The Preventative Effect of White Radish Roots Against Aflatoxins-Induced Oxidative Stress in Male Rats

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ABSTRACT

Aflatoxins (AFs) are deleterious components that bring about cancerogenic effects on tissues. It can produce oxidative stress provocation cells into evil. However, bioactive compounds from natural plant species might prevent toxic substances produced by a fungus, from causing sick effects. Radish white roots (RWRs), a source of active substances, have favorable indispensable effects and are the most known consuming part and utilized as vegetables by multiple communities. Total flavonoids and phenolics compounds and the total antioxidant capacity were identified in WRRs. The experiment was designed by using five rat groups to evaluate the potential WRRs effect to reduce the deleterious oxidative stress effects of AFs. The negative rat group was orally given distilled water and kept as a normal group. Treated groups were orally given aflatoxins (AFB_1) without RWRs (Positive group) and with RWRs in diets (treated groups). Diet fortification by 10, 15, and 20% of RWRs of AFB_1-treated rats exhibited an augmentation in body weight, and food efficiency ratio, as well as improvements in liver enzymes, kidney function, and serum lipids, in comparison to AFs treated-group only. In addition, the reduction in serum levels of albumin, total protein, globulin, and activities of antioxidant enzymes by AFs were ameliorated by RWRs attendance in diets. Histopathological inspection of the liver from AFs-treated rats alone showed multiple tissue alterations described by massive intrahepatic necrosis, fibrous tissue proliferation, mononuclear inflammatory cell infiltration, degradative hepatocytes, and macrovesicular steatosis.

Nevertheless, in AFs-treated groups fed on supplemented diets with RWRs, liver hepatocytes appeared to gradually improve with increasing the level of RWRs added, to be approximately normal, especially with higher levels (20%). In conclusion, results discovered that RWRs reduced the toxic effect of aflatoxins. Accordingly, regular intake of RWRs may be beneficial to prevent the consequences of injury from toxicity by AFs. This action may be due to RWRs wide range of content of flavonoids and phenolics as bioactive compounds.

Keywords: Liver Toxicity; Aflatoxins; Radish White Roots; Raphanus sativus L; Liver Functions; Kidney Functions; Oxidative Stress; Antioxidant Enzymes

1. INTRODUCTION

Most plants contain bioavailable compound components and are a critical curative proxy in medicine due to their substitute for chemical substances. Radish (*Raphanus subsp. sativus L*.) is a plant pertinence to the species *Raphanus* of the *Brassicaceae* family (**Nishio, 2017**). Radish is regarded as a root because its structure is the part of the stem of an embryo plant beneath the stalk leaves, its appearance is near to the real roots, and can store starch and other components (**Radovich, 2018**). Radish roots (RRs) have different surface colors, such as white, green, purple, red and black, although their pulp is white in most world regions (**Nishio, 2017**). Although the stalk and leaves are used as vegetables, the radish roots are the most consumed part. The radish roots are commonly eaten in fresh raw salads, cooked, salted with other types of vegetables, or dried or canned pickles in Asia (**Manivanan *et al.*, 2019**).

Several previous studies indicated the use of radish roots and leave as a useful source of biologically active components with medical and health involvement for cardiometabolic disturbance (**Manivanan *et al.*, 2019**). In addition, they're used as an antioxidant and anticarcinogenic (**Hecht *et al.*, 2000**), antimutagenic (**Nakamura *et al.*, 2001**), and antimicrobial

(**Aruna *et al.*, 2012**). Also, in traditional medicine, the roots are used to treat syphilis disease and bladder soreness, and the leaf juice is applied as a diuretic and laxative (**Ahmad *et al.*, 2012**). The biological effects of radish roots may be due to the existence of multiple classes of phytochemical constituents such as flavonoids, phenols, and alkaloids (**Shin *et al.*, 2015**). Flavonoid compounds are the most critical of these phytochemicals and are known for their health, nutritional, and pharmaceutical characteristics (**Ngoc *et al.*, 2017**). Additionally, radish benefit derives from its nutritional valuable constituents such as low-fat, high-fiber, and rich sources of several essential minerals and vitamins (**Manchali *et al.*, 2012**). Aflatoxins (AFs) are the derivative substances formed during the metabolism of Aspergillus flavus and parasitic fungi and are regarded as dangerous and toxic compounds that contaminate food and food products (**Bennett and Klich, 2003**). AFs are extremely the highest perilous health adversary for human and animal, they can motivate mutagenicity and carcinogenesis (**Shehata *et al.*, 2017**).

AFs are not just only carcinogenic substances (**Wangikar *et al.*, 2005**), but they are also related to multiple health problems such as causing developmental malformations, growth retardation, immunosuppress, hematologic disturbance, and hepatic and renal toxicity (**Fapohunda *et al.*, 2008**). The metabolization of AFs, especially AFB1in the liver by cytochrome P450 results in the production of reactive oxygen species (hydroxyl radical, hydrogen peroxide, and superoxide anion). And so, it attacks the cell membranes and soluble components, resulting in the deterioration of cell functions and cytolysis (**Lee *et al.*, 2005**).

