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The effect of white kidney bean fertilized with nano-zinc on nutritional and biochemical aspects in rats

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ABSTRACT

This study aims to estimate the safety of white kidney bean (WKB) fertilized by zinc oxide nanoparticles (ZnO-NPs) via studying changes of liver and kidney function, lipid profile and histological examination for the liver and kidney tissue in rats fed on it. Twenty Four male albino rats were used in this study divided into four groups; the first fed balanced diet (control group), the second fed WKB treated with normal ZnO (nWKB), the third fed WKB treated with 20 ppm ZnO-NPs (tWKB-1), and the fourth fed WKB treated with 40 ppm ZnO-NPs (tWKB-2). The results revealed that WKB treated with ZnO-NPs reduced body weight, food efficiency ratio, relative liver weight, and relative spleen weight were increased as well as the most biochemical parameters exhibited non-significant changes as compared to control group. Meanwhile, tWKB-2 group demonstrated a decrease in alkaline phosphatase and aspartate transaminase activities as compared to nWKB group.

1. Introduction

Phaseolus vulgaris L or white kidney bean (WKB) is a member of the leguminous family. Legumes are considered an important source of proteins, especially in developing countries that it is difficult to depend on animal proteins where people have low income . It considers as one of the most essential vegetable crops cultivated in Egypt for exportation and for the local market. Legumes are also good sources of complex carbohydrates, unsaturated fats, minerals, and vitamins. Moreover, they contain various amounts of non-nutrient phytochemicals such as polyphenols and flavonoids that have many health benefits against different ailments such as heart disease, cancer, and immune disturbance. Some of these compounds are termed as antinutrient factors since they are inhibitors for growth like tannins that inhibited the digestibility of the proteins, and phytic acid which decreased some minerals bioavailability [1].

Also, lectins from WKB have been proved to participate in the etiology of Parkinson's disease [2]. Besides were eating raw or uncooked WKB may result in intestinal inflammation due to the interaction between its lectins with the mucosa of the small intestine [3,4]. Moreover, raw bean significantly reduced water and electrolyte absorption that may be attributed to distortion of intestine morphology and disturbance of hydroelectrolytic flux induced by raw bean [5,6]. Legumes processing is important to eliminate or reduce such antinutritive factors [7].

Various cooking methods may enhance the nutritional property of edible legumes to different extents. WKB is a well- known legume that contains proteins, minerals, vitamins, carbohydrates and various kinds of antioxidants. It was documented that diets containing WKB significantly reduced plasma lipids in hyperlipidemic animals [8] and could reduce body weight [9]. Amylase inhibitor from WKB reduced hyperglycemia in diabetic rats [10]. Comparative evaluation between meals prepared from lentils or WKB in anemic rats showed that WKB extremely enhanced iron bioavailability and liver store than lentils [11]. WKB non-digestible fibers have been shown to improve an early stage of colorectal cancer by modulating signaling pathway genes in rats [12]. Also, WKB was demonstrated to modulate renal genes in diabetic rats [13]. In all studied trials about WKB, cooked bean showed the least

Abbreviations: ALT, Alanine transaminase; ALP, alkaline phosphatase; AST, Aspartate transaminase; HDL-ch, high density lipoprotein cholesterol; LDL, low density lipoprotein; LDL-ch, low density lipoprotein cholesterol; NPs, nanoparticles; nWKB, Normal white kidney bean; tWKB-1, treated WKB with 20ppm ZnO-NPs; tWKB-2, treated WKB with 40ppm ZnO-NPs; WKB, white kidney bean; ZnO-NPs, zinc oxide nanoparticles.

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interactions and inflammation with the intestinal mucosa as compared to the raw one [3,5]. Moreover, raw WKB causes detrimental effects on intestinal enzymes [6].

Nanotechnology has produced a wide range of nanoparticles (NPs) which are extremely used. NPs possess a large surface area and have high reactivity in the cell [14]. There are many varieties of NPs being produced today among which zinc oxide NPs (ZnO-NPs). They are extensively applied in sunscreens and in many cosmetics due to their powerful UV- absorption. Besides, they are used in the food industry as additive and preservative, in medicine; they are administered as anticancer drugs [15], and in agriculture, they are employed for their potential fungicidal effects and for fertilization, Where the zinc (Zn) element is one of the vital micronutrients for a plant that plays an essential role in carbohydrate and proteins metabolism in addition, it controls plant growth hormone. The plants need (230 g/ ha) of Zn element in normal size and its deficiency is serious [16].

