyde (MDA) in nmol/g tissue by HPLC according to.[20] Hepatic content of reduced glutathione (GSH) was assayed by HPLC, according to,[21] and the level of nitric acid (NO) was measured by HPLC, according to.[22]

Histopathological Examination

After fixation, specimens were prepared for stained with Ehrlich's hematoxylin and counterstained with eosin as a routine method[23] and Sirius red staining (0.1% Sirius red in saturated picric acid) were performed separately to evaluate the fibrotic level of liver tissues.[24]

Immunohistochemical Examination

The techniques of immunochemistry tints are applied to demonstrate the process of localizing proteins in tissues by exploiting antibodies associated with antigens. The antibody is specifically visualized by merging an enzyme with the antibody to produce a color-changing reaction. The advantage of this method is the ability to show exactly where a given protein is located. The techniques were performed using an avidin–biotin complex, immune-peroxidase to detect each of matrix metalloproteinase (MMP2) and transforming growth factor-β_1 (TGF-β_1) expressions according to the methods of [25,26] respectively.

The ultra-structure investigation

Liver tissues were prepared for TEM using the procedures described according to[27] stained grids were examined with a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

Statistical analysis

Data were expressed as means ± S.E (n = 6 rats) values in the different groups. Statistical differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey Test using Graph pad prism version 5.1. $P < 0.05$ was considered significant.

RESULT

Composition of fatty acid in wheat germ oil %

Table 1 summarizes the results of the fatty acids composition of WGO. In the course of the present study, 5 main components composed of 17.35% saturated fatty acid (palmitic acid) and 82.65% of unsaturated fatty acids. The major components of unsaturated fatty acid were linoleic acid (53.37%) and oleic acid (21.15%). While the minor components were Linolenic acid (6.53%) and Arachidic acid (1.57). The Total amount of Vit E. in WGO was measured by HPLC and equivalent to 640 µg/gm.

Effect of wheat germ oil on diagnostic markers of liver function

Table 2 illustrates the result of liver functions as manifested by measuring enzyme activity of ALT, AST, ALP, and T. Bil. The anti-TB intoxicated drugs group revealed a significant ($p < 0.05$) increase in ALT, AST, ALP and T. Bil by percentage change of 29.71%, 204.96 %, 54 %, and 104% respectively, and a significant decrease in TP and ALB with the percentage change of -13.36 % and -9.54 % respectively when compared with the control group. Pre-treatment with WGO before administration of anti-TB intoxicated drug showed a significant ($p<0.05$) improvement in all parameters on dependent-concentration manner when compared with anti-TB intoxicated drug only.

Effect of wheat germ oil on inflammatory mediators (IL-10 and NF- κB) in all experimental animals

Table 3 showed that. The level of IL-10 in serum was significantly ($p < 0.05$) decreased with the percentage change of -40.64 %. Meanwhile, the level of NF- κB in serum was highly

augmented ($p < 0.05$) with percentage change (201.5%) in rate treated with the anti- TB intoxicated drugs when compared with the control group. On the other hand, the rate treated with wheat germ oil ameliorates the level of inflammatory biomarker IL-10 and NF-κB when compared with the anti-TB intoxicated drug group.

Effect of wheat germ oil on oxidative stress (GSH, MDA) and NO in all experimental animals

Hepatic MDA levels, as a marker of lipid per oxidation and NO, were significantly increased in rate treated with the anti-TB intoxicated drugs with percentage change of 82.21 % and 48.1 % respectively as shown in Table 4. While the level of hepatic content of GSH was significantly ($p < 0.05$) declined in the same group with the percentage change of -51.45 %, when compared with the control group. Pre-treatment with WGO before administration of anti-TB intoxicated drugs showed a significant ($p < 0.05$) reduction in hepatic MDA, NO and increased in the content of hepatic GSH when compared with anti-TB intoxicated drugs group on dependent - concentration manner.