Several food substances are susceptible to being contaminated by AFs fungi (**Abdel-Razek *et al.*, 2018**). However, cereals, legumes, and grains incorporated into human diets are food items susceptible to contamination by AFs (**Caloni and Cortinovis, 2011**). In comparison with other food

contaminants, AFs, especially aflatoxin B1, is the most pollutant causing chronic human diseases, due to the presence of mycotoxin in body fluids (**Ramalho *et al.*, 2018**). The existing study was directed to the estimation of the radish root content of total phenolic and flavonoid compounds, as well as the total antioxidant capacity was identified in WRRs. Also, to explore the potential protective effect of WRRs against AFs-induced oxidative stress in rats.

2. MATERIALS AND METHODS

White Radish Roots and Preparation of Dried Powder:

A Fresh White Radish (*Raphanus subsp. sativus* L.) was harvested from the farm field during the winter seasons of 2021-2022 at Al-Qalyubia Governorate, Egypt. The general shape of the whole plant is shown in Picture **1**. The plant was scrubbed and cleaned under running tap water and all green leaves were separated. Then, the root were cut into thin slices (Picture **2**) and dried in a drying oven vacuum at 40 C° (Picture **3**). Thereafter, the dried slices of roots were milled and sifted via a 40 mesh sieve to get a roots powder. Then, the final powder was packaged in an enclosed bag and stored in the refrigerator at 5 ^{o}C till use.

Picture 1: Shape of Radish (*Raphanus subsp. Sativus*

Picture 2: Cleaned and Sliced

Picture 3: Dried Radish

Chemicals, Reagents and Aflatoxins B_1:

Chemicals and reagents for chemical and biochemical analysis were purchased from Sigma Chem., Co., Cairo, Egypt. AFB_1 was obtained as standard dry films from Agricul. Resea. Center (ARC), Giza, Egypt.

Components and Formularization of Rat-Diet (AIN-93M):

The fundamental components of the rat diet were getting from El-Gomhoriya Co., for Trad., Drugs and Chem., Egypt. While, soybean oil was derived from Agric. Res. Center, Giza, Egypt. Cornstarch and dextrinized cornstarch were derived from an Egyptian Manufacturer of Starch and Glucose Co. Fine white sucrose was purchased from a local trade market. Afterwards dietary components were formulated collectively to meet the desirable adequate dietary intake of rats as revealed by **Reeves *et al.*, (1993)** in Table**1**.

Table 1: Components of Rat-diet (AIN-93M) per 1 kg. Diet

Components	Amounts
Casein (85% protein)	140g
Cornstarch	465. 692 g
Dextrinized cornstarch	155 g
Sucrose	100g
Fiber	50g
Soybean oil	40g
Mineral mixture	35g
Vitamin mixture	10g
Choline chloride	2.5g
L-cysteine	1.8 g
Tert-butylhydroquinone	0.008g

Quantitative Determination of Total Flavonoids and Phenolics Compounds in WRRs:

The total content of flavonoid and phenolic compounds in WRRs was spectrophotometrically measured as described by

Kim *et al.*, (2016) and **Chun *et al.*, (2003)**, respectively. Quercetin and Gallic acid were used as a criterion for the standard curve of flavonoids and phenolics, respectively. The total content of flavonoids was stated as mg Quercetin equivalents per gram of sample (mg QE/g). The content of total phenolics was stated as mg Gallic Equivalent/gram of the sample.

Total Antioxidant of WRRs

The total antioxidant ability (TA) of WRRs samples was evaluated using the Trolox Equivalent Antioxidant Capability (TEAC) measured as mentioned by **Chakravartula *et al.*, (2021).** The results of TA were stated as an mM of Trolox equivalent antioxidant (TEA) / g of the sample.

Preparation of AFs Solution

The standard dry film of AFB_1 was used to provide an intentional concentration (ng mL^{-1}) by using acetonitrile: methanol (1:9) (V: V). Then, AF B_1 doses were dissolved in phosphate buffer saline and orally administered to rats daily at a dose of 850 ng/kg/b. wt. according to the description delineated by **Hussein *et al.*,** (**2019**).

Rats and Designed for Experimental Study

Fifty rats, Sprague Dawley weighing (180±5g), were attained from the Helwan Colony for Experimental Animals, Ministry of Health and Population, Egypt. After that, the rats were relocated to the experimental animal lab at the Faculty of Home Economics, Helwan Univ., and distributed into five equal groups in acrylic cages. Rats were maintained in a healthy environment regulated at 22±2 °C temperature, 12/12 hrs. of

light/dark cycle, and 50 ± 5% of humidity with the provision of water and food.

Following one week of the acclimatized in the lab surrounding, rat groups were named according to the type of treatment as follows:

Negative control group (G 1): Fed on the normal diet.

Untreated positive control group (G 2): Fed on the normal diet and treated orally with AFB_1.

A treated group at level 1 (G 3): Treated orally with AFB_1 and fed on the formulated diet with 10% of WRRs.

A treated group at Level 2 (G 4): Treated orally with AFB_1 and fed on the formulated diet with 15% of WRRs.

