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Research Article
Counteractive Role of White Pepper Extracts for Oxidative Stress and Hepatotoxicity Induced by Aflatoxin B_1 in Rats

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Abstract

Background and Objective: Aflatoxin B_1 (AFB_1), a pre-carcinogenic and toxic compound which contaminates foodstuffs and edible tissues, is associated with oxidative stress and hepatotoxicity. This investigation aimed to assess the counteractive role of ethanol (EWP), petroleum ether (PWP) and n-hexane (HWP) white pepper extracts for oxidative stress and hepatotoxicity induced by AFB_1 in a rat model. **Materials and Methods:** Concentrated white pepper extracts (WPEs) estimated for total phenolic, total flavonoids, anti-oxidant and anti-fungal activities. Otherwise, the fatty acids composition of white pepper was analyzed. Forty-eight male albino rats were divided into 8 groups, negative and positive AFB_1 groups and the other 6 groups were treated to evaluate the WPEs biological effects either in the AFB_1 presence or absence. **Results:** The results elucidated that WPEs suppressed both the raising of aminotransferases enzymes (alanine and aspartate) and alkaline phosphatase and the reduction of total protein. The WPEs combat the negative impact of AFB_1 on kidney functions and alleviated AFB_1 mediated oxidative stress either in plasma or liver. Also, it relieved the AFB_1 mediated lipid disturbance and hemoglobin reduction and exhibited antioxidant and antifungal activities. **Conclusion:** It was concluded that the extracts gave a counteractive role for oxidative stress which support the hepatotoxicity induced by AFB_1 presence.

Key words: Aflatoxin B_1, anti-fungal activity, hepatotoxicity, white pepper extracts, oxidative stress, antifungal activities

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aflatoxins, the secondary metabolites of fungi produced by *Aspergillus flavus* and *A. parasiticus*, are considered dangerous and toxic compounds (especially AFB_1) which contaminate foodstuffs[1]. Since cereals and food grains included in most human diets are susceptible for the aflatoxins contamination[2,3]. Aflatoxins are not only carcinogenic and teratogenic substances[4], but also it associated with several health problems such as growth retardation, hematologic disorders, hepato and nephrotoxicity and immunosuppression[5-8]. The metabolism of AFB_1 in the liver results in reactive oxygen species (ROS) production including superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2) by cytochrome P450. The soluble cell compounds and membranes can be attacked by these reactive oxygen species which results in the impairment of cell functions and cytolysis[9].

Various phytochemicals compounds are reported to detoxifying aflatoxins[10]. White pepper (*Piper nigrum* family Piperaceae) is the sun-dried stony seeds of ripen pepper fruits, it was reported as rich in several phytochemicals compounds[11]. It is utilized as a common spice in food preparation. Otherwise, it was included in several traditional medicine recipes particularly in China and Southeast Asia countries[12]. Several health benefits for white pepper are demonstrated among its anti-oxidant, anti-inflammation, anti-atherogenic as well as anti-platelet aggregation activities[13,14]. Piperine, as a phytochemical component, reported inhibiting aflatoxin production by toxigenic fungi[15]. Also a modern study reported the piperine genetic impact on aflatoxigenic fungi strains which leads to suppress aflatoxin production[16]. According to extraction type, piperine reported higher in the non-polar extraction[17]. Despite the microbial load and mycotoxin contamination on pepper fruits (black or white) were dealt with it in previous studies, while aflatoxin reduction by different WPEs types, also the oxidative stress of toxigenic fungi-producing strains in the presence of WPEs has not been evaluated.

The current research was designed to innovate a novel utilization of WPEs against the hazard biological effects occurred by AFB_1 and to evaluate the counteractive role of WPEs against the oxidative stress and hepatotoxicity induced by AFB_1 in biological systems. Otherwise, the changes in biochemical parameters and tissue enzymes system conjugated to utilize WPEs were also estimated to validate the safety application of WPEs in food products.

MATERIALS AND METHODS

The current study was carried out in the National Research Centre, Cairo, Egypt from January-June, 2018.

Materials

Plant materials: White pepper was purchased from the local market, sun-dried then ground using an electric grinder and directly extracted.

Animals: Male albino rats of 125.27±7.52 g as (Mean±SD) were obtained from the animal house of the National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel cages at room temperature. Water and food had been given *ad-libitum*.

Diets: Balanced diet was prepared to contain 10% protein supplemented from casein, 10% corn oil, 10% sucrose, 60.5% maize starch, 5% fiber, 3.5% salt mixture and 1% vitamin mixture provided by the AIN-93 formulation[18]. The oil soluble vitamins had given weekly to rats separately from the diet.

Methods

Extracts preparation: The EWP, PWP and HWP extracts were prepared using ethanol, petroleum ether 40/60 and n-hexane, respectively. The solvent was added to extract the fine powder (4:1 w/v). The mixture was stirred using an overhead ultrasonic probe (30°C/45 min), filtered through Whatman No. 4 filter paper for separating the extract. The filtrate was concentrated to 25% (v/v) at 40°C using a rotary evaporator system. Nitrogen gas was utilized to complete solvent removing then stored at -20°C until further analysis and applications.

Determination of total phenolic and total flavonoid contents of WPEs: Total phenolic contents (TPC) and total flavonoid contents (TFC) were determined according to Singleton *et al.*[19] and Chang *et al.*[20], respectively. The TPC was expressed as mg Gallic acid equivalents (GAE) per g extract while the TFC were expressed as mg quercetin equivalent (QE) per g extract.