Histopathological finding

The histological appearances of the hepatic tissue of control rat represented in Figure 2, which revealed normal lobular architecture with normal hepatic cells radiating from the central vein. Hepatocytes appeared with well-defined vesicular basophilic nuclei and preserved eosinophilic granular as showing in Figure 2-a. While the administration of anti-TB intoxicated drug- induced marked histopathological lesion, which was characterized by necrotic area, scattered fragmented apoptotic body with dilated and hemorrhage in central vein and blood sinusoid as shown in the Figure 2-b. Another field in this group, the Figure 2- c showing the damaged in portal triads with fibrotic appearance, pyknotic nuclei, and massively hyperplasia with elongated bile duct accompanied by lymphocytic inflammation. On the contrary, the photomicrograph of liver tissue in the group treated with WGO (500 mg /kg), illustrate the significant improvement in liver architecture except some hemorrhaged patch's and few lymphocytes infiltration as shown in Figure 2-d. Meanwhile, the liver tissue of the group treated with WGO (250 mg /kg), revealed milled (improvement with some inflammation around dilated portal vein with mild liver fibrosis and bile duct accompanied by dilated sinusoids as showing in Figure 2-e. To evaluate the fibrotic tissue distribution in the liver by Sirius red stain applied as showing in Figure 3. The photomicrograph represented control group in Figure 3-a, that illustrate normal fibrotic distribution. Otherwise the hepatic tissue of group-administered anti-TB intoxicated drugs contain stacked fibers clearly emerged as demonstrated in Figure 3-b, whilst the amount of fiber has been declining in the Figures 3-c and 3-d represented the groups treated with WGO (500 and 250 mg/kg) respectively. On the other side, the figure 4, bring to light the immunohistochemical investigation of the Mmp2 activity, the section of liver from control group the Figure 4-a manifested the normal distribution of (Mmp2) in the extracellular matrix of hepatocytes, while the hepatic tissue of anti-TB intoxicated drugs group as shown in Figure 4-b, that illustrates the intensity of the presence of this material ,While this density is defeated by treatment with WGO (500 and 250 mg /kg), respectively as shown in the Figures 4-c and 4-d. Another technique was performed to determine the Transforming growth factors β_1-(TGF-β_1) cytokine polypeptide demonstrated in Figure 5. The Figure 5-a illustrate the natural appearance of this protein in control tissue, while the anti-TB intoxicated drug group, showed the over-expression of TGF-β_1 are noticeable in Figure 5-b, but the treatment by WGO elucidate retreat effect of TGF-β_1 cytokine polypeptide and different distribution of TGF-β_1 immuno positively reactions in fibrosis hepatic represented in Figures 5-c and 5-d depending on concentration manner. While the Figure 6 illustrated the Ultra structure features of normal hepatocytes with rounded nucleus, well developed endoplasmic reticulum, and scattered many mitochondrial organelles manifested in Figure 6-a. Conversely, the Figure 6-b of liver tissue from rats administered anti-TB intoxicated drugs elucidate hemorrhagic inflammation, tortuosity in many nuclear envelop and disintegrated area around blood sinusoid, in another field in Figure 6-c showing minute structure of activated hepatic stellate cells (HSCs) or Ito (fat-storing) cell, fragmented endoplasmic reticulum, and mitochondrial malformation. Another partin Figure 6-d is showing a bundle of fibers and dissolution of mitochondrial folds. While The hepatic tissue of rat treated by the wheat germ oil 500 mg /kg and 250 mg /kg respectively, illustrate the marked improvement in high dose as demonstrated in Figure 6-e than the lower one evidenced by pyknotic nuclei and activated each of stellate and *Kupffer* cells as showing in Figure 6-f.