A treated group at Level 3 (G 5): Treated orally with AFB_1 and fed on the formulated diet with 20% of WRRs

Estimation of Food Intake, Body Weight Gain, and Feed Efficiency

The quantity of food intake every day (FI) by all rat groups was registered. The variance between the final (FBW) and the initial body weight (IBW) of the experimental period was used to calculate the gain in body weight (BWG) (FBW-IBW). The relative body weight gains (RBWG%) and feed efficiency (FER) were calculated using equations 1 and 2, respectively.

RBWG% = BWG/IBW X 100 (1)

FER= BWG (g/d)/FI (g/d) (2)

Preparation of Serum Samples

At the end of the sixth week of the experimental period, all rats were forbidden from food intake for 12 hr., except for water. Afterward, the rats were anesthetized, and the heart was perforated by drawing the blood samples with a 5 cm syringe, transferred into a centrifuge tube, and left to clot at lab temperature. Next, the clotted blood was centrifuged for 15 mins. at 4000 rpm to get serum samples. Separated serum

specimens were clearly taken by an automatic pipette, kept in the clean closed Eppendorf tubes, and retained at -20°C in a deep freeze once used for biochemical assay.

Biochemical Assay

Liver Enzymes

To investigate the effect of AFs and WRRs on liver function in rats, colorimetric ELISA-Elabscience Kits were used. Serum levels of AST and ALT enzymes were assessed as referred to by **Young (2000)**, and **Young, (1997)** for the assaying of serum level of ALP enzyme. Serum concentrations of Albumin (Alb) and total protein (TP) were analyzed as mentioned by **Buzanovskii, (2017)**, while the guidance of **Walker *et al.*, (1990)** was used for the assay of serum levels of globulin (Glb).

Kidney Functions

Serum concentrations of creatinine (Cr), urea nitrogen (BUN), and uric acid (UA) were measured according to the kit's instruction manual as mentioned by **Needleman *et al.*, (1992)**, **Mitrovic *et al.*, (2012)** and **Tietz *et al.*, (2005)**, respectively.

Serum Lipid Profile

Concentrations of serum triglycerides (TG), total lipids (TL), and total cholesterol (TC) were spectrophotometrically measured using EnzyChrom assay kit guidance as outlined by **Zhu *et al.*, (2000)**, **Lutzke and Brauler (1990)** and **Admundson and Zhou (1999)**, respectively.

Serum MDA Levels and Activities of SOD, CAT and GPx Enzymes

The effect of AFs and WRRs on lipid peroxidation (LP) and antioxidant activity (AA) in rats was tested. Serum levels of MDA and the activities of antioxidant enzymes (SOD, CAT, and GPx) were measured colorimetrically using Invitrogen kits. Serum MDA levels were determined as described by **Rio *et al.*,**

(2005), while instructions by **Cristiana *et al.*, (2014), Glorieux and Calderon (2017),** and **Chu *et al.*, (1993)** were utilized for the assay of serum activities of the above-tested enzymes, respectively.

Histopathological Screening of Liver:

The histopathological screening process for the liver tissues of all rats was carried out as referred procedures by **Bancroft and Gamble, (2002)**. Briefly, liver samples were carefully washed in an isotonic solution, dried on a filter paper, and immersed in buffer formalin (10%). Afterward, the fixed liver specimens were dehydrated in graded ethyl alcohol from 50 to 100%. Subsequently, specimens were cleared by Xylol, immersed in paraffin bulk, sliced to 4-6 μm thickness and colored with Hematoxylin (HX) and eosin (E) for the inspection.

Statistical Evaluation of Data

Results of chemicals and biochemical inspection were statistically analyzed using the SPSS computerized statistics program for Windows, version 22.0. Descriptive data were compared using a one-way variance (ANOVA) test. Comparison of differentiate among groups was stated as Mean ± Standard Division (SD) at $p<0.05$.

3. RESULTS

The results of the total contents of flavonoids (TFs) and phenolics (TPs), and total antioxidants (TAs) of fresh WHRs are shown in Figure 1. The quantitative estimate revealed that TFs and TPs content as well as TAs were 40.88±2.01mgQE/g, 111.53±1.30 GAE/g, and 40.88±1.25 mM TEA/g, respectively.

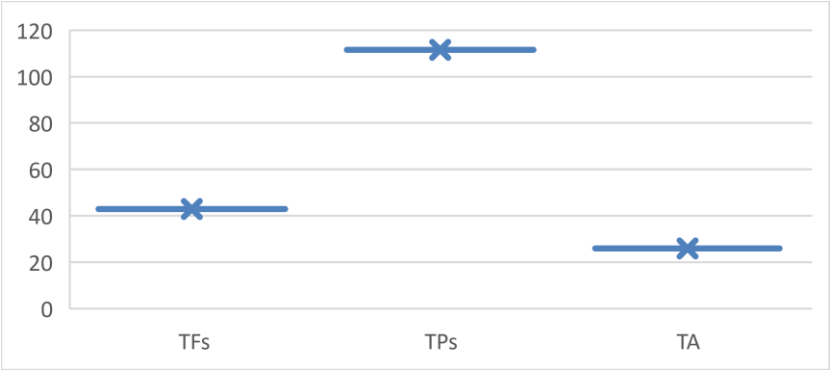


Figure 1: Total of Flavonoids (TFs) and Phenolics (TPs) Content, and Total Antioxidants (TAs)