DPPH radical scavenging activities of WPEs: The free radical scavenging activity was measured by spectrophotometer using the DPPH method as described by Teke *et al.*[21].

ABTS cation decolonization assay of WPEs: The ability to scavenge free ABTS radicals was applied based on the protocol declared by Re *et al.*[22]. Results were expressed as µmol trolox equivalents (TE)/g sample.

Ferric reducing ability (FRAP) assay of WPEs: The FRAP assay was performed according to Hwang and Thi[23]. The results were expressed as µmol trolox equivalent (TE)/g sample.

Fatty acid composition evaluation: Fatty acid methyl esters of HWP extract were prepared according to AOAC[24] to be subjected to GLC analysis of fatty acids. Identification of the fatty acid methyl ester was carried out by direct comparison of retention times of each of the separated compounds with authentic standards and the results were reported as weight percentages after integration and calculation using Chem. Station (Agilent Technologies).

White pepper extracts effect on fungal growth: Yeast extract sucrose (YES) was utilized to evaluate fungal growth inhibition[25]. The inhibition was represented as a loss in dry weight of fungal growth in the presence of extracts against the control.

Agar diffusion test: Disk diffusion was evaluated according to the method described by Badr *et al.*[25]. The inhibition effect was determined as a ratio of the calculated as the equation follows:

$$\text{Growth inhibition (\%)} = \frac{Ac - At}{Ac} \times 100$$

Where:

Ac = Fungal diameter growth in control plate
At = Fungal diameter growth in treatment plate

Wells diffusion test: Well diffusion was evaluated according to the method described by Badr *et al.*[25]. Each well in a plate media was loaded by 250 µL of WPEs, one plate used for one fungus and one extract type, the plates were incubated at 25°C/5 days. The inhibition zone was determined as a ratio of decreasing in fungal growth compared to the control.

Determination of minimal fungicidal concentration of white pepper: The inhibition was used to determine the anti-fungal activity of the extracts against fungi as previously described by Shehata *et al.*[26]. The concentration required to give 50% inhibition of growth IC_{50} was calculated from the regression equation. Nystatin was used as a positive control.

Preparation of standards for aflatoxin: The dry-film standard of aflatoxin was applied to prepare a calculated concentration as ng mL^{-1} using a volume of methanol: acetonitrile (9:1).

Preparation of dosage form: White pepper extracts were dispersed separately in phosphate buffer saline (pH = 7.2) to be given orally to rats.

Design of the animal study: Forty-eight rats were used in the present study. After one week of acclimation, rats were divided into 8 groups (n = 6) as follows:

Group1 (G1) : Normal healthy group (Normal control)
Group 2 (G2) : Rats were treated orally with AFB_1
Group 3 (G3) : Rats were treated orally with AFB_1 and ethanol extract of white pepper (AFB_1+EWP)
Group 4 (G4) : Rats were treated orally with ethanol extract of white pepper (EWP)
Group 5 (G5) : Rats were treated orally with AFB_1 and petroleum ether extract of white pepper (AFB_1+PWP)
Group 6 (G6) : Rats were treated orally with petroleum ether extract of white pepper (PWP)
Group 7 (G7) : Rats were treated orally with AFB_1 and n-hexane extract of white pepper (AFB_1+HWP)
Group 8 (G8) : Rats were treated orally with n-hexane extract of white pepper (HWP)

The daily oral dose of aflatoxin B_1 adjusted at 850 ng kg^{-1} b.wt./day. Moreover, the daily oral dose of each extract of white pepper was 250 mg kg^{-1} b.wt./day. All rats were fed on a balanced diet all over the study period (4 weeks). During the experiment, body weight and food intake recorded weekly. However, the total food intake, body weight gain and food efficiency ratio calculated at the end of the experiment. Blood samples were collected from all rats after an overnight fast. A portion of the whole blood was analyzed for hemoglobin (Hb) concentration[27]. The remaining blood was centrifuged and the plasma was analyzed for plasma levels of total protein using the method of Rheinhold[28], the activities of aspartate transaminase (AST) and alanine transaminase (ALT) according to Reitman and Frankel[29] and alkaline phosphatase (ALP) according to Bessey *et al.*[30]. The levels of creatinine, urea, blood urea nitrogen (BUN), albumin and uric acid were determined depending on Larsen[31], Fawcett and Scott[32], Christian *et al.*[33], Doumas *et al.*[34] and Watts[35] in succession as indicators of kidneys function. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein

cholesterol (LDL-C) and triglycerides were determined according to Watson[36], Burstein *et al*.[37], Schriewer *et al*.[38] and Megraw *et al*.[39] in succession. Cholesterol/HDL-C ratio and VLDL-C were calculated. Plasma total anti-oxidant capacity was determined according to Koracevic *et al*.[40]. Plasma and liver malondialdehyde (MDA) was determined according to Ohkawa *et al*.[41]. Liver catalase, glutathione-s-transferase (GST) and superoxide dismutase (SOD) activities were determined according to Beers and Sizer[42], Habig *et al*.[43] and Nishikimi *et al*.[44] in succession. Rats were dissected and liver, kidney were immediately separated from each rat and weighed. This study has been carried out according to the Ethics Committee, National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Statistical analysis: The results were expressed as the Mean±SE. Results of animal experiments were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases; $p<0.05$ was used as the criterion of statistical significance.