Exposure to ZnO-NPs has been progressively increased, with the increased applications of ZnO-NPs with concomitant elevated toxicity that may be cytotoxic, inflammatory, and genotoxic effects. These particles can enter our bodies through different routes such as injection, inhalation, and ingestion; the latter route is dominant. They may enter the bloodstream and cause many adverse reactions in various body organs. Omidi et al. [17] found that ZnO-NPs have comparable properties when compared nano zinc-oxide eugenol sealer with conventional sealer. Also, ZnO-NPs showed more analgesic effects than macro-sized ZnO that may be probably attributed to its interaction with the opioidergic system in the body [18]. Besides, ZnO-NPs exert antioxidant properties and improvement for plasma testosterone level, sperm count and DNA damage against doxorubicin-induced toxicity [19]. Amara et al. [20] reported that ZnO-NPs administration neither affects cognitive capacity nor neurotransmitters levels in the animal. However, Torabi et al. [21] proved that ZnO-NPs treats anxiety in rats. Although Kim et al. [22] proved that bulk ZnO particles may have high bioavailability than ZnO-NPs.

Moreover, Many studies have documented that high doses of ZnO-NPs can cause apoptosis in liver and produce oxidative stress [23] and also they can induce liver DNA damage [24]. Moreover, a high dose of ZnO-NPs can induce bone resorption in rats [25]. In addition, Han et al. [26] proved that ZnO-NPs may have an adverse effect on the rat's hippocampus. Therefore, we aimed to explore the safety of WKB fertilized by ZnO-NPs on rats via evaluating nutritional parameters, liver and kidney functions. In addition, we tried to determine its effects on the plasma lipid profile. Furthermore, histological examination was performed for the liver and kidney tissue to confirm our results.

2. Material and methods

2.1. Experiment layout

At the first week of March, seeds of WKB were sown at rate 120 kg/ha in clay soil in Shebin El-Kom, El-Monifia governorate, Egypt, during two seasons 2016 and 2017 and then sown at rate of 2 seeds per hill and 30 cm distance between hills on one side of ridge (60 cm at distance between ridges). Seeds of WKB were provided from Agricultural Research Centre, Ministry of Agricultural and Land Reclamation.

Organic manures (47.6 m^3/ha) were added during the soil preparation with calcium super phosphate at rate 476 kg/ha (15.5% P_2O_5) +238 kg/ha Agricultural sulfur +119 kg/ha potassium sulphate (48% K_2O) +119 kg/ha ammonium sulfate (20.6% N) before the first irrigation (after seeds germination) ammonium sulfate (20.6% N) at rate 238 kg/ha were applied, then potassium sulphate (48% K_2O) 119 kg/ha + ammonium sulfate (20.6% N) 238 kg/ha were supplied before the second irrigation.WKB plants were sprayed with normal ZnO with concentration 200 ppm and ZnO-NPs with concentration 20 and 40 ppm after 20 days from the seeds planting as reported in our previous work [27].

2.1.1. Data recorded

- At harvesting stage (after 90 days from sowing), the plants were harvested to determine seeds and shoot residues per plant and weight of (Seeds and shoot residues ton/ha and Kg/ ha, respectively).
- Fresh samples of WKB (leaves and seeds) were dried in an oven at 60°C till constant weight, and then dried sample was taken to determine the zinc by atomic absorption according to the method described in the AOAC [28].

2.2. Nutritional experiment

2.2.1. Plant materials

The seeds of WKB were divided into three types according to the type and dose of ZnO used during cultivation of the plants; 1st type normal seeds of WKB which their plant was supplied with normal ZnO and 2nd and 3rd type were treated seeds of WKB that obtained from plant that was sprayed with 20 ppm and 40 ppm of ZnO-NPs during cultivation, respectively. All types were cleaned to remove dust and foreign matter manually and then were carefully washed. One kilogram of each sample was soaked in 5 liters of distilled water for 4 h at room temperature. Then the seeds were removed from the soaking water and then cooked by boiling with five times their weight of distilled water (ordinary cooking) [29]. After that WKB were taken and dried in a hot air drier at 60 °C and then ground to pass a 40 mesh screen. This flour was used to prepare experimental diets.