The effect of oral administration of AFs alone and with feeding supplemented diet with the different levels (10, 15, and 20%) of WRRs on FBW, BWG, FI, RBWG (%), and FE is recorded in Table **3**. Rats treated with AFs alone (positive control group) had a significant ($p<0.05$) lower in FBW, BWG, FI, RBWG (%) and FE, comparable to the negative control group. Co-combined oral administration of AFS with the supplemented diets with the different levels of WRRs results in a significant augmentation in FBW, BWG, FI, RBWG (%), and FE, compared to the rat-group who received AFs orally and fed on the basal diet alone. It was also noted that the development rates in FBW, BWG, FI, RBWG (%) and FE increased with the rise in WRRS in diets.

The attained results in Table **4** exhibit the effect of provision AFs orally without or with feeding on the complemented diet with WRRS on liver function in rat groups. Delimited results showed that the AFs-treated group feeding on the basal diet alone had a significant ($P<0.05$) increment in activities of liver enzymes (AST, ALT, and ALP), and a decrease in the serum levels of Alb, TP, and Glb, compared to a normal control group. However, combining a supplemented diet with the different levels of WRRS with the administration of AFs orally, results in a significant ($p<0.05$) lowering in the liver enzymes and

increasing serum levels of Alb, TP, and Glb in treated rat groups, compared with the positive control group. As exhibited, there is better improvement in liver function by the enhancement in the tested parameters with increasing levels of WRRs taken in the diet.

Table 3: Effect of diet-supplemented with WRRs on BWG, FI, RBWG (%) and FE in AFs treated rats

Groups / Parameters	Negative group (G1)	Positive group (G2)	Treated rats with the WRRs at a level of:		
			10% (G3)	15% (G4)	20% (G5)
IBW (g)	180.30±1.30	180.40±1.10	181 ± 1.79	180.35±1.11	181±1.22
FBW (g)	236.50±2.70^{a}	218.00±2.20^{d}	226.20±1.80^{c}	230.50±1.44^{b}	236.48±.12^{a}
BWG (g)	56.30±2.74^{a}	37.60± 2.33^{e}	45.20± 3.54^{d}	50.15± 2.63^{c}	55.48±2.41^{b}
FI (g/d)	16.81±0.23^{a}	15.81±0.11^{d}	16.08±0.28^{c}	16.07± 0.16^{c}	16.15±0.14^{b}
RBWG (%)	31.23±0.98^{a}	20.84±1.03^{e}	24.98±1.03^{d}	27.81±1.13^{c}	30.65±1.11^{b}
FE	0.07±0.03^{a}	0.05±0.02^{c}	0.06±0.04^{b}	0.07±0.02^{b}	0.08±0.04^{a}

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

Table 4: Effect of the supplemented diet with WRRs on activities of liver enzymes (AST, ALT and ALP) and serum levels of Alb and TP and Glb in AFs treated rats.

Groups / Parameters	Negative group (G1)	Positive group (G2)	Treated rats with the WRRs at a level of:		
			10% (G3)	15% (G4)	20% (G5)
AST (u/ml)	28.27±1.21^{e}	47.41±1.39^{a}	42.60±1.27^{b}	35.43±1.44^{c}	30.25±2.04^{d}
ALT (u/ml)	23.01±0.19^{e}	51.54±0.54^{a}	41.35±0.37^{b}	34.50±0.36^{c}	32.29±0.34^{d}
ALP (u/ml)	47.41±0.21^{e}	83.24±0.57^{a}	66.20±0.31^{b}	55.12±0.37^{c}	49.64±0.50^{d}
Alb (g/dl)	5.12±0.21^{a}	3.27±0.12^{d}	4.28±0.16^{c}	4.87±0.23^{b}	4.96±0.12^{b}
TP (g/dl)	8.92±0.32^{a}	3.35±0.12^{d}	6.32±0.22^{c}	7.55±0.14^{b}	7.57±0.13^{b}
Glb (g/dl)	4.43±1.20^{a}	1.63±0.52^{d}	3.44±1.04^{c}	3.68±1.01^{c}	4.14±0.21^{b}

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

The end outcome in Table **5** shows that rats treated with AFs alone had a significant ($p < 0.05$) increase in serum levels of Cr, BUN and UA, as compared to that of the negative control rats. Feeding rats, the supplemented diet with WRRs at the three different levels for protection against AFs toxicity generated almost a significant ($p<0.05$) decrease in the serum Cr, BUN and UA levels. As a performance, the superior improvement in the serum levels of the tested variables were showed in the treated group with the highest level (20%) of WRRS, which was near the levels of normal rats.