RESULTS

The represented data in Table 1 declared that the petroleum ether extract of white pepper seeds recorded the highest values of anti-oxidant activities (AA) for the three applied assays (DPPH, ABTS and FRAP). Whereas, n-hexane extract of white pepper showed the lowest values of anti-oxidant activities. The TPC of WPEs was also ordered ascendingly as HWP<EWP<PWP. Moreover, the highest value of total flavonoid content was recorded by petroleum ether extract.

The fatty acid composition of white pepper (n-hexane extract) was illustrated in Table 2. As shown white pepper n-hexane extract recorded high content of oleic acid (13.22%) and linoleic acid (8.26%). The major saturated fatty acid was luric acid (17.69%). Erucic acid was not detected in the white pepper n-hexane extract.

Evaluation of minimal fungicidal concentration of white pepper extracts: The results presented in Table 3 showed the minimal fungicidal concentration values of white pepper extracts compared to Nystatin as a standard anti-fungal material. It was obvious that petroleum ether extract of white pepper recorded the best results of minimal fungicidal concentration against the four toxigenic fungi strains.

Table 1: Total anti-oxidant, total phenolic and total flavonoid of white pepper extracts

Parameters	EWP extract	PWP extract	HWP extract
DPPH (μmol TE g^{-1})	5.27±0.02	11.26±0.03	2.17±0.06
ABTS (μmol TE g^{-1})	42.28±0.62	104.65±1.22	21.37±0.18
FRAP (μmol TE g^{-1})	9.62±0.41	14.79±0.81	2.56±0.31
TPC (mg GAE g^{-1})	4.33±0.63	7.57±0.42	2.41±0.12
TFC (mg QE g^{-1})	12.47±0.74	52.33±1.05	32.66±1.1

TPC: Total phenolic content, TFC: Total flavonoid content

Table 2: Fatty acid composition of white pepper n-hexane extract

Synonym	Fatty acids	Percentage
C12:0	Lauric acid	17.69±0.04
C14:0	Myristic acid	6.82±0.24
C16:0	Palmitic acid	12.37±0.65
C16:1	Palmitoleic acid	10.22±0.28
C17:0	Margaric acid	ND
C18:0	Stearic acid	15.72±0.42
C18:1	Oleic acid	13.22±0.56
C18:1 trans	Vaccenic acid	6.34±0.28
C18:2	Linoleic acid	8.26±0.48
C18:3	Linolenic acid	4.84±0.71
C18:3 n5	Punicic acid	ND
C20:0	Arachidic acid	ND
C20:1	Gondoic acid	ND
C22:0	Behenic acid	ND
C22:1	Erucic acid	ND
C24:0	Lignoceric acid	4.52±0.23
C24:1	Nervonic acid	ND
SFA	Saturated fatty acids	57.12
MUSFA	Mono unsaturated fatty acids	29.78
PUSFA	Poly unsaturated fatty acids	13.10
USFA/SFA	Unsaturated fatty acids/ saturated fatty acids	0.75

ND: Not detected

Anti-fungal inhibition of white pepper extracts: The data in Table 4 presented the inhibition percentage of each extract against the control. The highest inhibition (%) recorded by the PWP extract, it was ranged from 39.2-48.6% of the control. The highest inhibition (%) of toxigenic fungi showed for *F. oxysporum* ITEM 12591 growth (using well diffusion assay), while the lowest inhibition (%) recorded for *Aspergillus parasiticus* ITEM 11 (on desk diffusion assay). However, the differentiation between the agar desk diffusion and agar well diffusion was not so far which may indicate honesty for the WPEs anti-fungal effect against the toxigenic fungi.

As shown in Table 4, the highest reduction of mycelial growth was accomplished by PWP. The highest reduction value recorded for *F. oxysporium* ITEM 12591, while the lowest reduction value was recorded for *A. pairasticus* ITEM 11.

Effect on body weight gain or total food intake: Results (Table 5) revealed that the administration of aflatoxin B_1 to rats lead to reduction in the body weight gain compared to the normal rats. Also orally treated rats with aflatoxin B_1 recorded

Table 3: Minimal fungicidal concentration of white pepper extracts

	Minimal fungicidal concentration (MFC)			
Parameters	EWP (μg mL^{-1})	PWP (μg mL^{-1})	HWP (μg mL^{-1})	Nystatin (ng mL^{-1})
Aspergillus flavus ITEM 698	440	370	1600	180
Aspergillus ochraceus ITEM 5117	400	370	1640	180
Fusarium oxysporum ITEM 12591	380	350	1580	150
Penicillium chrysogenum ATCC 10106	400	370	1600	180

Table 4: Fungal inhibition effect of white pepper extracts on solid and liquid media growth