2.3. Experimental procedures

Twenty four male albino rats weighing120-150 g were purchased from Animal House of National Research Centre (NRC), Dokki, Giza, Egypt. Animals were kept individually in stainless steel cages at a temperature (25 ± 2 °C) under a 12 h dark-light cycle. Water and food were supplied ad-libtium. For one week prior to the experiment for acclimatization. Experimental procedure and animal comfort were managed according to the guidelines of the Animal Care and Ethics Committee of NRC (registration number 16 457). Rats were randomly assigned to four groups, six animals per group. First, normal control group fed a balanced diet was prepared according to AOAC [28] salt and vitamin mixtures were prepared according to Briggs and Williams [30] and Morcos [31] respectively, the second group was a positive control that fed a balanced diet with 10% of normal WKB on the expense of carbohydrates (nWKB), the third group fed a balanced diet with 10% of treated WKB sprayed with 20 ppm of ZnO-NPs (tWKB-1) and the last group fed a balanced diet with 10% of treated WKB sprayed with 40 ppm of ZnO-NPs (tWKB-2). The experiment continued for 28 days (four weeks). During this period, animals were monitored for mortality, clinical signs, body weight, body weight gain, food consumption, and food efficiency, after experimental period, rats were fasted overnight (12 h) and blood samples were collected from retro-orbital vein under light anesthesia with ether. Plasma was separated and kept at -20 °C for various biochemical analyses. Liver and kidney were immediately separated, washed with saline, dried and weighed, then, they were preserved in 10% neutral buffered formalin for histopathological examination.

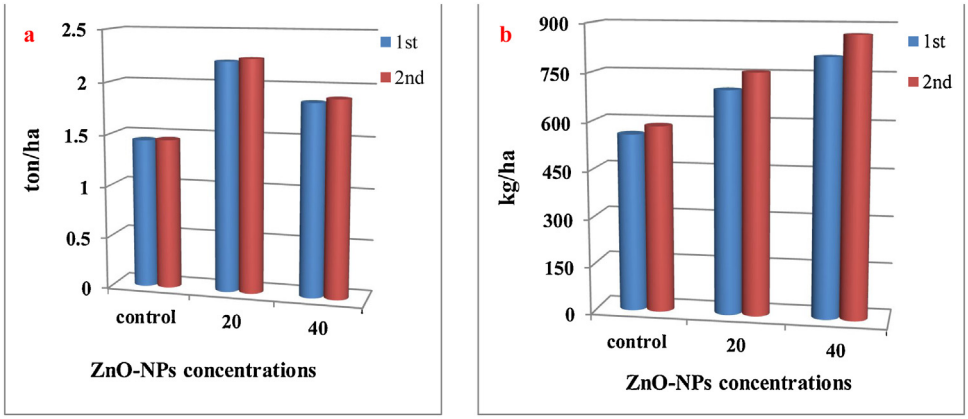

Fig. 1. Effect of ZnO-NPs concentrations on (a) seeds yield (ton/ha) and (b) shoot residues (kg/ha) of WKB plant during two successive seasons 2016 (1st) and 2017 (2nd).

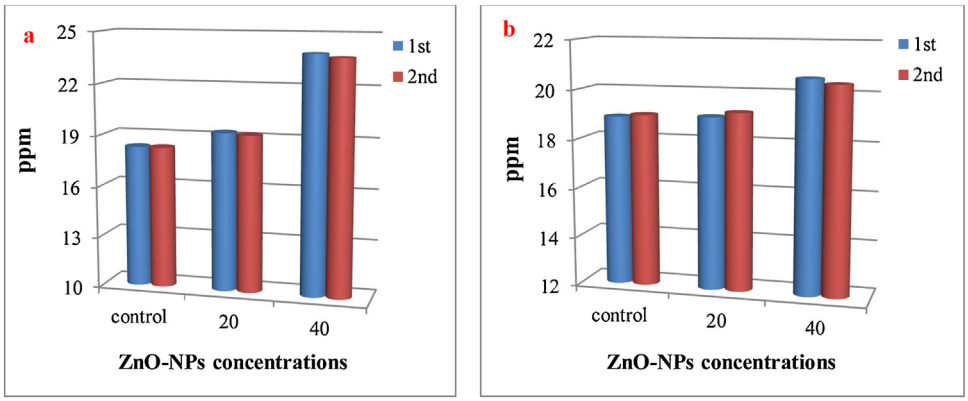

Fig. 2. Effect of ZnO-NPs concentrations on the zinc content in WKB leaves (a) and seeds (b) during two successive seasons 2016 (1st) and 2017 (2nd).

