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MAXIMIZE THE POTENTIAL BENEFITS OF WHEY PROTEIN CONCENTRATE IN FORMULA BY SUPPLEMENTATION GINSENG EXTRACT

Gad, A. S. [1]; Sahar A. Abdelaziz[2], Fathia A. Mannaa[3], Nabila S. Hassan[4], and M. A. Abdel-Wahhab[5]

[1] Dairy Science Dept., [2]Food Science and Nutrition Dept., [3]Medical Physiology Dept, [4]Pathology Dept., [5]Food Toxicology and Contaminants Dept., National Research Center, Dokki, Cairo, Egypt

ABSTRACT

Whey protein is a potent ally to the general immune system**.** Supplementation whey protein with a combination of another source of antioxidant may be more effective than supplementation with single nutrient to strengthen the antioxidant defense. Ginseng is well known to have antioxidant activity**.** The aim of this research was study the role of ginseng to support the antioxidant properties of whey protein and increase its benefits. The antioxidant and hepatoprotective effects of whey protein concentrate (WPC) and/or Korean ginseng extract (KGE) were evaluated *in vitro* in *in vivo*. In the *in vitro* study we tested five concentrations (e.g. 20, 40, 60, 80 and 100 mg/100 ml) of WPC and/or KGE for their antioxidant activity using ABTS and TBARS assays. In *vivo* study, male Sprague-Dawley rats were divided into eight groups including the control group, the group fed AFs-contaminated diet (2.5 mg/kg diet) and the groups treated with WPC (500 mg/kg b.w) and/or KGE (20 mg/kg b.w) with or without aflatoxins for 30 days. The results indicated that both WPC and KGE exhibit antioxidant activity in vitro and the combined treatment showed the potential effect. Both agents showed a potential hepatoprotective effects against aflatoxins-induced liver damage and oxidative stress. They succeeded to restore the biochemical parameters and improve the histological and histochemical picture of the liver. This improvement was pronounced in the group received the combined treatment of WPC and KGE. It could be concluded that WPC can maximize its potential benefits by supplementation ginseng extract as a healthy ingredient and can be used in formulating the functional foods.

Keywords: Whey protein concentrate; ginseng, aflatoxin; mycotoxins;; antioxidants activity.

INTRODUCTION

Today, nutritionists describe foods that are rich in certain “ingredients” that may provide a health benefit beyond the traditional nutrients it contains as functional foods. Recently, the functional and biological properties of individual whey proteins have become a focus of commercial interest as potential ingredients of so-called functional or health-promoting foods. Whey protein has the ability to act as an antioxidant (Counous, 2000), antihypertensive, antitumor (Yoo, *et al*., 1998), hypolipidemic, antiviral (Low, *et al*. 2003), antibacterial (Levay and Viljoen, 1995; Ajello, *et al*. 2002), and chelating agent (Weinberg, 1996). Reduced glutathione levels have been associated with some disease, and accompanied with weakened immune system. Glutathione (GSH) is the precursor for glutathione peroxidase (GSH-Px), a major free radical scavenging enzyme. The use of whey protein concentrates in formulating products is increasing due to the nutritional and health benefits attributed to these proteins (Onwulata, *et al*. 2004). It is most

important when considering “which whey to go” that if one wants to maximize the potential benefits of the products in a whey protein formula, one chooses other ingredients that have other health benefits. Ginseng constituent is supporting antioxidant defense mechanisms; by involve direct stimulation of cell defense mechanisms. Ginseng extract scavenges hydroxyl radicals and protects unsaturated fatty acids from oxidation, effects which may contribute to stabilizing the structure of the lipid membrane perturbed by free radical attack (Zhang, *et al*., 1996). The medicinal efficacy of ginseng is closely linked to its protective properties against free radical attack (Chen X. 1996; Maffei Facino, *et al*., 1999; Lee, H.J. *et al*., 1999). Recently, 20-O-(h-D-glucopyranosyl)-20(S)-protopanaxadiol (IH- 901), a novel ginseng saponin metabolite, formed from ginsenosides Rb1, Rb2, and Rc was isolated and purified after giving ginseng extract p.o. to humans and rats (Hasegawa *et al*., 1996). IH-901 has been shown to enhance the efficacy of anticancer drugs in cancer cell lines previously resistant to several anticancer drugs (Lee, S.J *et al*. 1999), to exhibit antigenotoxic and anticlastogenic activity in rats concurrently treated with benzo (a) pyrene (Lee *et al*., 1998), and to induce apoptosis (Choi *et al*., 2003). These studies found that the antitumor activity of IH-901 is attributable to the induction of apoptosis.

The aim of the current study was to evaluate the antioxidant and radical scavenger properties of whey protein which use in formulating products and ginseng extract alone or in combination *in vitro* and to evaluate their protective effects against aflatoxin-induced oxidative stress and in rats.

MATERIALS AND METHODS

Materials

Chemicals

Free stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH·), TPTZ (2,4,6-Tris(2- pyridyl)-s-triazine), $FeSO_4.7H_2O$ and Trolox (6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Kits

Transaminase (ALT and AST), alkaline phosphatase (ALP), Total protein and albumin kits were purchased from Randex Laboratories (San Francisco, CA, USA). Total and direct bilirobin kits were purchased from Biomerieux, Laboratory of Reagents and Products (Marcy Létoile, France). Cholesterol kits was purchased form Biodiagnostics Company (Cairo, Egypt). Malondialdehyde (MDA) was obtained from Eagle Diagnostics (Dallas, TX, USA). Total antioxidant capacity kit was purchased from Biodiagnostic Co. (Cairo, Egypt). Other chemicals were of the highest purity commercially available.

Whey protein concentrates*:*

Concentrated whey powder containing 80% proteins (WPC80) was purchased from Davisco Foods International, Inc. (Eden Prairie, MN, USA).