Table 5: Effect of the supplemented diet with WRRs on serum levels of Cr and BUN and UA in AFs treated rats

Groups / Parameters	Negative group (G1)	Positive group (G2)	Treated rats with the WRRs at a level of:		
			10% (G3)	15% (G4)	20% (G5)
Cr (mg/dl)	1.41±0.77^{d}	3.57±0.20^{a}	2.70±0.15^{b}	1.97±0.18^{c}	1.34±0.17^{d}
BUN(mg/dl)	31.63±1.65^{d}	70.34±1.67^{a}	52.87±1.97^{b}	45.37±1.82^{c}	31.03±1.24^{d}
UA (mg/dl)	1.40±0.27^{d}	3.51±0.43^{a}	2.89±0.17^{b}	1.65±0.32^{c}	1.35±0.49^{d}

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

Table 6: Effect of the supplemented diet with WRRs on serum levels of TG, TL, and TC in AFs treated rats

Groups / Parameters	Negative group (G1)	Positive group (G2)	Treated rats with the WRRs at a level of:		
			10% (G3)	15% (G4)	20% (G5)
TG (mg/dl)	84.77±2.92^{e}	259.93±1.9^{a}	124.97±1.30^{b}	89.80±0.85^{d}	94.36±0.72^{c}
TL (mg/dl)	208.23±1.38^{c}	474.26±0.77^{a}	307.43±2.05^{b}	208.56±1.54^{c}	208.44±1.11^{c}
TC (mg/dl)	78.03±0.80^{c}	102.53±1.11^{a}	89.47±1.7^{b}	78.04±0.73^{c}	78.87±0.92^{c}

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

Results in Table **6** demonstrate the effect of orally giving of AFs and feeding on a supplemented diet with 10, 15 and 20%of WRRs on the serum levels of TG, TL, and TC in rat groups. In comparison to the negative control group, oral administration of AFs induced a significant increase in serum concentrations of

TG, TL, and TC. However, in comparison to the positive control group, feeding on the supplemented diet with WRRs resulted in significantly lower serum levels of TG, TL and TC. A better improvement in serum levels of TG, TL and TC was discovered with increasing levels of WRRs in treated rats.

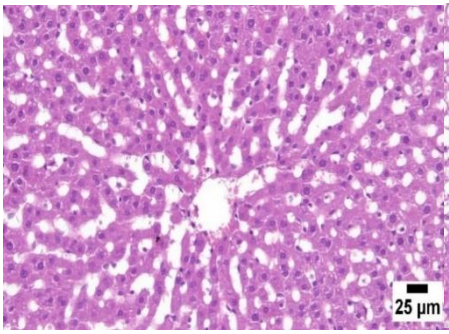
Table **7** represents the effect of WRRs on the protection of rats from an oxidative imbalance resulting from toxicity caused by AFs. Serum levels of MDA and the activities of antioxidant enzymes (SOD, CAT, and GPx) were used as indicators of this effect. Our results revealed that oral administration of AFs induced a significant ($p<0.05$) rise in serum MDA level, and lower activity of SOD, CAT, and GPx enzymes, in comparison to the negative control group. Combining the different levels of WRRs in the diet with the oral administration of AFs, significantly reduced serum levels of MDA and increased the activity of the mentioned antioxidant enzymes, compared to positive control rats fed on the basal diet alone. Also, the results revealed that an increasing added level of WRRs increased added protection for treated rats as shown by the eminent amelioration of the tested parameters.

Table 7: Effect of the supplemented diet with WRRs on serum levels of MDA and activities of SOD, CAT, and GPx enzymes in AFs treated rats

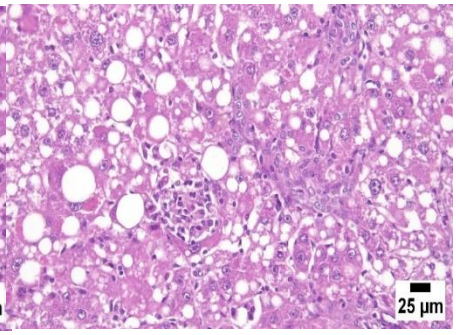
Groups / Parameters	Negative group (G1)	Positive group (G2)	Treated rats with the WRRs at a level of:		
			10% (G3)	15% (G4)	20% (G5)
MDA (mmol/L)	0.45 ± 0.14^{e}	1.54 ± 0.12^{a}	0.87 ± 0.04^{b}	0.61 ± 0.19^{c}	0.54 ± 0.21^{d}
SOD (mmol/L)	34.10 ± 1.10^{a}	14.71 ± 1.20^{e}	26.55 ± 0.81^{d}	30.39 ± 1.30^{c}	32.73 ± 1.12^{b}
CAT (mmol/dl)	79.84 ± 0.52^{a}	32.94 ± 0.57^{d}	68.13 ± 0.61^{c}	73.51 ± 0.52^{b}	79.86 ± 0.35^{a}
GPx (mmol/dl)	35.65 ± 0.33^{a}	10.59 ± 0.40^{d}	26.75 ± 0.33^{c}	32.49 ± 0.29^{b}	35.69 ± 0.21^{a}