	Desk diffusion inhibition zone (5 days incubation)			
Parameters	*Aspergillus flavus* ITEM 698	*Aspergillus parasiticus* ITEM 11	*Fusarium oxysporum* ITEM 12591	*Penicillium chrysogenum* ATCC 10106
Control growth (mm)	77.00±3.17	79.00±2.41	61.00±2.15	72.00±2.90
EWP inhibition (%)	29.13±1.05	25.44±1.71	28.27±2.25	31.28±2.11
PWP inhibition (%)	41.55±1.41	39.24±2.17	47.34±1.12	44.75±1.53
HWP inhibition (%)	12.31±2.16	12.15±1.91	14.94±2.57	11.77±2.89
	Well diffusion inhibition zone (5 days incubation)			
Parameters	*Aspergillus flavus* ITEM 698	*Aspergillus parasiticus* ITEM 11	*Fusarium oxysporum* ITEM 12591	*Penicillium chrysogenum* ATCC 10106
Control growth (mm)	85.00±2.31	90.00±1.90	72.00±1.81	81.00±2.45
EWP inhibition (%)	27.55±1.67	26.18±2.11	30.57±3.20	32.37±1.88
PWP inhibition (%)	44.28±2.85	41.73±3.42	48.61±2.48	47.16±2.94
HWP inhibition (%)	14.07±2.74	12.99±2.53	15.87±3.08	13.11±3.47
	Mycelial dry weight inhibition (7 days incubation)			
Parameters	*Aspergillus flavus* ITEM 698	*Aspergillus parasiticus* ITEM 11	*Fusarium oxysporum* ITEM 12591	*Penicillium chrysogenum* ATCC 10106
Control growth (g)	12.51±3.42	14.31±3.71	8.57±2.44	12.98±1.99
EWP inhibition (%)	9.43±1.37	11.13±2.41	6.73±1.22	9.88±1.41
PWP inhibition (%)	7.74±1.11	9.21±0.98	5.19±2.05	8.15±1.17
HWP inhibition (%)	10.84±1.23	12.87±1.07	7.13±1.16	11.03±0.61

Table 5: Nutritional parameters of different experimental groups (Mean±SE)

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Food intake (g/day)	Feed efficiency ratio
Normal control	125.17±4.40^{a}	189.17±5.76^{b}	64.00±3.10^{b}	479.17±2.70^{b}	17.11±0.10^{b}	0.13±0.01^{a}
AFB_1	125.33±2.98^{a}	173.67±4.45^{a}	48.33±5.97^{a}	455.00±6.78^{a}	16.25±0.24^{a}	0.11±0.01^{a}
AFB_1+EWP	125.17±3.47^{a}	178.33±4.56^{ab}	53.17±3.52^{ab}	466.17±6.06^{ab}	16.65±0.22^{ab}	0.11±0.01^{a}
EWP	125.33±2.80^{a}	187.33±4.03^{ab}	62.00±5.99^{ab}	478.33±3.13^{b}	17.08±0.11^{b}	0.13±0.01^{a}
AFB_1+PWP	125.33±2.68^{a}	180.50±2.92^{ab}	51.17±2.89^{ab}	465.33±3.68^{ab}	16.62±0.13^{ab}	0.12±0.01^{a}
PWP	125.33±3.54^{a}	187.17±3.26^{ab}	61.83±5.69^{ab}	477.00±2.84^{b}	17.04±0.10^{b}	0.13±0.01^{a}
AFB_1+HWP	125.17±2.41^{a}	179.50±4.64^{ab}	54.33±4.38^{ab}	476.17±3.13^{b}	17.01±0.11^{b}	0.13±0.01^{a}
HWP	125.33±3.87^{a}	188.00±4.08^{b}	62.67±3.85^{ab}	477.50±5.22^{b}	17.05±0.19^{b}	0.11±0.01^{a}

In each column, same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability

Table 6: Liver and kidney weight of different experimental groups (Mean±SE)

Groups	Liver (g)	Liver (%)	Kidney (g)	Kidney (%)
Normal control	4.17±0.25^{a}	2.20	1.28±0.08^{a}	0.68
AFB_1	5.04±0.07^{c}	2.92	1.09±0.07^{a}	0.63
AFB_1+EWP	4.76±0.13^{bc}	2.68	1.16±0.06^{a}	0.66
EWP	4.30±0.19^{a}	2.30	1.28±0.09^{a}	0.68
AFB_1+PWP	4.40±0.15^{ab}	2.44	1.12±0.05^{a}	0.62
PWP	4.19±0.19^{a}	2.24	1.27±0.11^{a}	0.68
AFB_1+HWP	4.50±0.17^{ab}	2.52	1.33±0.12^{a}	0.74
HWP	4.28±0.20^{a}	2.28	1.27±0.05^{a}	0.67

In each column, same letters means non-significant difference, different letter means the significance among the tested groups at 0.05 probability

the lowest value of food intake(16.25 g/day) and total food intake (455 g). On the other hand, orally treated rats with ethanol, petroleum ether or n-hexane extracts of white pepper along with aflatoxin B_1 showed improvement in the nutritional parameters either body weight gain or total food intake.

Effect on liver and kidney weight: With regard to liver and kidney weight of the studied groups, results (Table 6) declared that the liver weight of orally treated rats with aflatoxin B_1 significantly increased in comparison to the normal control rats. Rats administrated with of ethanol, petroleum ether, or n-hexane extracts of white pepper along with aflatoxin B_1 recorded liver weight lower than rats treated with aflatoxin B_1 only. It was obvious that the liver weight

Table 7: Liver functions of different experimental groups (Mean±SE)