DISCUSSION

Tuberculosis (TB) is a highly infectious disease in developing countries. Isoniazid (INH) and Rifampicin (RIF) are the two major chemotherapeutic drugs used for the treatment of TB, are associated with hepatotoxicity[2]. It is well known that both INH and RIF produce lipid peroxides, inducing the formation of MDA, and develop oxidative insult in the cell. As a result, the integrity of the liver cell membrane is lost and the liver is damaged[28,4]. ALT and AST in the systemic circulation is a clear marker of hepatocellular injury. In the current study, WGO reduces the formation of lipid peroxide and maintains liver cell integrity, thus reducing the ALT and AST levels in the serum.[4] During hepatic cellular damage, the liver cell is unable to excrete bilirubin; hence bilirubin accumulated in the serum.[29] In the present work, WGO prevents the liver cell damage and retains the normal bilirubin excretion. ALP is a membrane-bound glycoprotein enzyme, with high concentrations in sinusoids and endothelium and is excreted into the bile and its elevation in serum occurs in hepatobiliary diseases.[30,10] In the present study, pre-treatment with WGO caused a decrease in the activity of ALP when compared with anti-TB intoxicated drugs group, showing its anti-hepatotoxic potential. TP and ALB levels are indicators of liver function since the majority of plasma proteins and ALB are synthesized in the liver.[12] Reduction of serum TP and ALB level indicate alarming liver damage in rats treated with INH and RIF. In the present study, WGO restores the normal function of the liver by increasing the TP and ALB levels. Furthermore, the hematoxylin with eosin and Sirius red staining also showed that WGO can obviously improve the hepatic morphology and architecture and markedly ameliorate the degree of liver fibrosis. These results indicated that WGO exerts anti-fibrotic effects, which is compatible with the previous report.[31] Lipid per oxidation is an autocatalytic process, which is a common consequence of cell death. The level of MDA indicates an enhanced lipid per oxidation leading to liver tissue injury and failure of antioxidant defense[10]. In the present study, a significant decrease in the MDA level was observed in the WGO treatment group when compared to the anti-TB intoxicated drugs model. Hydrazine, the principal metabolite of INH is highly reactive with a sulfhydryl group, which results in depletion of hepatic content of reduced GSH and induces oxidative stress of the hepatic cell[12]. In the current study, liver injury has been observed when hepatic content of reduced GSH is markedly depleted in anti-TB intoxicated drugs administered rats while WGO increases the hepatic content of reduced GSH. Tissue injury induced by anti-tubercular drugs has also been reported to be associated with induction of the pro oxidant enzyme iNOS[32,4].

Table 1: Composition of fatty acid in wheat germ oil %

Fatty acid	%
Palmitic acid (C16:0)	17.35 %
Oleic acid (C18:1)	21.15 %
Linoleic acid (C18:2)	53.37 %
Linolenic acid (C18:3)	6.53
Arachidic acid (C20:1)	1.57 %

Table 2: Effect of wheat germ oil on diagnostic markers of liver function

Groups	Parameters											
	ALT (U/L)		AST (U/L)		ALP (U/L)		T. Bilirubin (mg/dl)		TP (g/dl)		Albumin(g/dl)	
	Mean ± S. E	% change	Mean ± S. E	% change	Mean ± S. E	% change	Mean ± S. E	% change	Mean ± S. E	% change	Mean ± S. E	% change
Control	27.43 ± 0.68	---	25.43 ± 0.89	-----	159.5 ± 3.02	---	0.5 ± 0.02		8.68 ± 0.12		4.61 ± 0.09	
Anti- TB intoxicated drug	35.58 ± 0.67$^{\#}$	29.71 %	77.55 ± 0.84$^{\#}$	204.96 %	245.67 ± 5.86$^{\#}$	54 %	1.02 ± 0.05$^{\#}$	104%	7.52 ± 0.09$^{\#}$	13.36 %	4.17 ± 0.01$^{\#}$	-9.54%
WGO (500 mg/kg) + Anti- TB intoxicated drug	27.64 ± 0.49*	-22.3 %	28.58 ± 0.69*	63.15 %	164.54 ± 3.33*	-33 %	0.59 ± 0.03*	-42.2%	7.94 ± 0.13	-5.58 %	4.29 ± 0.04	2.88%
WGO (250 mg/kg) + Anti -TB intoxicated drug	28.89 ± 0.88$^{\text{ϫ}}$	-18.80 %	30.72 ± 0.61$^{\text{ϫ}}$	-60.38 %	184.25 ± 2.6 $^{\text{ϫ}}$	-25 %	0.64 ± 0.04	-37.3 %	7.85 ± 0.17	4.4 %	4.10 ± 0.06	-1.67%

Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p < 0.05$. Data are analyzed with one- way ANOVA followed by Tukey Test at $p < 0.05$, # Significant difference from control at $p < 0.05$, * and ϫ significant difference from anti-TB intoxicated drug at $p < 0.05$.