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

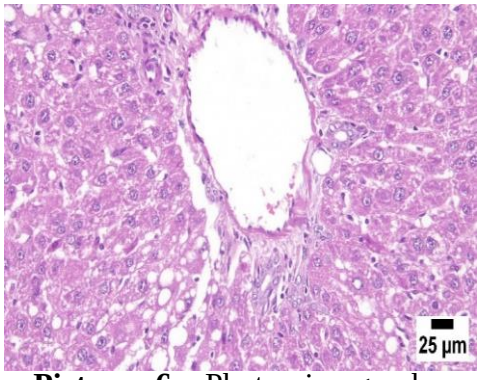
Microscopic investigation of liver sections of the negative control group (normal rats) discovered a normal histological arrangement without any pathological amendment (Picture **4**). On contrary, liver sections from AFs-treated rats (positive control group) and fed a normal diet pronounced several tissue modifications pronounced by massive hepatocellular necrosis, fibrous tissue proliferation with mononuclear inflammatory cell infiltration, degradative changes of hepatocytes and steatohepatitis (Picture **5**). On the other hand, liver sections from AFs-treated rats and fed on supplemented diet with 10% of WRRs exhibited mild portal fibroplasia, mononuclear inflammatory cell infiltrate and moderate steatohepatitis, (Picture **6**). Nevertheless, liver sections from AFs-treated rats and fed on supplemented diet with 15 and 20% of WRRs presented slight steatohepatitis with apparently normal hepatocytes as demonstrated in Pictures **7** and **8**, respectively.



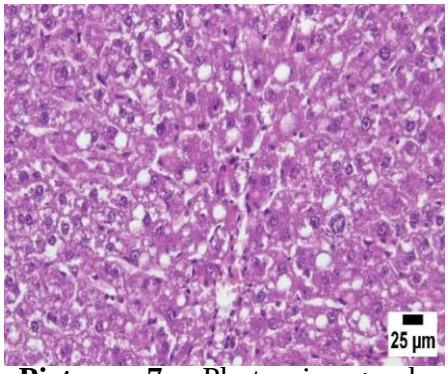
Picture 4: Photomicrograph of liver section from negative control group showing normal hepatocytes with the normal histological arrangement (H&E).



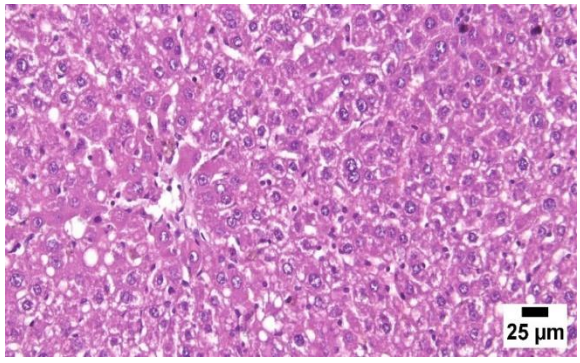
Picture 5: Photomicrograph of liver section from positive control group showing massive hepatocellular necrosis, fibrous tissue proliferation with mononuclear inflammatory cell infiltration, degradative changes of hepatocytes, and steatohepatitis (H&E).



Picture 6: Photomicrograph from AFs-treated rats and fed on supplemented diet with 10% of WRRs showing mild portal fibroplasia, mononuclear inflammatory cell infiltrate, and moderate steatohepatitis (H&E).



Picture 7: Photomicrograph from AFs-treated rats and fed on supplemented diet with 15% of WRRs showing slight steatohepatitis with apparently normal hepatocytes(H&E).



Picture 8: Photomicrograph from AFs-treated rats and fed on supplemented diet with 20% of WRRs showing slight steatohepatitis with apparently normal hepatocytes(H&E).

4. DISCUSSIONS

Aflatoxins (AFs) are an input of mycotoxins related to the metabolites of fungal produced by the Aspergillus flavus and parasitic fungi in foods and their products. AFs are responsible

for occurring toxicity and many biological changes and carcinogenesis in both humans and animals. Therefore, the existing study was directed to estimate the conceivable protective effect of WRRs against AF-induced oxidative stress in rats. The results of the biochemical measurements indicated an increase in the production of AST, ALT and ALP enzymes, as well a reduction in total protein (TP) globulin (Gbl) levels. Histopathological investigation of liver tissues also showed several pathological changes that have been clarified previously, which suggest the attendance of liver injury. In addition to the elevation in serum levels of Cr, BUN and UA which points out the presence of kidney damage. The altitude serum levels of triglycerides (TG), total lipids (TL), and total cholesterol (TC) and MDA, and the reduction in the activities of SOD, CAT and GPx enzymes indicate inducing oxidative stress by oral administration of AFs in rats. The obtained results were in accordance with **Abdel-Wahhab *et al.*, (2010)** who noted the reduction in serum protein and its portions, and significant changes in all serum biochemical variables associated with intense histological alteration of the liver tissue in AFs-treated rats. Also, the present results agreed with **Hathout *et al.*, (2011)** and **Badr and Naeem (2019)** who exhibited that AFs-treated rats have a significant reduction in FBW, BWG, FI and FE and serum levels of Glb, TP, Alb, as well activities of SOD, GSH and CAT enzymes. However, there is a significant increase in liver enzymes (AST, ALT, ALT), TG and TC and MDA levels. Recently, **Hatipoğlu and Keskin (2022)** showed significantly lower BW and higher serum BUN, UA, and Cr in the AF treatment group than in the normal group.