Groups	ALT (U L^{-1})	AST (U L^{-1})	Alkaline phosphatase (U L^{-1})	Total protein (g dL^{-1})
Normal control	37.90±1.33^{a}	62.37±2.39^{a}	52.60±1.42^{a}	6.37±0.16^{d}
AFB_1	69.70±1.24^{d}	99.27±2.28^{d}	89.42±0.61^{d}	3.06±0.18^{a}
AFB_1+EWP	54.40±2.42^{c}	79.25±2.15^{c}	86.17±0.70^{d}	3.12±0.15^{a}
EWP	37.60±1.14^{a}	64.10±1.29^{a}	54.70±1.40^{a}	6.27±0.16^{d}
AFB_1+PWP	42.64±1.17^{b}	68.90±2.69^{ab}	67.22±1.38^{b}	5.31±0.18^{c}
PWP	37.50±1.36^{a}	62.75±1.28^{a}	53.20±1.28^{a}	5.36±0.12^{c}
AFB_1+HWP	52.40±1.22^{c}	72.07±2.03^{b}	77.10±1.74^{c}	4.88±0.14^{b}
HWP	38.20±1.77^{a}	63.71±2.29^{a}	53.70±1.36^{a}	6.11±0.15^{d}

In each column, same letters means non-significant difference, different letter means the significance among the tested groups at 0.05 probability

Table 8: Kidney functions of different experimental groups (Mean±SE)

Groups	Urea (mg dL^{-1})	BUN (mg dL^{-1})	Uric acid (mg dL^{-1})	Creatinine (mg dL^{-1})	Albumin (g dL^{-1})
Normal control	28.76±0.69^{a}	13.42±0.32^{a}	1.17±0.02^{a}	0.97±0.01^{a}	4.13±0.12^{a}
AFB_1	33.09±1.35^{b}	15.44±0.63^{b}	2.45±0.14^{d}	1.13±0.02^{c}	2.28±0.22^{e}
AFB_1+EWP	31.56±1.59^{ab}	14.73±0.74^{ab}	2.06±0.08^{c}	1.02±0.08^{c}	2.89±0.27^{d}
EWP	28.91±0.89^{a}	13.49±0.42^{a}	1.28±0.04^{a}	0.99±0.02^{b}	4.08±0.26^{a}
AFB_1+PWP	30.26±1.11^{ab}	14.12±0.52^{ab}	1.69±0.05^{b}	0.97±0.07^{a}	3.73±0.24^{c}
PWP	28.15±0.94^{a}	13.14±0.44^{a}	1.22±0.14^{a}	0.97±0.02^{a}	4.11±0.12^{a}
AFB_1+HWP	30.85±1.18^{ab}	14.40±0.55^{ab}	2.07±0.13^{c}	0.99±0.01^{b}	2.54±0.29^{b}
HWP	28.64±0.77^{a}	13.37±0.36^{a}	1.33±0.37^{a}	0.98±0.14^{b}	4.07±0.18^{a}

In each column, same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability

didn't affected by the administration of WPEs only without the aflatoxicosis induction. There weren't significant differences in kidney weight of the studied groups.

Effect on liver functions: As illustrated in Table 7, the impact of aflatoxin B_1 on the liver functions was evident. Since the activities of ALT, AST and alkaline phosphatase significantly elevated in orally treated rats with aflatoxin B_1 (69.70, 99.27 and 89.42 U L^{-1} in succession) compared to the normal control rats. The total protein value significantly decreased in orally treated rats with aflatoxin B_1 (3.06 g dL^{-1}). The extracts of white pepper suppressed either the raising in ALT, AST and alkaline phosphatase activities or the reduction in total protein. The petroleum ether extract of white pepper exhibit the most promising effect followed by white pepper n-hexane extract. The rats given ethanol, petroleum ether or n-hexane extracts of white pepper only without the aflatoxicosis induction didn't show significant differences neither in ALT, AST and alkaline phosphatase activities nor in total protein values in comparison to normal control rats.

Effect on kidney functions: Kidney functions were not immune to the negative effect of aflatoxin B_1. Since as noticeable in Table 8, orally treated rats with aflatoxin B_1 exhibited the highest values of urea, uric acid and creatinine (33.09, 2.45 and 1.13 mg dL^{-1} in succession) while the albumin value decreased significantly in these rats in comparison to the normal control rats. The extracts of white pepper combat the negative effect of aflatoxin B_1 on kidney functions. The most promising effect on kidney functions was achieved by the petroleum ether extract of white pepper.

Hemoglobin concentration and oxidative stress impacts: It was manifested from Fig. 1a that the lowest value of hemoglobin concentration was recorded by the group given aflatoxin B_1 only (10.82 g dL^{-1}). The extracts of white pepper suppressed the reduction of hemoglobin concentration value induced by aflatoxin B_1.

Figure 1b and c declared that the intake of aflatoxin B_1 caused oxidative stress which was clearly demonstrated throughout the elevation of plasma malondialdehyde value as well as the reduction of plasma total antioxidant capacity value for the group given aflatoxin B_1 only in compared to the other groups. On the other hand, the extracts of white pepper reduced the oxidative effect of aflatoxin B_1 when they administrated together.

As shown in Fig. 2a-c, no significant differences in the oxidative stress markers (malondialdehyde, catalase, SOD and GST) in liver tissues were observed between the normal control group and the groups treated with each extract of white pepper only. On the other hand, the oxidative stress markers in liver tissues affected by the intake of aflatoxin B_1. While the extracts of white pepper alleviated the raising of liver malondialdehyde and the reduction of liver catalase, SOD as well as GST activities. The petroleum ether extract of white pepper exhibited the most promising effect.