2.4. Biochemical analysis

The following parameters were measured in plasma by spectrophotometric method using commercial kits provided from Biodiagnostic, Egypt; aspartate transaminase (AST) and alanine transaminase activity [32], alkaline phosphatase activity (ALP) [33], urea level [34], uric acid [35], creatinine concentration [36], total protein [37], albumin [38], triglycerides concentration [39], total cholesterol [40], high-density lipoprotein cholesterol (HDL-ch) [41], and low-density lipoprotein cholesterol (LDL-ch) [42].

2.5. Histopathological study

Liver and kidneys were dissected out and fixed instantaneously in 10% formalin saline for 24 h. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point 55–60 °C). Sections of 6μm thickness were prepared and stained with Haematoxylin and eosin for histopathological examination [43].

2.6. Statistical analysis

Data were designated as a mean ± standard error of the mean (SE). Statistical analysis of the results was performed using SPSS-PC software. One-way ANOVA test was used to compare the statistical difference between various groups. $p \leq 0.05$ was used as the criterion of statistical significance.

3. Results

3.1. The yield of WKB plant

Data presented in Fig. 1**a** reveals that the yield of seeds per hectare of WKB plant showed the highest value by using 20 ppm of ZnO-NPs during two seasons 2016 and 2017. On the other hand, the WKB plants sprayed with 40 ppm ZnO-NPs recorded the maximum weight of shoot residues per hectare (Fig. 1b).

3.2. Zinc content in leaves and seeds of WKB

Data in Fig. 2 **a&b** illustrated that foliar application of ZnO-NPs at a concentration of 40 ppm on WKB plants recorded the greatest value of zinc content in the leaves and the seeds, on a dry weight basis during two successive seasons 2016 and 2017.

3.3. Nutritional and biochemical parameters

Table 1 and 2 demonstrate the changes in various nutritional parameters. nWKB, tWKB-1 and tWKB-2 showed a decreased of final body weight, body weight change, total food intake, food efficiency ratio, and relative liver weight as compared to normal control.

Table 1
Nutritional changes for various studied groups.

groups	Normal control	nWKB	tWKB-1	tWKB-2
Initial body weight (g)	130 ± 2.6	120 ± 4.2	131 ± 4.9	131 ± 4.2
Final body weight (g)	138 ± 2.8	90 [a*]± 3.3	120 [ab*] ± 6.9	112 [b*]± 6.3
body weight changes (g)	8 ± 3.7	−30 [a*]±2.9	−11 [ab*]± 4.9	−19 ±4.1
Total food intake (g)	508 ±19.1	369 [a*]±16.6	453 ± 47.4	436[b*]±21.1
Food intake /Day (g)	15.9 ± 0.6	11.5 ±0.5	14.2 ± 1.5	13.6[b]± 0.7
Food efficiency ratio	0.0186 ± 0.004	−0.0845[a*]± 0.01	−0.02 [b*]± 0.005	−0.045 [ab*]± 0.01

Values are expressed as mean ± S.E.
[a*]Values are significantly different at $p \leq 0.05$ as compared to normal control.
b*Values are significantly different at $p \leq 0.05$ as compared to nWKB.

Table 2
Relative organ weight for different groups.

groups	Normal control	nWKB	tWKB-1	tWKB-2
Relative liver weight (g%)	4.02 ± 0.152	3.31[a*]± 0.133	3.45[b*]± 0.119	3.66 ± 0.26
Relative kidney weight (g%)	0.897 ± 0.03	0.909 ± 0.06	0.868 ± 0.03	0.856 ± 0.02
Relative spleen weight (g%)	0.478 ± 0.046	0.571 ± 0.074	0.346[b*]± 0.0 6	0.375[b*]± 0.02

Values are expressed as mean ± S.E.
[a*] Values are significantly different at $p \leq 0.05$ as compared to normal control.
[b*] Values are significantly different at $p \leq 0.05$ as compared to nWKB.