Ginseng (Panax ginseng)

Ginseng (Panax ginseng**)** used in the present work was Korean crude red ginseng extract supplied by Korean society of ginseng, Soul, Korea and it was used as ginseng water extract.

Methods

Preparation of whey protein concentrates solution:

Concentrated whey powder that contains 80% proteins [Davisco Foods International, Inc., U.S.] was used as nutritional and functional ingredients. Whey protein concentrate powder (10 g) was weighed accurately and taken in a beaker. Then 100 ml of water was added to it was stirred with a magnetic stirrer, and left in refrigerator over night to fully hydrate. This gave (50 mg/0.5 ml) like used in vivo.

Perpatration of Korean ginseng extracts (KGE)

Crude ginseng (400 mg) was dissolved in 100 ml distilled water to give dose (20 mg/kg body weight). This gave (2 mg/0.5 ml) like used in vivo.

In vitro study

Determination of whey protein concentrates solubility:

Protein Solubility (PS) was determined in duplicate by the method of Bera and Mukherjee (1989). Two hundred milligrams of proteins were dispersed in 10 mL of deionized water. The pH of suspensions was adjusted to different levels (2.0 to 8.0) by using 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH. The suspensions were stirred at room temperature for 30 min and then centrifuged at 10000x g for 30 min (Kika Ultra Turrax T18 basic, Germany). Protein contents in supernates were determined by Kjeldahl method (Ceirwyn, 1995). The percentage of protein solubility in each suspension was calculated by the ratio of protein in the supernate to protein in 200 mg

Determination of the major component of WPC and KGE content*:*

To verify the manufacturer's claim of WPC, we determined the Thiol (SH) as major components which are responsible for the antioxidant activity. Thiol (SH) content was determined using a modification Ellman's assay (Anema and Lioyd, 1999) and was expressed as cysteine equivalents µM. Whereas, the ginsenoside content in the KGE (Korean Ginseng Extract) was determined as follows. Briefly, an aliquot of KGE dissolved in distilled water was passed through Sep-Pak C_{18} cartridge, and the cartridge was washed with distilled water. Subsequently, ginsenosides were eluted with 90% methanol and then analyzed by high performance liquid chromatography (Ko *et al*., 1989).

Preparation of working solution

Five concentrations (20, 40, 60, 80 and 100 mg/100 ml) of WPC and KGE alone or in combination were tested for their antioxidant activity. The antioxidant potential of these ingredients as a free radical scavenging activity was determined by 2,2'-azinobis-[3-ethylbenzthiazoline-6-sulphonic acid **(**ABTS) assay and thiobarbituric acid reactive substances assay the thiobarbituric acid assay (TBARS).

Antioxidant activity test:

Various concentrations (20.0, 40.0, 60.0, 80.0, 100.0 mg/100 ml) of WPC, KGE and WPC-KGE were employed in this study and evaluate its antioxidant potential.

ABTS (2,2'-azinobis-[3-ethylbenzthiazoline-6-sulphonic acid]) assay:

The antioxidant activity of WPC and KGE alone or in combination was carried out using ABTS assay according to the method described by Re *et al*. (1999). ABTS was dissolved in deionised water at 7 mM concentration

and potassium persulphate was added at a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature for 12 h in the dark before use. The resultant intensely-colored $ABTS^{\bullet+}$ radical cation was diluted with 0.01 M phosphate buffered saline (PBS), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. The tested materials were diluted 100x with the $ABTS^{\bullet+}$ solution to a total volume of 1 ml and the absorbance was measured spectrophotometrically. The assay was performed in triplicates. To allow for any absorbance of the tested materials themselves, 990 µl of PBS was added to the controls instead of $ABTS^{\bullet+}$**.** Trolox, the water-soluble α-tocopherol (vitamin E) analogue was used as a standard. The results of the assay were expressed relative to Trolox in terms of Trolox equivalent antioxidant capacity (TEAC).

The thiobarbituric acid assay (TBARS):

Lipid peroxidation measured on the basis of malonyldialdehyde (MDA) levels was carried out using the method described by Huerta *et al*. (2007). The concentration of the tested materials showed that the best antioxidant activity in ABTS assay was used in this test. Briefly, 1 ml reaction mixture was incubated at 95 °C for 1 h with 250 ml of thiobarbaturic acid (TBA) (0.67%) and 100 ml of H_3PO_4 (0.44 M) then 150 ml of trichloroacetic acid (TCA) (20%) were added. The mixture was centrifuged and the lipid peroxidation products in the tested samples (WPC and/or KGE) were estimated by the formation of thiobarbaturic acid reactive substances (TBARS) and quantified in term of malonyldialdehyde (MDA) as described by Haraguchi *et al.* (1997). Inhibition of TBARS formation by the KGE and/or WPC was calculated compared to control, which did not contain the extracts and reference antioxidant substance [0.01% Butylated hydroxyanisole (BHA)]. TBARS were assayed as previously described (Hseu *et al*., 2002) and were expressed in terms of MDA equivalents in µmol/L.

In vivo study

Aflatoxin Production:

The aflatoxin (AFs) was produced via fermentation of rice by *Aspergillus parasiticus* NRRL 2999 as described by Demet *et al*. (1995). The fermented rice was autoclaved, dried and ground to a powder, and the aflatoxin content was measured by the use of HPLC (Hustchins and Hagler, 1983). The aflatoxins of the rice powder consisted of 83.1% B_1, 12.9% B_2, 2.8% G_1 and 1.2% G_2 based on the total aflatoxins in the rice powder. The rice powder was incorporated into the basal diet to provide the desired level of 2.5 mg/kg diet. The diet containing aflatoxins was analyzed and the presence of parent aflatoxins was confirmed and determined as mentioned above.