Table 3: Effect of wheat germ oil on inflammatory mediators (IL-10 and NF- κB) in all experimental animals

Parameters / Groups	IL-10 (pg/ml)		NF-Kb (pg/ml)	
	Mean ± S. E	% change	Mean ± S. E	% change
Control	109.75 ± 5.30	-	4.12 ± 0.27	-
Anti-TB intoxicated drug	65.15 ± 2.92$^{\#}$	-40.64 %	12.42 ± 0.67$^{\#}$	201.5%
WGO (500 mg/kg) + Anti-TB intoxicated drug	102.42 ± 4.56*	57.2 %	7.69 ± 0.25*	-38.1 %
WGO (250 mg/kg) + Anti-TB intoxicated drug	80.90 ± 2.04 $^{\text{ϫ}}$	24.17 %	9.34 ± 0.46 $^{\text{ϫ}}$	-24.79%

Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p < 0.05$. Data are analyzed with one-way ANOVA followed by Tukey Test at $p < 0.05$. # Significant difference from control at $p < 0.05$, * and ϫ significant difference from anti-TB intoxicated drug at $p < 0.05$

Table 4: Effect of wheat germ oil on oxidative stress (GSH, MDA) and NO in all experimental groups

Parameters / Groups	GSH (µmol/g tissue)		MDA (nmol/g tissue)		NO (µmol/g tissue)	
	Mean ± S. E	% change	Mean ± S. E	% change	Mean ± S. E	% change
Control	51.02 ± 1.34	--	19.47 ± 0.5	-	6.23 ± 0.23	-
Anti- TB drug	24.77 ± 0.78$^{\#}$	-51.45%	35.46 ± 0.85$^{\#}$	82.12 %	9.23 ± 0.33$^{\#}$	48.1 %
WGO (500 mg/kg) + Anti TB intoxicated drug	36.25 ± 1.31*	46.35 %	20.1 ± 0.46*	-43.32 %	6.27 ± 0.31*	-32.1%
WGO (250 mg/kg) + Anti TB intoxicated drug	30.18 ± 2.06 $^{\text{ϫ}}$	21.8 %	24.97 ± 0.85 $^{\text{ϫ}}$	-29.58 %	8.22 ± 0.24	-10.9 %

Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p < 0.05$. Data are analyzed with one-way ANOVA followed by Tukey Test at $p < 0.05$. # Significant difference from control at $p < 0.05$, * and ϫ significant difference from anti-TB intoxicated drug at $p < 0.05$

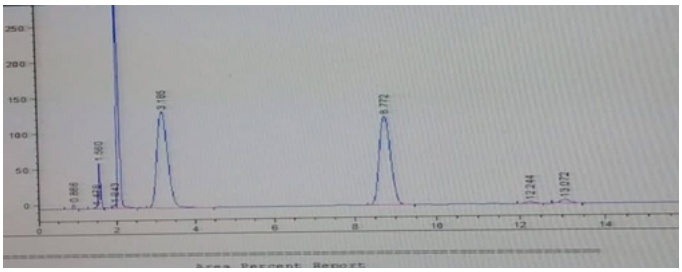


Figure (1): HPLC Chromatogram of Vitamin E.

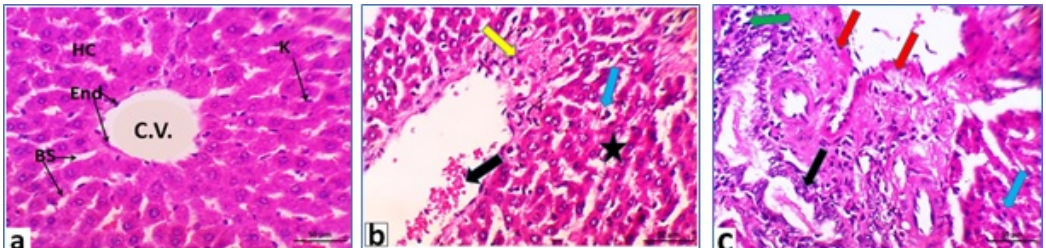
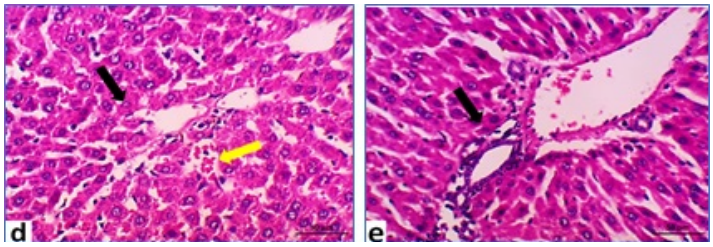