Table 3
Liver and kidney markers for various experimental groups.

groups	Normal control	nWKB	tWKB-1	tWKB-2	ANOVA	
					F-ratio	P-value
AST (U/ml)	127 ± 5.46	122 ± 4.01	119 ± 5.04	110[a,*]±5.23	2.065	NS
ALT (U/ml)	49.3 ± 2.19	50 ± 2.22	56.7[a,*]±2.82	47.5[c,**]±2.17	2.861	–
ALP (U/L)	437 ± 47.9	516 ± 17.97	447 ± 42.9	481 ± 29.9	0.958	–
Urea (mg/dL)	33.3 ± 2.87	30.6 ± 2.67	42.4[b,*]±4.35	36 ± 3.63	2.155	–
Uric acid (mg/dL)	1.25 ± 0.195	1.2 ± 0.163	1.23 ± 0.123	1.1 ± 0.132	0.186	–
Creatinine (mg/dL)	0.92 ± 0.072	0.72 ± 0.067	0.82 ± 0.071	0.88 ± 0.085	1.438	–

Values are expressed as mean ± S.E.
[*] Values are significantly different at $p \leq 0.05$.
[**] Values are significantly different at $p \leq 0.01$.
[a] Values are significantly different as compared with control group.
[b] Values are significantly different as compared with nWKB group.
[c] Values are significantly different as compared to tWKB-1.

Final body weight and food efficiency ratio were significantly increased in both tWKB-1 and tWKB-2 groups as compared to nWKB group. Also, body weight changes (g) was significantly increased in tWKB-1 than nWKB. Total food intake was noticeably increased in tWKB-2 group as compared to nWKB group. Meanwhile, Relative liver weight was significantly increased in tWKB-1 than nWKB while relative weight of spleen was significantly decreased in both tWKB-1 and tWKB-2 groups as compared to nWKB group.

Table 3 demonstrated that both renal function tests including plasma uric acid and creatinine and liver function tests including plasma ALP, of both tWKB-1 and tWKB-2 groups were not significantly different than those of normal control. Meanwhile, plasma AST level in tWKB-2 group showed a significant decrease than that of normal control. Also, plasma ALT level in tWKB-1 group showed a significant increase than that of normal control. While ALT was significantly decreased in tWKB-2 than tWKB-1

The effects of different dietary supplements on plasma protein and lipids were presented in Table 4. Data revealed that nWKB, tWKB-1 and tWKB-2 groups considerably significantly elevated plasma albumin level as compared to that of normal control. LDL-ch was markedly elevated in tWKB-2 group as compared to nWKB group. Other parameters showed non-significant changes as compared to that of normal control.

3.4. Histopathological examination

Examination of liver sections of the normal control group showed the normal structure of the hepatic lobules. Each lobule was formed of cords of hepatocytes and blood sinusoids in-between. The hepatocytes are polyhedral cells with one or rarely two spherical nuclei and abundant cytoplasm. The cytoplasm of such cells was granular and strongly eosinophilic. The nuclei of the hepatocytes were large with peripherally dispersed chromatin and prominent nucleoli (Fig. 3A).

Sections of liver of rats of nWKB group showed lymphocytic infiltration in the portal and periportal areas with dilated and congested veins. Haop of edema in the periportal area was compressed the surrounding hepatocytes. These sections also showed dilated and congested portal vessels. The hepatocytes around the dilated congested vessels appeared variable in shape and size (Fig. 3B). In some cases, fatty changes appeared in the form of micro or macro vesicular (Fig. 3C).

Examination of the liver of rats in tWKB-1 group showed the normal architecture of the hepatic lobule with granulated cytoplasmic hepatocytes (Fig. 3D). On the other hand, some sections showed the normal hepatic lobules structure and portal areas except foci of necrotic hepatocytes (Fig. 3E).

Examination of the liver of tWKB-2 group showed the normal architecture of the hepatic lobules with granulated cytoplasmic

Table 4
Plasma protein, albumin, and lipids for various experimental groups.

groups	Normal control	nWKB	tWKB-1	tWKB-2	ANOVA	
					F-ratio	P-value
Protein (g/dL)	6.49 ± 0.42	6.63 ± 0.61	5.88 ± 0.44	6.68 ± 0.45	0.572	NS
Albumin (g/dL)	3.16 ± 0.11	3.98[a,***] ±0.17	3.55[a,b,*] ±0.07	3.77[a,**] ±0.14	7.716	0.001
Triglycerides (mg/dL)	66.1 ± 4.11	62.1 ± 5.34	57.6 ± 3.92	61.4 ± 5.34	0.545	NS
Total cholesterol (mg/dL)	117 ± 8.6	109 ± 9.04	110 ± 5.61	116 ± 12.1	0.202	–
HDL.ch (mg/dL)	55 ± 4.48	61.2 ± 7.23	53.7 ± 6.22	50.3 ± 3.89	0.651	–
LDL.ch (mg/dL)	38 ± 3.73	35.5 ± 2.39	39.3 ± 5.04	43[b,*] ±2.02	0.796	–

Values are expressed as mean ± S.E.
[*] Values are significantly different at $p \leq 0.05$.
[**] Values are significantly different at $p \leq 0.01$.
[***] Values are significantly different at $p \leq 0.001$.
[a] Values are significantly different as compared with control group.
[b] Values are significantly different as compared with nWKB group.