Mostly, AFs are subjected to bio-alteration in the hepatic cells in two stages. In stage 1, reactions are usually oxidation, and fungal reduction, and/or hydrolyzed. After that, AFs are transformed to the reactive epoxied creator (8,9-epoxide), which by cytochrome P450 enzymes is able to bind to proteins and DNA (**Kumar, 2018**). In stage 2, reactions depend on

integrated cellular components, involving the metabolism of glutathione, glucuronide, and sulfate, as well as amino acid conjugation (**Yilmaz *et al.*, 2017**). In addition, the reactive aflatoxin epoxied tie for the N7 place of guanines (**Santini and Ritieni 2013**). Thus, aflatoxin leads to oxidative stress that acts a main role in aflatoxicosis due to the immediate effect of AFs either independently or through their metabolites. Metabolized aflatoxin increases free radicals and lipid peroxides production, causing cell injury (**Josse *et al.*, 2012**). CYP450 enzymes create superoxide hydrogen peroxide (H_2O_2) as an intermediate component which can attack cell membranes and their soluble components, after all, leading to the deterioration of cell functions and cytolysis and cell death, and other cell disorders (**Shimamoto, 2013**). The most target organs for ROS formed from AFB1 are the liver and kidney as shown by the significant increase in liver enzymes, and lipid peroxidation in the hepatic and renal tissues, with a notable decline in total antioxidant capability in rats (**El-Nekeety *et al.*, 2011**). Therefore, the oxidative damage caused by AF is considered to be the main mechanism leading to hepatonephro-toxicity (**Preetha *et al.*, 2006**).

Rats fed with an AFs-polluted diet suffer from oxidative stress as indicated by the considerable augmentation of lipid peroxidation and the decrease of antioxidant enzymes (SOD and GSH-Px) (**Kanbur *et al.*, 2011**). Free radicals produced as a result of AFs toxicity can induce protein damage. In addition, AFs can prohibit some proteolytic enzymes accountable for protein synthesis, and thereby, inhibit protein synthesis, altering serum protein composition (**Peng *et al.*, 2007**). This explains the reduction in serum levels of TP, ALB, and Glb in AFs-intoxicated rats. The effect of AFs intoxication on the kidney may be accompanied by AF's oxidative stress. Therefore, oxidative stress is a considerable risk factor for AF-induced nephrotoxicity that is principal to the degeneration of the antioxidant system and oxidative detriment in the kidneys

(**Abdel-Hamid and Firgany, 2015**). After that, it leads to the constriction of renal tubules, followed by a decline in the nephrotic capillary filtration stage. In addition, the forming of several vasculomotor intermediaries can immediately affect kidney function due to a reduced nephrotic filtration rate (**Garcia-Cohen *et al.*, 2000**). Lipids play a critical role in metabolic pathways and the most important clinical and physiological lipids are TG, TC and phospholipids (**Rolim *et al.*, 2015**). Disruption in the metabolic process of these lipids and lipoproteins results in dyslipidemia characterized by hyperlipidemia, hypertriglyceridemia, lower HDL-c, and raised LDL-c (**Katsiki *et al.*, 2016**). AFB1 induced alteration in the levels of blood and liver (**El-Nekeety *et al.*, 2014**). Also, **Rotimi *et al.*, (2017)** demonstrated that AFB1 markedly increased plasma TG, TC, and TL. The results may be related to that AFB1-induced hepatic deterioration and result in the expression of metabolizing gene alteration of lipids and lipoproteins with concomitant dyslipidemia (**Lu *et al.*, 2013**). Radish root exhibited significant amounts of total flavonoids (TF) and phenolic (TP), as well as total antioxidants (TA). Although there is a difference in the quantities of TF, TP and TA, several studies (**Kim *et al.*, 2013**, **Eugenio *et al.*, 2017** and **Noman *et al.*, 2021**) agreed that radish have biological and antioxidant properties. The present variations may be due to several factors, including soil type, irrigation source, harvest time, post-harvest processing, storage conditions, and method and chemo-diversity of the plant.

Radish is a cheap vegetable widespread in all governors of Egypt. Therefore, the object of the current study was to identify the potential protective effect of radish against the deterioration effect from AFs as a food poisonous. The co-combination of complemented diet by the different levels (10, 15 and 20%) of WRRs and oral administration of AFs caused a significant result in an improvement in the rate of FI, FBW, BWG and RBWG%. In addition, the amelioration of the tested biochemical variables