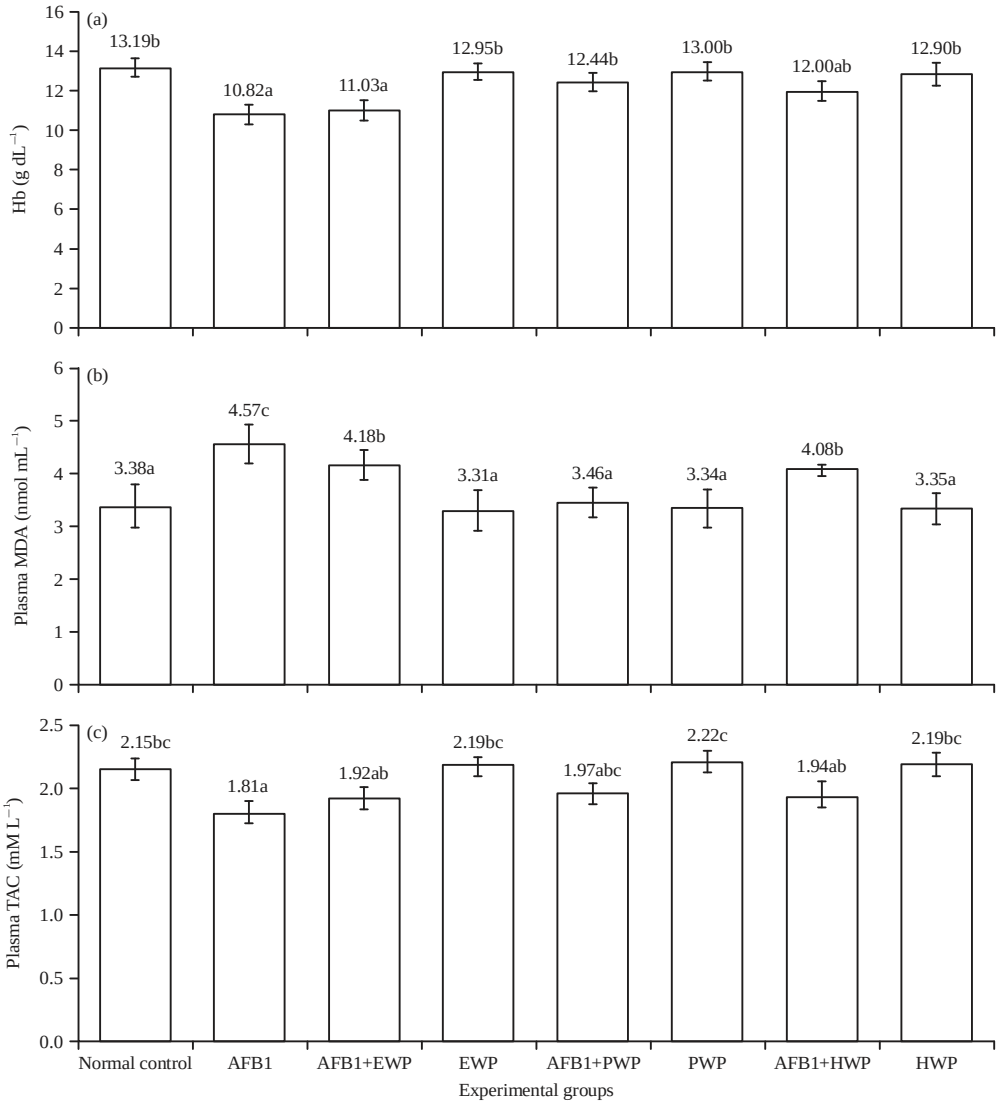

Fig. 1(a-c): (a) Hemoglobin, (b) Plasma malondialdehyde and (c) Total anti-oxidant capacity of different experimental groups

Same letters means non-significant difference, different letter means the significance among the tested groups at 0.05 probability

Table 9: Lipid profile of different experimental groups (Mean±SE)

Groups	T-Ch (mg dL^{-1})	TG (mg dL^{-1})	HDL-Ch (mg dL^{-1})	LDL-Ch (mg dL^{-1})	VLDL-Ch (mg dL^{-1})	T-Ch/ HDL-Ch
Normal control	71.60±3.30^{a}	83.25±3.15^{a}	33.26±1.37^{c}	21.69±3.67^{a}	16.65±0.63^{a}	2.18±0.16^{a}
AFB1	98.41±2.69^{c}	99.53±2.84^{b}	25.87±1.20^{a}	52.63±2.09^{c}	19.91±0.57^{b}	3.82±0.11^{c}
AFB1+EWP	85.03±3.77^{b}	90.28±3.52^{a}	27.99±1.23^{ab}	38.99±3.86^{b}	18.06±0.70^{a}	3.06±0.17^{b}
EWP	70.20±4.21^{a}	82.75±3.69^{a}	33.76±1.44^{c}	19.89±4.75^{a}	16.55±0.74^{a}	2.10±0.16^{a}
AFB1+PWP	83.31±3.33^{b}	88.40±3.21^{a}	31.42±1.16^{bc}	34.21±3.44^{b}	17.68±0.64^{a}	2.66±0.12^{b}
PWP	70.03±3.32^{a}	82.52±3.39^{a}	34.38±1.09^{c}	19.14±4.25^{a}	16.50±0.68^{a}	2.05±0.13^{a}
AFB1+HWP	80.38±3.73^{ab}	88.07±3.05^{a}	28.23±1.06^{ab}	34.54±3.21^{b}	17.61±0.61^{a}	2.85±0.13^{b}
HWP	69.92±4.32^{a}	81.37±2.44^{a}	35.14±1.28^{c}	18.51±4.47^{a}	16.27±0.49^{a}	2.00±0.15^{a}

In each column, same letters means non-significant difference, different letter means the significance among the tested groups at 0.05 probability

Plasma lipid profile: The plasma lipid profile also was affected by the intake of aflatoxin B_1. Results (Table 9) revealed that the highest values of plasma total cholesterol, triglycerides, LDL-Ch and VLDL-Ch as well as T- Ch/ HDL-Ch ratio were recorded by the group given aflatoxin B_1 only whereas, this group showed the lowest value of HDL-Ch. Significantly the extracts of white pepper improved the lipid profile when they administrated along with aflatoxin B_1.