Experimental animals:

Three-months old Sprague-Dawley male rats (100-120 g, purchased from animal house colony, Giza,) were maintained on standard lab diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolizable energy 12.08 MJ) purchased from Meladco Feed Co. (El Aubor City, Cairo, Egypt). Animals were housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled, at the Animal House Lab., National Research Centre, Dokki, Cairo. After an acclimatization period of

one week, the animals were divided into eight groups (10 rats/group) and housed in filter-top polycarbonate cages. All animals were received human care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo.

Experimental design:

Animals within different treatment groups were maintained on their respective diets for 30 days as follows: group 1, untreated control; group 2, fed AFs-contaminated diet (2.5 mg/kg diet); group 3, treated orally with the aqueous solution of WPC (500 mg/ kg b.w); group 4, treated orally with KGE (20 mg/kg b.w); group 5, treated orally with WPC plus KGE (at the same doses); group 6, fed AFs-contaminated diet and treated orally with WPC; group 7, fed AFs-contaminated diet and treated orally with KGE and group 8, fed AFs-contaminated diet and treated orally with WPC plus KGE. The animals were observed daily for signs of toxicity. At the end of experimentation period (i.e. day 31), blood samples were collected from all animals from retro-orbital venous plexus for biochemical analysis. The following biochemical methods were performed according to the kits instructions ALT and AST, ALP, TP, albumin, cholesterol, TB and DB.

Determination of lipid peroxidation and total antioxidant capacity in liver tissues

Samples from liver tissues (approximately 0.05-0.1 g) were homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate (Lin *et al*., 1998). This homogenate was centrifuged at 1700 rpm and 4 °C for 10 min; the supernatant was stored at -70 °C until analysis. This supernatant (20%) was used for the determination of hepatic lipid peroxidation according to the method described by Ruiz-Larrea *et al.* (1994). In brief, 4.5 ml working reagent (1 volume of 0.8 g thiobarbuturic acid dissolved in 100 ml of 10% perchloric acid) and 3 volume of (20% trichloroacetic acid) were added to 0.5 ml sample and incubated for 20 min in boiling water bath then left to cool at room temperature before centrifuge at 3000 rpm for 5 min at 0 ^{O}C. the pink color was measured at wavelength 532 nm, against blank solution which was prepared by the addition of 0.5 ml of distilled water to 4.5 ml working reagent, using V- 530 UV/Vis spectrophotometer. The level of lipid was expressed as nmol malondialdehyde (MDA)/g liver tissue. The homogenate was further diluted to give 5% (w/v), centrifuged at 3000 rpm for 5 min at 0 ^{O}C and used for the determination of total antioxidant capacity (TAC) according to the method described by Koracevic *et al.* (2001).

Samples of the liver from all animals within different treatment groups were excised and fixed in 10% formal saline followed by dehydration in ascending grades of alcohol, clearing in xylene and embedding in paraffin wax. Liver sections (5 µm thickness) were stained with hematoxylin and eosin (H&E) for the histological examination (Drury and Wallington, 1980). Another liver section from all groups was stained with Bromophenol blue technique to demonstrate total protein contents (Mazia *et al*., 1953). The Optical Density (O.D.) of total protein was measured using computerized image analyzer.

Statistical analysis:

All data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System (SAS, 1982). The significance of

the differences among treatment groups was determined by Waller-Duncan k-ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P \leq 0.05$.

RESULTS AND DISCUSSION

In vitro results

Whey protein concentrates solubility*:*

The whey protein solubility was measured in the pH range from 2 to 8 (the pH-protein solubility profiles of WPC 80. Results showed that WPC 80 had minimum solubility at pH 4.0-5.0. The samples contained between 74 and 78% protein. Solubility in the isoelectric point (pI) range was 75.0 and the highest solubility values were at the both acidity pH range between 2.0 and 3.0 and alkaline pH range between 7.0 and 8.0. Whey proteins have high solubility over a wide range of pH, (lower the pH, higher the solubility). The solubility of whey protein is considered as the most important one because it affects the other functional properties like gelling, foaming and emulsifying properties. Examination commercially available whey protein concentrates is important because it related to protein functionality. The level of insoluble denatured proteins in WPC products affects solubility, and solubility determines functionality (Puyol *et al*., 1999). The results showed that, pH influenced in the protein solubility. The solubility value of whey protein was minimum at the pH value of 4.5, which is the isoelectric point of whey proteins (Pelegrine and Gasparetto, 2005). The results indicated that the major components including Thiol (SH) groups in WPC and confirmed the manufactures claim. According to Onwulata *et al*. (2004) the solubility of commercial WPC has been shown to be highly variable. The extremely high solubility index reported in the current study revealed that the proteins present are in the tested WPC is in undenatured form which consequently, when ingested, exhibit higher level of tissue glutathione the major intracellular antioxidant (Lands *et al*. 1999).

The major component of WPC and KGE content

The determination of the major components of the tested materials indicated that WPC contained 523 µM cysteine equivalents. Where as, the HPLC analyses of the KGE is presented in (Table 1). These results showed that individual ginsenoside content in KGE was 0.54, 0.95, 1.02, 0.88, 3.16, 3.72, 1.89, 1.71, 1.32, 4.04, and 0.11 mg/g for Rg1, Re, Rf, Rh1, Rg2, Rb1, Rc, Rb2, Rd, Rg3 and Rh2, respectively. The sum of ginsenosides contents was 19.3 mg/g (1.93%) The principle active ingredients in natural ginseng are a unique class of bioactive natural compounds known as Ginsenosides. These extremely interesting substances identified as (Rh, Rg, Rg1, Rg2, Ra,Rd, Rc, Rb1 & Ro) are attracting increasing attention and respect from the scientific community.. In the current study, we evaluated the antioxidant and radical scavenging properties of WPC and/or KGE in vitro and their protective role in vivo during aflatoxin exposure.