Figure (2): A Photomicrograph of Liver tissue in control group revealed normal structure of hepatocytes **(a)** The Photomicrographs of Liver tissues in group administered (Anti-TB intoxicated drug.),in **(b)** illustrate the hepatocytes' necrotic area (yellow arrow), other fragmented apoptotic body (blue arrow) ,dilated and hemorrhage in central vein (black arrow) and dilated blood sinusoid **(star). (c)** damaged portal triads with fibrotic appearance (red arrow), pyknotic nuclei (blue arrow), and massively hyperplasia, elongated bile duct (black arrow), and lymphocytic inflammation (green arrow). (H & E x 400).



(d) A Photomicrograph of Liver tissue of group treated by wheat germ oil (500mg/kg), illustrate significant improvement liver architecture (black arrow) except some hemorrhaged patch's (yellow arrow).and few lymphocytes infiltration (H&E x400) **(e)**A Photomicrograph of Liver tissue of group treated by wheat germ oil (250 mg /kg), showing milled improvement with some inflammation around dilated portal vein with mild liver fibrosis and bile duct (black arrow), and dilated sinusoids (H&E x400)

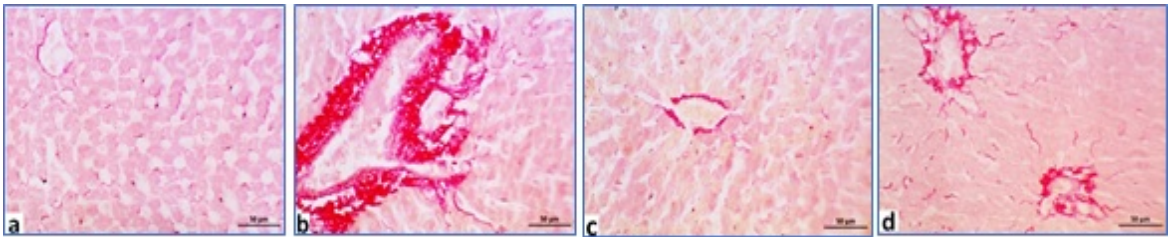


Figure (3): A Photomicrograph of Liver tissue sections were stained with Sirius red to evaluate fibrotic level occurred in hepatocytes control rat **(a)**,A group administered (Anti-TB intoxicated drug) induced liver fibrosis and group treated by wheat germ oil (500 mg /kg),and (250 mg /kg), respectively showing the gradually improvement in the amount of fibrous tissue scattered within hepatic tissue **(b,c,d)** respectively (SR . X400)

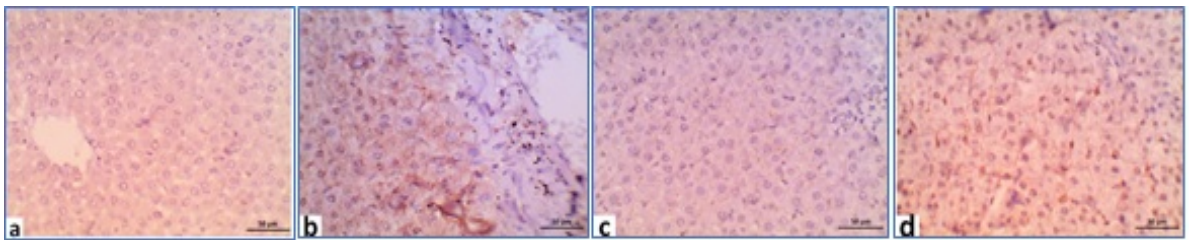


Figure (4): A Photomicrograph of Liver tissue sections were stained with Mmp2 immune – reaction in hepatocytes, **(a)** control, group administered ((Anti-TB intoxicated drug)) induced liver fibrosis, group treated by wheat germ oil (500 mg /kg),and (250 mg /kg), respectively ,showing The distribution levels of Mmp2 immune - reactions in intercellular ground of hepatocyte **(b,c,d)** . (Mmp2. X400)