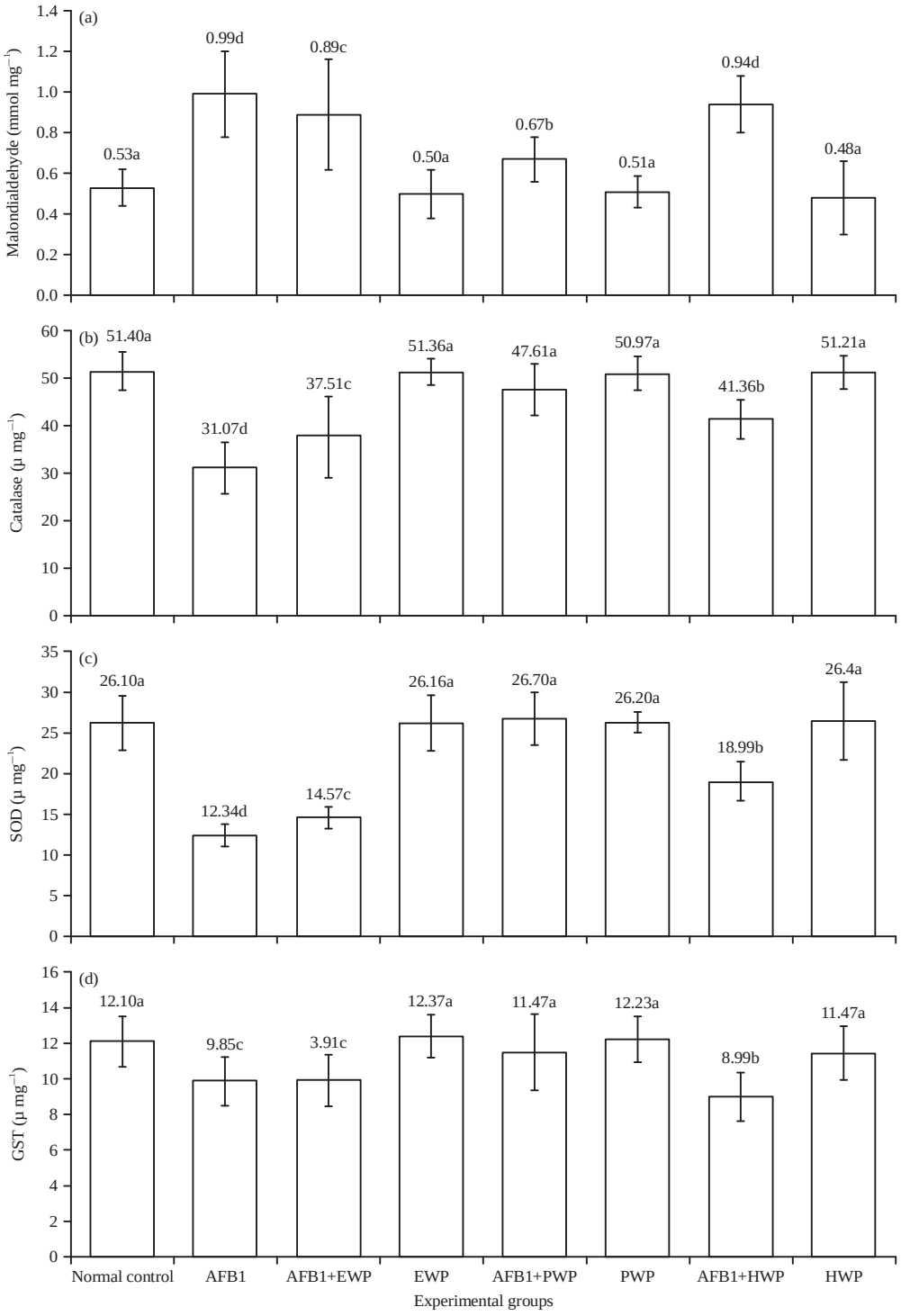

Fig. 2(a-d): Oxidative stress markers in liver, (a) Malondialdehyde, (b) Catalase, (c) SOD and (d) GST of different experimental groups