and the development in liver tissues as a response to protection are contrary to the toxic effect of aflatoxin. Thus, Radish has the ability to protect rats from hepatic nephrotoxicity, dyslipidemia and an imbalanced antioxidant system. The enhanced effect of WRRS on FI and BWG might be due to the hepatic and renal function improvement which were shown in the consequent results. The current results concur with the revealed results of **Salah-Abbès *et al.*, (2008)** who indicated radish roots (RRs) extract proved precautionary effects against Zen intoxication and oxidative stress in rats. They confirmed that by the improvement in biochemical variables, and the histological anatomy of the liver and kidney tissues. Also, the hepatoprotective belongings of the RRs extract against CCl_4 have been demonstrated by **Lee *et al.*, (2012)** who showed that radish markedly decreased the raised levels of liver enzyme. **Jin *and* Kyung (2001)** reported that Radish is a rich source of flavonoids and vitamin C, and feeding rats with radish powder significantly increased CAT, SOD and GPx activities in red blood cells and the liver. In addition, the significant decreased the serum lipid levels by increasing the secretion of TL, TG, and TC in rats fecal. Recently, **Hwang *et al.*, (2022)** demonstrated that Radish extract significantly improved the histological textures of liver tissue, markedly diminished the levels of ALT, AST, and MDA, and increased the activities of GSH, SOD and CAT enzymes in the Acetaminophen-induced hepatic injury in mice. According to the study by **Lee, *et al.*, (2012)** which showed that radish extract regulated the manifestation of CYP2E1, CYP450, Heme oxygenase-1 and nuclear factor Nrf-2. Therefore, the potential mechanism with regard to the hepatoprotective effects pronounced by radish might be due to the rehabilitation of the Nrf-2/HO-1 antioxidant pathway.

Al-Timimi *et al.*, (2019) discovered that radish root juice (RRJ) caused a significant reduction in BUN, serum Cr and UA in rats with nephrotoxicity. **Aziz and Hassan (2020)** also

indicated that RRJ significantly improved kidney function against urolithiasis induced by ethylene glycol. Recently, **Mushtaqa *et al.*, (2022)** confirmed that RRJ substantially lowers serum levels of Cr and UA and UN with the enhancement activities of SOD, Gpx, GSH and CAT enzymes in rats with chronic nephritic disease (CND) caused by ethanol. Accordingly, RR has powerful antioxidant properties against CND due to its content of 1,1-diphenyl-2-picrylhydrazyl.

Earlier research by **Satoh *et al.*, (1993)** reported that mice treated with RRs alone had a significant reduction in serum TG and TC levels due to its higher content of a particular protein which prevents the intestinal absorption of bile acids and cholesterol. Also, radish is rich in selenium, essential for T3 production (**Pedrero *et al.*, 2006**), which furthermore has an essential function in the upkeep of lipid biotransformation (**Dhingra and Bansal, 2006**). Therefore, **Suh *et al.*, (2006)** proved that radish extract has anti-restenosis and antiatrterosclerosis activities and, therefore, may protect against the progress of vascular and heart diseases.

Manivanan *et al.*, (2019) suggested the beneficial use of radish roots as a neutral source of bioavailable components which have health involvement in metabolic disturbance and antioxidant disorders. Radishes have natural antioxidant properties regard to the existence of multiple phytoconstituents such as flavonoids, alkaloids, and phenolic components **(Shin *et al.*, 2015).** The flavonoid components possess the capability to diminish the production of ROS, suppress the synthesis of protein, DNA and the apoptosis caused by AFs and have scavenging properties as indicated by inhibited production of NO production (**Guerra *et al.*, 2005**). Flavonoids are the generality substantial of these phytochemicals due to their nutritional and medicinal properties (**Ngoc *et al.*, 2017**). **Lugasi *et al.*, (2001)** confirmed the antioxidant properties of RRs extract, in vitro. Also, **Sipose *et al.*, (2002)** proved that RRs have a suppressant effect on membrane alteration and helpfully

affect the natural scavenging action of colon mucosa and protect the cell membranes from lipid peroxidation as a consequence of a high-fat diet in rats. **Takya *et al.*, (2003)** discovered that radish extracts have antioxidant enzyme activities such as glutathione reductase (**Vitoria *et al.*, 2001**), superoxide dismutase (**Jin and Kyung 2001**), and peroxides (**Wang *et al.*, 2004**). **Lee, *et al.*, (2012)** enzyme extract of RR effectively provided defense against membrane instability and reduced the outflow of AST and ALT. In addition to **You, *et al.*, (2015)** reported that isolated bioavailable components from RR, such as 3-(E)-(methylthio) -methylene-2-pyrrolidinethione-methylene-2-pyrroli-dinethione and indole-3-carbinol-indole-3-carbinol, 3-[ethoxy- (methylthio) methyl]-pyrrolidinethione significantly decreased the intensity sever of hepatostetosis disease in rat models.

5. CONCLUSION

In conclusion, the attained results in the current study obviously stated a protective effect of white Radish roots (WRRs) on Aflatoxins (AFs) oxidative stress. WRRs actively protect from AFS-induced oxidative stress by diminishing serum levels of MDA and augmenting activities of antioxidant enzymes, which may constitute a derivative safeguarding actionable against oxidative stresses. In addition to the alleviation effect of WRRS on augmented hepatic and renal functions and a serum lipid profile. WRRs, through their higher content of flavonoids, phenolics, and total antioxidants ability, inhibits the toxicity effect of AFs and can be considered as an active food against oxidative stress caused by AFs toxicity.

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