Table (1): Concentrations of ginsenosides in KGE as determined by HPLC

Ginsenosides	Concentration (mg/g)
Rg1	0.54
Re	0.95
Rf	1.02
Rh1	0.88
Rg2	3.16
Rb1	3.72
Rc	1.89
Rb2	1.71
Rd	1.32
Rg3	4.04
Rh2	0.11
Total	19.3 (1.93%)

Antioxidant activity

ABTS (2,2'-azinobis-[3-ethylbenzthiazoline-6-sulphonic acid]) assay:

The method of scavenging ABTS free radicals was used to evaluate the antioxidant activity of different concentrations of WPC-KGE (20-100 mg/100ml) of oral dose. WPC-KGE dose displayed antioxidant activities as they were able to scavenge the ABTS**•+** radical cation. As shown in Fig. (1), this activity increased by increasing the concentration of the ingredients. KGE-WPC compound gave the highest ABTS radical scavenging activity followed by ginseng solution extract then WPC solution which showed the least scavenging activity. In comparing WPC solution and WPC-KGE solution scavenging activity of WPC-KGE was significantly more than absolute WPC with different concentration. The highest scavenging activity of WPC-KGE solution was at concentration 100 mg/100ml (TE 172.8 mg/L) whereas WPC solution at concentration 100 mg/100ml was (TE 140.6 mg/L). The current in vitro antioxidant results showed that WPC exhibit a weak scavenger to acid radical ($ABTS^{\cdot+}$) compared to KGE which indicate that the ability of WPC to donate hydrogen to reduce free radicals was low (Colbert and Decker, 1991). Whey has potent antioxidant activity, likely by contributing cysteine-rich proteins that aid in the synthesis of glutathione (GSH), a potent intracellular antioxidant (Walzem, *et al*. 2002). The higher radical scavenging activity for KGE reported in the current study may be due to not only the ginenosides but also to the phenolic substances (maltol, salicylic acid, vanillic acid) which have the ability to scavenge free radicals (Zhang *et al*., 1996; Kim *et al*., 2002). The potential of radical scavenging activity of WPC plus KGE at all tested concentrations demonstrated a possible synergistic action.

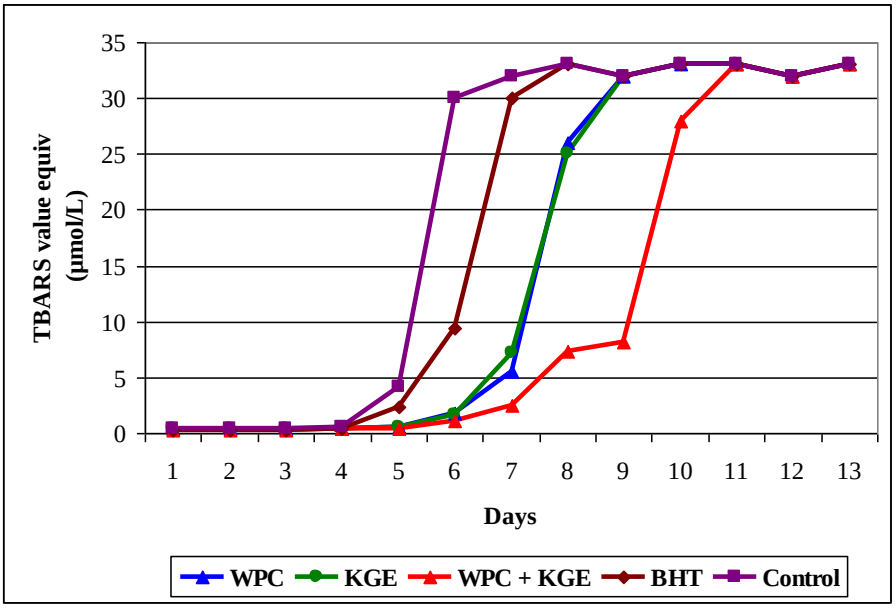

Fig.(1): Antioxidant activity using ABTS method in different concentration of ingredients additives. Data are expressed as Trolox equivalent mg/L.

Thiobarburic acid reactive substances (TBARS) assay:

Thiobarbituric acidreactive species (TBARS) assay was used to measure the lipid peroxide formed. Malondialdehyde (MDA), produced by the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm which was measured. Samples test concentration used is 100 mg/ml. Results showed that there is no significant difference between the antioxidant activity for both WPC and KGE at all concentrations (Fig. 2). However both had more significant activity than BHA and that is obvious started after 6 days as TBARS formed where the lipid peroxidation in the presence of BHA, WPC and KGE were 9.4, 1.75 and 1.6 µmol/L respectively, whereas the mixture WPC-KGE was formed 1.1 µmol/L TBARS. At 7th d TBARS compound formed in the presence of BHA, WPC, KGE and WPC-KGE were 30, 5.5, 7.2 and 2.5µmol/L respectively. WPC-KGE succeeded to inhibit the lipid oxidation significantly until 9 d which was 8.2 µmol/L TBARS, whereas all samples TBARS formed was 32 µmol/L. Lipid peroxidation had provided important information regarding free radical activity in disease states.

In the present study, TBARS was used as markers of lipid peroxidation which is a useful assay for the measurement of antioxidant activity of several compounds (Villa-Caballero *et al.*, 2000) to protect unsaturated fatty acids from oxidation. Using this model system to measure the antioxidant activity of WPC plus KGE is considered to be antioxidant. The results also showed that the combination of WPC and KGE had more ability

to restrict the unsaturated fatty acids from oxidation than BHA. WPC which are rich in sulfur amino acids, cysteine and methionine that can inhibit lipid oxidation had the same ability of ginsenosides in KGE which were shown previously to protect against lipid peroxidation and low density lipoprotein (LDL) oxidation *in vitro* (Zhang *et al*., 1996). These authors also suggested that hydroxyl radical formed by the Fenton reaction were completely inhibited by ginseng extract. Generally, the combination of WPC and KGE was the most effective to protect unsaturated fatty acids from oxidation which may contribute to stabilizing the structure of the lipid membrane perturbed by free radical attack.