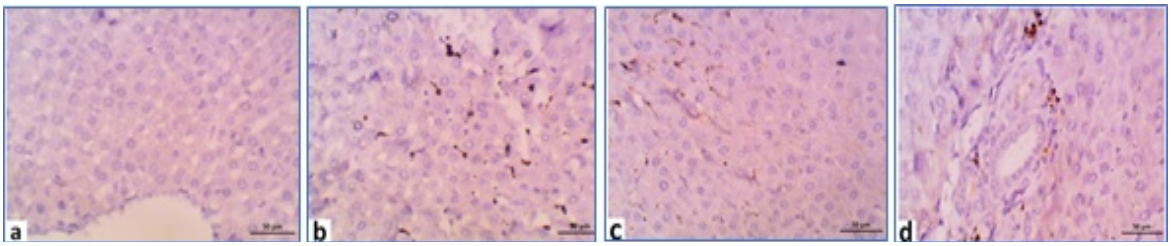


Figure (5): A Photomicrograph of Liver tissue sections were stained with TGF-β immune – reaction in hepatocytes , control, group administered ((Anti-TB intoxicated drug)) induced liver fibrosis , group treated by wheat germ oil (500 mg /kg),and (250 mg /kg), respectively ,illustrates positive reaction of the different distribution of TGF-β immune - positive reaction in fibrosis hepatic tissue **(a,b,c,d**). (TGFβ. X400)