Same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability

DISCUSSION

Taking into consideration the contamination of food stuffs and edible tissues with mycotoxin, especially aflatoxin B_1 which exhibit several hazards on the health, there is a need to natural products to suppress the negative effects and hazards of aflatoxin B_1. Anti-oxidant and anti-fungal activities of ethanol and petroleum ether extracts as well as n-hexane

extract of white pepper were evaluated also the protective effect of these items against hepatotoxicity induced by aflatoxin B_1 in rats was studied. Logically, the suppression of fungal growth results in prevention of mycotoxin production. The anti-fungal activities of ethanol and petroleum ether extracts as well as n-hexane extract of white pepper, may be attributed to its anti-oxidant activities and contents of phenolic compounds. Since, El Khoury *et al.*[45] reported that; there was a relation between the total phenolic and anti-oxidant activity of plant extracts and fungal growth degradation. The results reported by Grintzalis *et al.*[46] referred to an inhibition effect of anti-oxidant on mycotoxin production more than its effect on the sclerotial of fungi. Mahoney *et al.*[47] reported that phenolic compounds could inhibit aflatoxin production and fungal growth of *A. flavus*. Moreover, the synthetic anti-oxidant like butylated hydroxyanisole (BHA) also showed a similar impact[48]. It was reported in a previous studies that the materials with a higher minimal inhibitory concentration and minimal fungicidal concentration values represented more potent role in fungal growth inhibition[25,49]. This effect may refer to the change of oxidative stress in the fungal growth media which cause a reduction not only in fungal growth amount, but also in the mycotoxin secreted by the fungi in the media[50]. In addition to anti-oxidant activities and phenolic compounds of ethanol and petroleum ether extracts of white pepper, the antifungal activities of these items may be attributed to the alkaloid compound known as piperine (1-peperoyl piperidine) which possesses anti-fungal and antioxidant effects as mentioned by Gurinderdeep[17]. Although Kanaki *et al.*[51] disclosed that petroleum ether one of the solvents utilized for piperine extraction also ethanol one of the solvents utilized for piperine extraction[52] but Raman and Gaikar[53] found that the highest extraction efficiency of piperine (94% with a purity of 85%) was achieved using petroleum ether as a non-polar solvent while the lowest extraction efficiency of piperine (75-80% with a purity of 72%) was achieved using polar solvent such as ethanol. This finding may interpret the superiority of the effect of white pepper petroleum ether extract as anti-fungal and anti-oxidant agent. In the same pattern, results of the current study demonstrated that the white pepper petroleum ether extract showed the superiority as a hepatic protective agent against aflatoxin B_1 in rats. This superiority may be due to its higher content of piperine which possesses hepatoprotective activity[15]. Aflatoxin B_1 mediated weight gain reduction is related to the anorexia[54]. Choi *et al.*[55] reported that the hepatic damage caused by aflatoxin B_1 is associated with the generation of reactive oxygen species and aflatoxin B_1- 8,9-epoxide. ALT, AST as well as ALP are considered biomarkers which indicate to the hepatic injury shown to be increased in hepatotoxicity induced by aflatoxin[56-59] B_1. The elevation of these biomarkers may be due to the disruption of plasma membrane by reactive oxygen species produced during the metabolism of aflatoxin[60] AFB_1, Galvano *et al.*[61] also mentioned that; the metabolic processing of aflatoxin B_1 by cytochrome P450 in the liver results in reactive oxygen species. These reactive oxygen species are associated with aflatoxin B_1 mediated oxidative stress which clearly observed in the results of the present study via the elevation of plasma and liver MDA, reduction of plasma TAC and decreasing of liver catalase, SOD as well as GST of rats given aflatoxin B_1 alone. White pepper extracts potentially reversed the oxidative stress (either in plasma or in the liver) with an elevation of elevation of liver functions due to its antioxidant activities. In addition to that, the beneficial effect of white pepper extracts may be attributed not only to piperine which has an important role in free radical damage and can reduce the expressions of MDA and SOD activity[62,63] but also to other bioactive compounds such as flavonoids which exhibit protective effect of cells against aflatoxin[64] AFB_1 and can improve the detoxification of aflatoxin B_1 possibly by enhancing the activities of ROS detoxifying enzymes, thus the redox imbalance caused by aflatoxin B_1 and the cellular macromolecules oxidation and fragmentation can be prevented[65,66]. The results of the current study indicated that rats treated with aflatoxin B_1 alone showed elevation of total cholesterol, triglycerides and low density lipoprotein levels as well as the reduction of high density lipoprotein. Abdel-Wahhab *et al.*[67,68] reported that a disturbance in the lipid metabolism causes by the toxic action of aflatoxin B_1 on liver functions. So, the improvement of lipid profile which accomplished by white pepper extracts may be due to its beneficial effect on liver functions. In addition to the presence of hypolipidemic bioactive compounds such as phenolic compounds and flavonoids in these extracts. Aflatoxin B_1 mediated hemoglobin reduction is related to not only the anemia caused by aflatoxin[54] B_1 but also to the red blood cells haemolysis by elevated lipid peroxides[69]. Thus, white pepper extracts potentially reversed the hemoglobin reduction due to its antioxidant activities.

CONCLUSION

The results of the current investigation indicated to the potency of white pepper extracts as an anti-oxidant and anti-fungal agent. The most promising effect was accomplished by the petroleum ether extract of white pepper, either for its bioactive components contents or its anti-fungal potency. The counteractive role of white pepper extracts for

oxidative stress and hepatotoxicity induced by aflatoxin B_1 in rats was demonstrated. These results directing to recommend implementing WPEs in safe foodstuffs production, mycotoxin health hazard avoidance in food products, besides the possibility to extend food shelf-life.

SIGNIFICANCE STATEMENT

This study confirmed that the white pepper extracts can act as anti-oxidant and antifungal agents. The counteractive role of white pepper extracts for oxidative stress and hepatotoxicity induced by aflatoxin B_1 in rats was demonstrated. The results of the current study directing to recommend implementing WPEs in safe foodstuffs production, mycotoxin health hazard avoidance in food products, besides the possibility to extend food shelf-life.

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