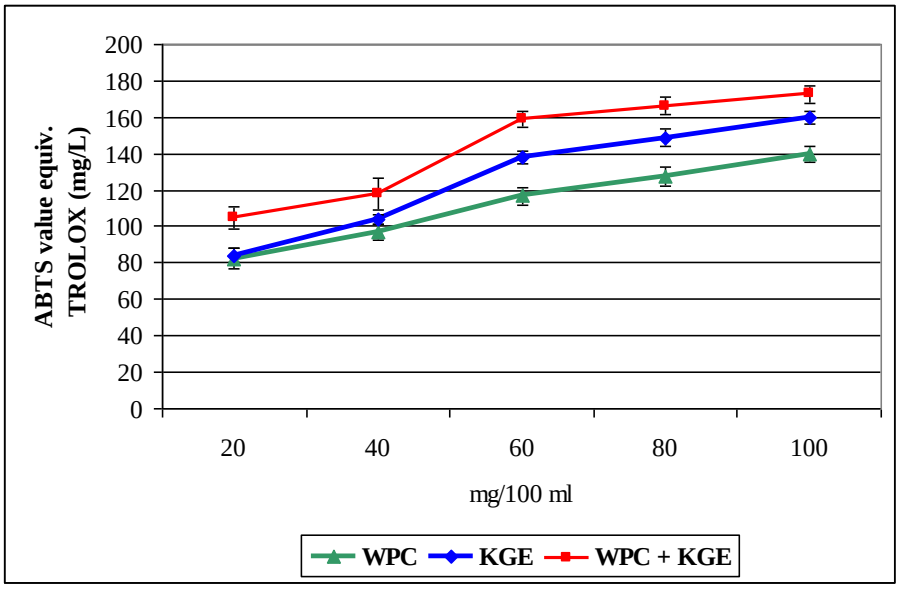

Fig.(2): Antioxidant activity using TBARS method in different concentration of ingredients additives. Data are expressed as µmol MDA/L.

In vivo results

In the current study we further evaluated the antioxidant and radical scavenging properties of WPC and/or KGE *in vivo* in rats fed AFs-contaminated diet. The selected doses of

AFs and KGE were based on our previous work (Abdel-Wahhab and Aly, 2005; Abdel-Wahhab and Ahmed, 2004) whereas, the selected dose of WPC was based on the results of the current *in vitro* study.

The results of the biochemical parameters (Table 2) revealed that rats fed aflatoxin-contaminated diet showed a significant increase in ALT, AST, ALP, TB, DB, cholesterol and lipid peroxidation accompanied with a significant decrease in TP, albumin and TAC. Animals treated with KGE alone showed a significant increase in ALT and DB accompanied with a significant decrease in AST, ALP and cholesterol whereas; TP and albumin

were comparable to the control. Animals fed AFs-contaminated diet and treated with KGE showed a significant improvement in all biochemical parameters towards the control values although it did not normalize them but it succeeded to normalize TB and cholesterol. Animals treated with WPC alone showed a significant increase in ALP and DB accompanied with a significant decrease in cholesterol whoever the other tested parameters were not significantly affected. The combined treatment with WPC and KGE increased ALT and decreased AST, cholesterol and lipid peroxidation but not significantly affect on the other parameters. Animals fed AFs-contaminated diet and treated with WPC and/or KGE showed a significant improvement in all biochemical parameters toward the control values although these treatments failed to normalize these levels. Moreover, the recorded improvement was more pronounced in the group fed AFs-contaminated diet and received the combined treatment. These results indicated that the increase in ALT, AST, ALP, cholesterol, TB and DB in animals fed AF-contaminated revealed degenerative changes and hypofunction of liver (Abdel-Wahhab *et al.*, 2006). However, the decreasing in serum levels of TP and albumin may indicate protein catabolism and/or kidney dysfunction (Abdel-Wahhab *et al*., 2007). These results clearly showed that aflatoxin has a harmful and stressful influence on the hepatic tissue consistent with those reported in the literature of aflatoxicosis (Miller and Willson, 1994). The decreasing in TAC increasing in LP reported herein in the animals fed AFs-contaminated diet revealed that aflatoxins induced oxidative stress in liver tissue.

Table (2): Effect of WPC and KGE alone or in combination on different biochemical parameters in rats fed AFs-contaminated diet