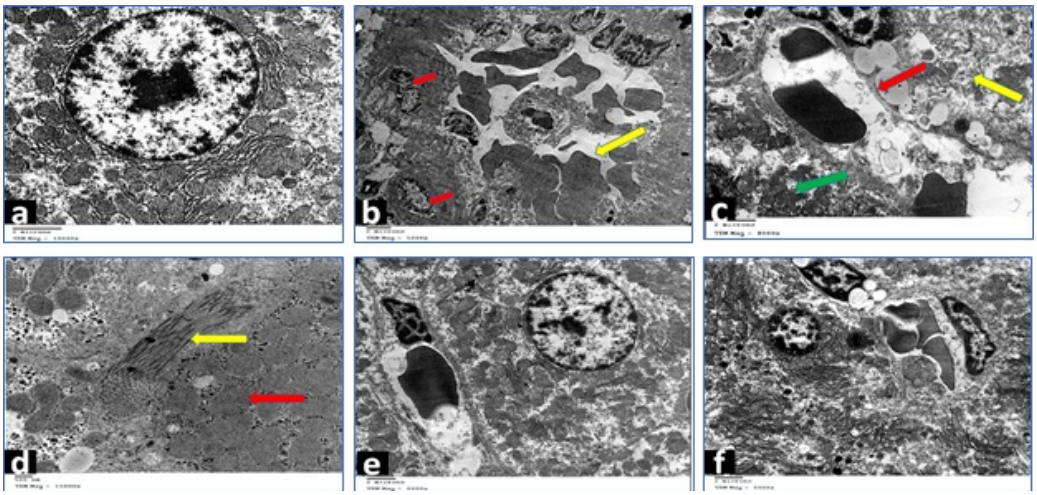


Figure (6): The Ultrastructure Photomicrograph of liver tissue normal structure of hepatocytes with rounded nucleus, endoplasmic reticulum, and many mitochondrial organelles scattered around **(a)**. (TEM 10000X)
(b) is Photomicrograph of liver tissue group administered ((Anti-TB intoxicated drug) illustrate hemorrhagic inflammation (yellow arrow), tortuosity in many nuclear envelop (red arrow), and disintegrated area around blood sinusoid (TEM 5000X)**. (c)** showing activated stellate cell (red arrow), fragmented endoplasmic reticulum (yellow arrow) mitochondrial malformation (green arrow) (TEM8000x), Whereas in **(d)** showing a bundle of fibers (yellow arrow) and dissolution of mitochondria folds (red arrow) (TEM15000x).While **(e , f)** The Ultrastructure Photomicrographs of liver tissue group treated by wheat germ oil 500 mg /kg and 250mg /kg respectively, illustrates marked improvement in high dose **(e)** than lower one evidenced by pyknotic nuclei and activated each of stellate and *Kupffer* cells as showing in section **(f)**. (TEM 6000X).

Furthermore, the highly reactive per oxynitrite that is usually produced by the reaction between nitric oxide (NO) and superoxide (O_2) has been demonstrated to be involved in cellular[33]. Our study illustrated that WGO oil- induced a reduction in the level of NO when compared with anti-TB intoxicated drugs group. The NF-κB pathway has emerged as one of the best-characterized signaling pathways in the pathogenesis in many diseases, including liver diseases, inflammatory disorders and tumor development[34]. Many reports have demonstrated that NF-κB is considered to play a major role in HSCs activation leading to fibrogenesis[35,10] which agrrement with current histological and E.M findings. In the present study, anti-TB intoxicated drugs induce NF-kB expression indicating that anti-TB intoxicated drugs can induce the expression of inflammatory genes. Meanwhile, WGO inhibits the activation of NF-κB. IL-10, one of the major pro-inflammatory cytokines, with important roles in counterbalancing hyperactive immune responses to protect the body from excessive cell and organ damage.[36] IL-10-deficient shows higher liver fibrosis with larger inflammatory infiltration[37] this was reinforced by histological examination. In thioacetamide-induced liver fibrosis, IL-10 gene therapy reverts hepatic fibrosis and prevents cell apoptosis after fibrosis has already been established, suggesting a therapeutic potential for treatment with IL-10[38]. Consistent with those aspects, the current results relived that, WGO improves the level of IL-10 and protects the liver from fibrosis which was occurred when the hepatic level of MMP-2, MMP-9, and TGF-β1 elevated, that increases the production of matrix glycoprotein and glycoglycans and led to the activation and proliferates stellate cell and transforming to myofibroblasts that performing to tissue fibrosis.[11] The present study revealed that the hepatoprotective effect of WGO may be attributed to the high amount of unsaturated fatty acids including linoleic acid C18:2 (omega 6), linolenic acid C18:3 (omega 3) which stimulate fatty acid oxidation and high content of Vit. E which acts as a strong antioxidant.[12,14] This result agrees with[39] Who reported that WGO intake results in a rapid increase in the content of Vit. E in the liver and gives powerful antioxidant protection to liver tissues. WGO is also rich in unsaturated fatty acids, mainly oleic, linoleic, and α-linolenic acids that may exert inhibition of oxidative stress[13]. The previous investigation showed that WGO is one of the most important protective factors for hepatotoxicity that works efficiently against disease infections. The daily dose of WGO is thought to be the ideal way to enrich the diet with a powerful natural antioxidant. In addition, it contains vitamin E, which protects the liver through its unique properties. Finally; the present study confirmed that WGO possess antioxidant and anti-inflammatory properties. Consequently, it leads to the improvement referred to current investigation on the biochemical, histological and ultra-structure levels.

CONCLUSION

WGO has a hepatoprotective effect against the toxicity induced by combined therapy of INH+RIF in the experimental animal model of hepatotoxicity.

Abbreviation

WGO	Wheat germ oil
INH	Isoniazid
RIF	Rifampicin
TB	Tuberculosis
PZA	Pyrazinamide
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
T. Bil	Total bilirubin
TP	Total protein
ALB	Albumin
Vit. E	Vitamin E
IL-10	Interleukin -10
NF-Kb	Necrosis factor kappa B
GSH	Reducing glutathione
MDA	Malonaldehyde
NO	Nitric oxide
ROS	Reactive oxygen species
CYP 450	cytochrome P450
HSCs	Hepatic Stella cell

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