Groups Parameters	Control	AFs	WPC	KGE	WPC + KGE	WPC + AFs	KGE + AFs	WPC + KGE + AFs
ALT (U/L)	24.43 ± 0.84^{a}	105.57 ± 3.96^{b}	22.43 ± 2.55^{a}	23.29 ± 2.71^{a}	24.14 ± 3.29^{a}	44.86 ± 3.17^{c}	40.57 ± 1.60^{c}	35.00 ± 0.76^{d}
AST (U/L)	59.29 ± 4.7^{a}	124 ± 4.1^{b}	57.49 ± 3.51^{a}	52.71 ± 3.68^{c}	50.14 ± 3.7^{c}	68.43 ± 1.11^{d}	69.43 ± 1.63^{d}	57.14 ± 1.67^{a}
ALP (U/L)	53.88 ± 4.34^{a}	99.04 ± 3.03^{b}	57.56 ± 3.79^{a}	50.96 ± 6.34^{a}	51.83 ± 5.09^{a}	74.71 ± 3.65^{c}	76.91 ± 1.69^{c}	73.64 ± 1.59^{c}
TP (mg/dl)	7.01 ± 0.28^{a}	4.09 ± 0.19^{b}	7.03 ± 0.10^{a}	6.98 ± 0.13^{a}	6.96 ± 0.09^{a}	5.56 ± 0.20^{c}	5.64 ± 0.11^{c}	6.45 ± 0.31^{a}
Alb (mg/dl)	3.01 ± 0.19^{a}	1.64 ± 0.21^{b}	2.95 ± 0.15^{a}	3.10 ± 0.15^{a}	2.95 ± 0.17^{a}	2.54 ± 0.23^{a}	2.84 ± 0.23^{a}	2.74 ± 0.15^{a}
TB (mg/dl)	3.35 ± 0.1^{a}	7.57 ± 0.23^{b}	3.16 ± 0.23^{a}	3.80 ± 0.44^{a}	2.98 ± 0.18^{a}	4.33 ± 0.19^{c}	3.34 ± 0.32^{a}	3.95 ± 0.41^{a}
DB (mg/dl)	1.76 ± 0.09^{a}	5.24 ± 0.38^{b}	2.00 ± 0.28^{c}	2.06 ± 0.25^{c}	1.70 ± 0.16^{a}	3.45 ± 0.11^{d}	3.26 ± 0.41^{d}	2.17 ± 0.23^{c}
Cho (mg/dl)	82.8 ± 4.91^{a}	208.27 ± 20.18^{b}	59.30 ± 7.82^{c}	59.61 ± 3.25^{c}	56.86 ± 6.08^{c}	75.74 ± 6.10^{a}	85.37 ± 7.15^{a}	41.43 ± 2.94^{d}
MDA (ng/g liver tissue)	90.16 ± 0.83^{a}	203.28 ± 15.08^{b}	89.35 ± 0.93^{a}	90.18 ± 1.29^{a}	83.63 ± 1.49^{c}	111.44 ± 4.05^{d}	118.3 ± 4.97^{d}	96.98 ± 6.67^{a}
TAC (µmol/g liver tissue)	47.42 ± 47.4^{a}	27.5 ± 1.89^{b}	47.01 ± 0.13^{a}	47.27 ± 0.16^{a}	47.01 ± 0.13^{a}	42.24 ± 1.65^{a}	44.25 ± 1.39^{a}	45.88 ± 0.66^{a}

Within each row, means superscript with different letters are significantly different (P≤ 0.05)

The aflatoxin-induced alterations in the hepatic antioxidant status may therefore be considered as manifestation of increased oxidative stress caused by aflatoxin and its metabolites. It is well known that TAC includes enzymes such as SOD, catalase and GPX. TAC may provide more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids. Both GPX and SOD are considered enzymatic free-radical scavengers in cells. The decreasing in TAC and increasing in LP reported in the current study in AFs-treated leading to an indirect increase in oxidative DNA damage. These results were in agreement with those reported by Meki *et al.* (2004) and Abdel-Wahhab *et al*. (2010).

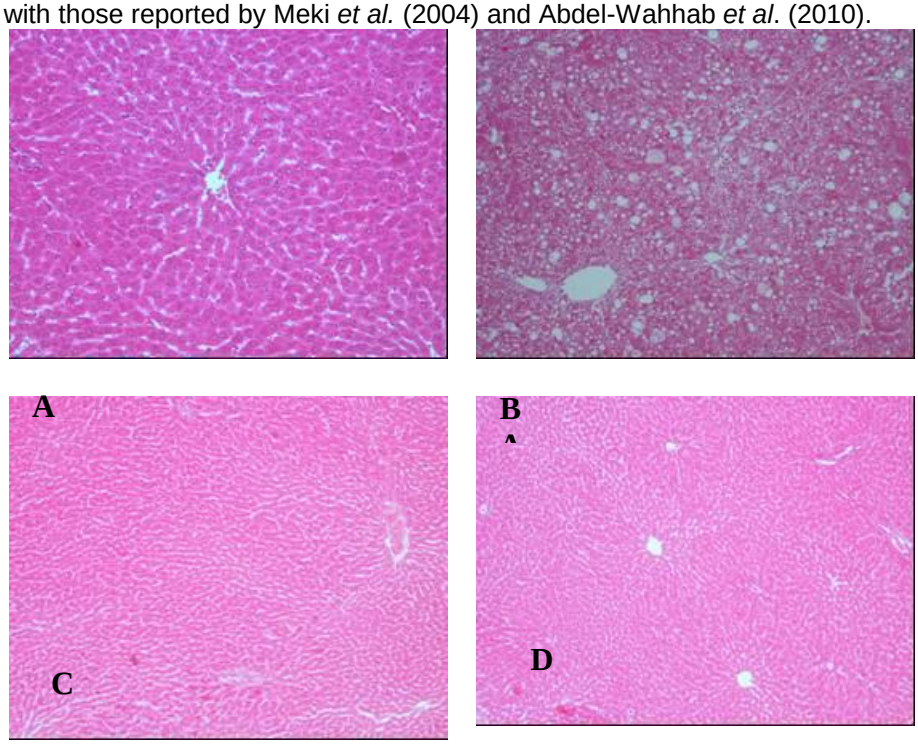

Fig. (3). A photomicrograph in a liver section of: (A) Control rat showing normal hepatocytes and central vein, (B) Rats treated with AFs alone showing hepatocytes with fatty degeneration and necrosis scattered in all liver tissue, (C) Rats treated with WPC alone showing normal hepatocytes architecture, central veins, portal tracts and hepatic cords separated with blood sinusoids and (D) Rats treated with KGE alone showing marked improvement in hepatocytes structure. (H&E X150).

The histological examination of the liver sections of the control animals revealed normal hepatocytes and central vein (Fig 3a). Microscopic examination of the liver sections of animals fed AFs-contaminated diet

showed that hepatocytes with fatty degeneration and necrosis scattered in all liver tissue (Fig. 3b). The liver sections of rats treated with WPC alone showed normal hepatocytes architecture, central veins, portal tracts and hepatic cords separated with blood sinusoids (Fig. 3c). Livers of rats treated with KGE alone showed nearly normal hepatocytes structure (Fig. 3d). Animals treated with WPC plus KGE raveled more or less normal hepatocytes structure (Fig. 4a). Animals fed AFs-contaminated diet and treated with WPC showed decrease in different featuers of degenerative changes and improvement in hepatocytes in central zone and around blood vessels (Fig. 4b) however; those fed AFs-contaminated diet and treated with KGE alone or plus WPC showed prominent improvement in all hepatocytes, blood vessels and connective tissue and the liver sections become nearly normal (Fig. 4c, 4d).

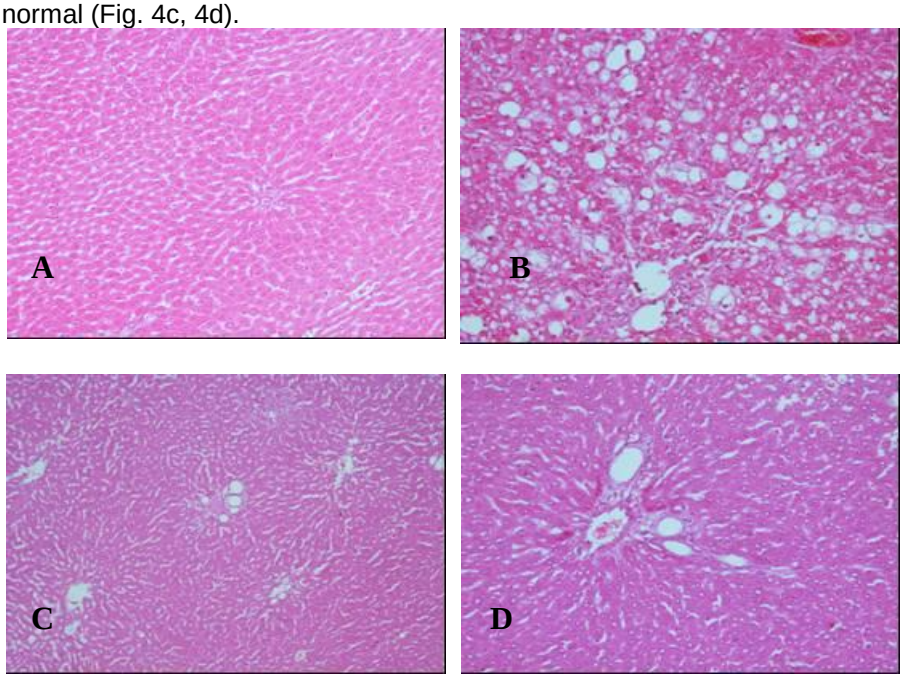

Fig. (4): A photomicrograph in a liver section of: (A) Rats treated with WPC plus KGE alone showing normal hepatocytes structure, (B) Rats treated with AFs plus WPC showing decrease in different features of degenerative changes and improvement in hepatocytes in central zone, (C) Rats treated with AFs plus KGE showing prominent improvement in all hepatocytes, no inflammation or connective tissue around blood vessels and it is nearly normal . (D) Rats treated with AFs plus WPC and KGE showing prominent improvement in all hepatocytes, blood vessels and connective tissue; the section become nearly normal (H&E X150).

The histochemical inspection of total protein content in the liver tissue as determined by the optical density (Fig. 5) revealed that strong protein materials in the cytoplasm and nuclear membrane of the control rats. Rats fed AFs-contaminated diet showed moderate decrease in the distribution of

protein materials in the cytoplasm and nuclear membrane. Rats treated with WPC alone showed a marked increased in protein materials in the hepatocytes whereas those treated with KGE alone showed a marked increase in protein content of the majority of hepatocytes. Rats treated with WPC plus KGE showed a remarkable restoration in the stain of protein particles in the majority of hepatocytes. Rats fed AFs-contaminated diet and treated with WPC showed a moderate restoration in the stain of protein particles in the majority of hepatocytes. Rats fed AFs-contaminated diet and treated with KGE showed a decrease in protein particles in the majority of hepatocytes which still abnormal whereas, those fed AFs-contaminated diet and treated with WPC plus KGE showing mild improvement in protein contents.

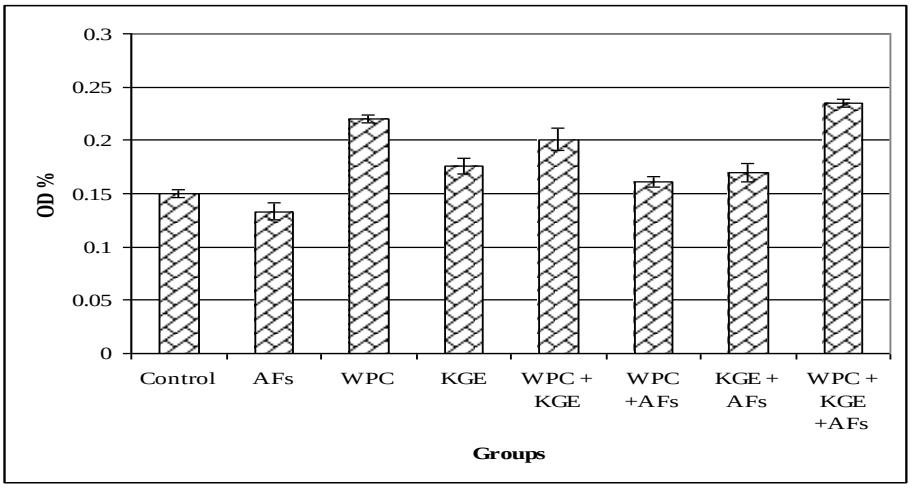

Fig. (5): Optical density (O.D) of total protein content of liver in rats fed AFs-contaminated diet and treated with WPC and/or KGE

In the current study, animals fed AFs-contaminated diet and treated with WPC and/or KGE showed a significant improvement in the biochemical parameters, the histological and the histochemical picture of the liver. Moreover, this improvement was more pronounced in the group received the combined treatment of WPC and KGE. This group showed a significant decrease in LP compared with the control group.

Previous reports indicated that WPC has a potential antioxidant activity due to its ability to increase glutathione levels (Peng *et al.,* 2009; Bayram *et al.,* 2008). WPC is well known to be rich in cysteine, α-lactalbumin, β-lactoglobulin, and bovine serum albumin (Morr and Ha, 1993). Cysteine is an amino acid regulates *in vivo* concentrations of GSH and supplementation of the diet with whey protein high cysteine may promote GSH biosynthesis (Kent *et al*., 2003). GSH was reported to be an antioxidant and anticarcinogenic tripeptide, and thereby improving protection against oxidant-induced cell damage (Peng *et al*., 2008). The increased TAC production reported herein in rats treated with WPC accompanied with the decrease

level of LP supported the earlier findings of Watanabe *et al*. (2000). The results of the current study and others suggested that cysteine content is responsible, in part, for the observed increase TAC via the increase in GSH. According to Bounous (2000), the suggested mechanism by which WPC induced its protection has been attributed to the increase in blood and tissue GSH concentration, which in turn increased the scavenger of the free radicals produced by AFs.

Ginseng also was found to protect against liver toxicity induced by CCL_4 through the inhibition of cytochrome P450-associated monoxygenase activities (Kim *et al*., 1997). Therefore, the protective effect of KGE in the current study is attributable to its free radical scavenging activity (Mannaa *et al*., 2006). These antioxidant effects of ginseng may be responsible for its wide pharmacological actions in clinical practice by a free radical reaction-inhibition mechanism consequently decreased risk for most cancers including carcinomas of the esophagus, stomach, colon, pancreas, lung and liver (Jeong *et al*., 1997; Abdel-Wahhab *et al*., 2010). Ginsenoside Rh2 (GS-Rh2) is one of the ginsenosides presented in KGE plays an essential role in the prevention and treatment of liver cancer by mechanisms postulated in a serious of reports. The panaxadiol fraction and its ginsenosides could induce the antioxidant enzymes which are important for maintaining cell viability by lowering the level of oxygen radical generated from intracellular metabolism (Chang *et al*., 1999).

Taken together, these results indicated that KGE have protective effects against liver injury induced by aflatoxins and it plays a role in increasing the antioxidant status as well as lowering the oxidative damage of nucleic acids in the body (Abdel-Wahhab and Ahmed, 2004; Mannaa *et al*., 2006).

Conclusion

In conclusion, the current study revealed that WPC and KGE exhibited a potential antioxidant and radical scavenging properties in vitro and in vivo. They were able to protect against the oxidative stress of aflatoxins singly or in combination. KGE was effective than WPC however, the combined treatment was found to be more effective than the single treatment. It could be concluded that WPC should be incorporated with KGE when used as functional foods to maximize the potential benefits of whey protein concentrate

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تعظيم فوائد بروتينات الشرش المركزة المستخدمة فى التركيبات الغذائية بأضافة مستخلص الجينسنج

أحمد سعد جاد* [١] ، سحر عبد الجيد عبد العزيز [٢] ، فتحية عبد الواحد مناع [٣] ، نبيلة صلاح حسن [٤] و مسعد عطية عبد الوهاب [٥]

١ قسم علوم وتكنولوجيا الالبان، ٢قسم التغذية وعلوم الاطعمة ، ٣ قسم علم وظائف الأعضاء ، ٤قسم الباثولوجى ، ٥قسم علوم السموم والملوثات - المركز القومي للبحوث ، الدقي ، القاهرة

تعتبر بروتينات الشرش من العناصر الهامة التى تدعم بها الاغذية لاهميتها فى تقوية جهاز المناعة٠ وجود عنصر اخر يتميز بنشاطه كمضاد للاكسدة ربما يعزز من تقوية الجهاز المناعى٠ الهدف من البحث هو دراسة دور الجينسنج فى دعم خصائص بروتينات الشرش المضادة للاكسدة لزيادة الاستفادة منها٠ تم تقييم بروتينات اشرش و مستخاص الجينسنج الكورى كل على حده ، وكذلك وهما مختلطين٠ اشتملت الاختبارات على اختبارات معملية و اختبارات على فئران التجارب (ذكور)٠ فى التجارب المعملية تم عمل خمس تركيزات (٢٠، ٤٠ ، ٦٠ ، ٨٠ ، ١٠٠ ملليجرام/ ١٠٠ مل) واستخدمت الاختبارات ABTS ، TBARS لتقدير النشاط كمضادات للاكسدة أما بالنسبي لفئران التجارب فقد تم تقسيمها الى ثمان مجموعات واستخدم الغذاء الملوث الأفلاتوكسين ليحدث ضرر بالكبد ثم دراسة تأثير الجرعات المستخدمة على حماية الكبد٠ اشتملت الجرعات على بروتينات الشرش المركزة (٥٠٠ ملليجرام/كجم من وزن الجسم) ، مستخلص الجينسنج (٢٠ملليجرام من الجينسنج المركز/كجم من وزن الجسم) وبعد ٣٠ يوم من التغذية تمت الاختبارات الاتية: biochemical parameters, histological and histochemical picture of the liver٠

أوضحت النتائج على أن للجينسنج تأثير ايجابى فى تعظيم دور بروتينات الشرش على جهاز المناعة٠ وظهر ذلك فى الارقام الناتجة لل biochemical parameters وفى تحسين الصورة النسيجية للكبد مقارنة بالتغذية على بروتينات الشرش المركزة على حده٠ هكذا نخلص فى أنه يمكن تعظيم دور بروتينات الشرش المركزة فى حماية الكبد والمحاظة عليه عند استخدامها فى تصميم الاغذية الوظيفية باضافة الجينسنج لها٠

قام بتحكيم البحث

أ.د / الطاهرة محمد أحمد عمار	**كلية الزراعة – جامعة المنصورة**
أ.د / محمود حمزة السنيطى	**المركز القومى للبحوث**