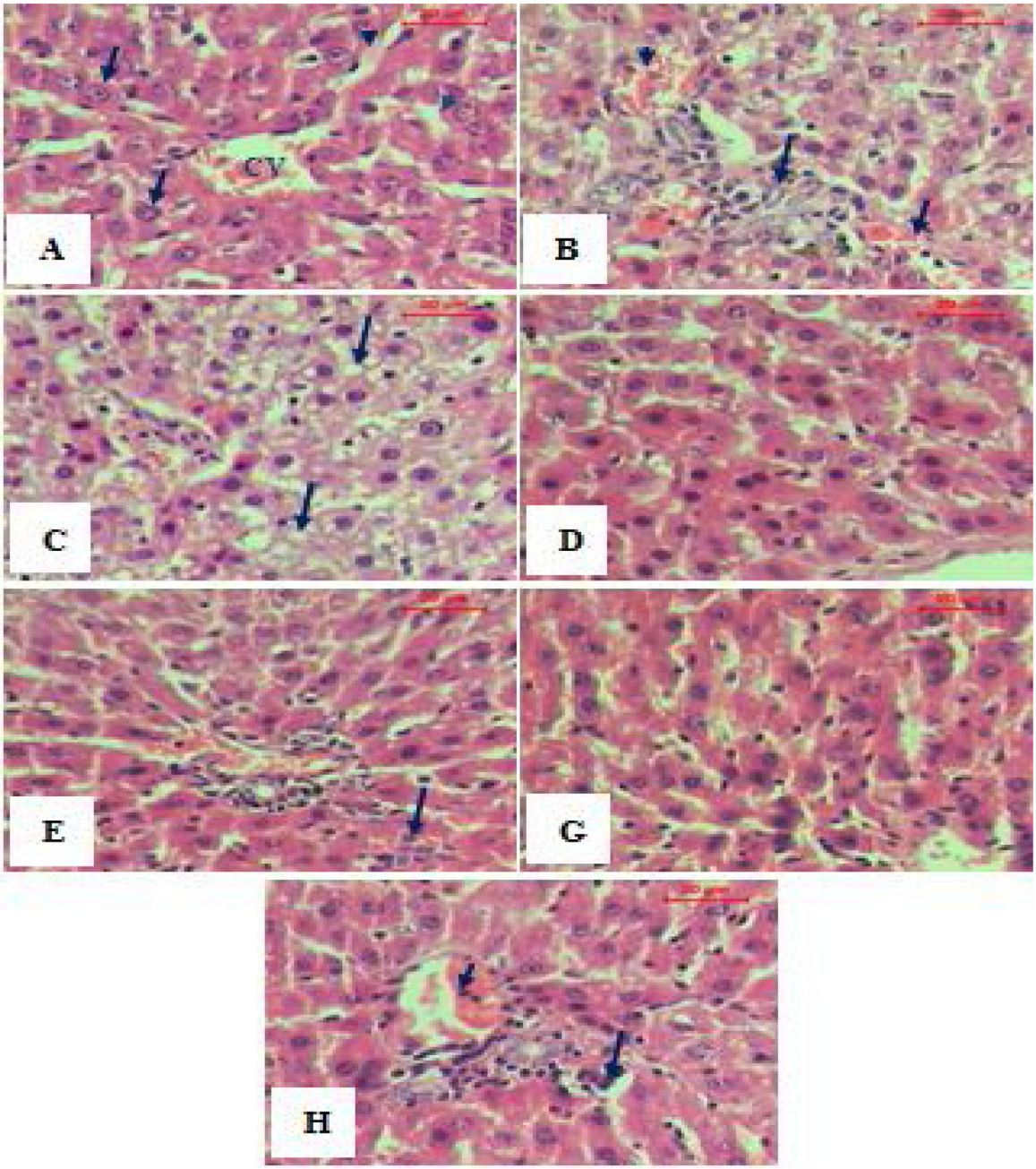

Fig. 3. A photomicrograph of section of liver of (A) normal control group, (B & C) nWKB goup, (D & E) tWKB-1group, and (G & H) tWKB-2 group (H & E stain, Scale Bar: 20 μm).

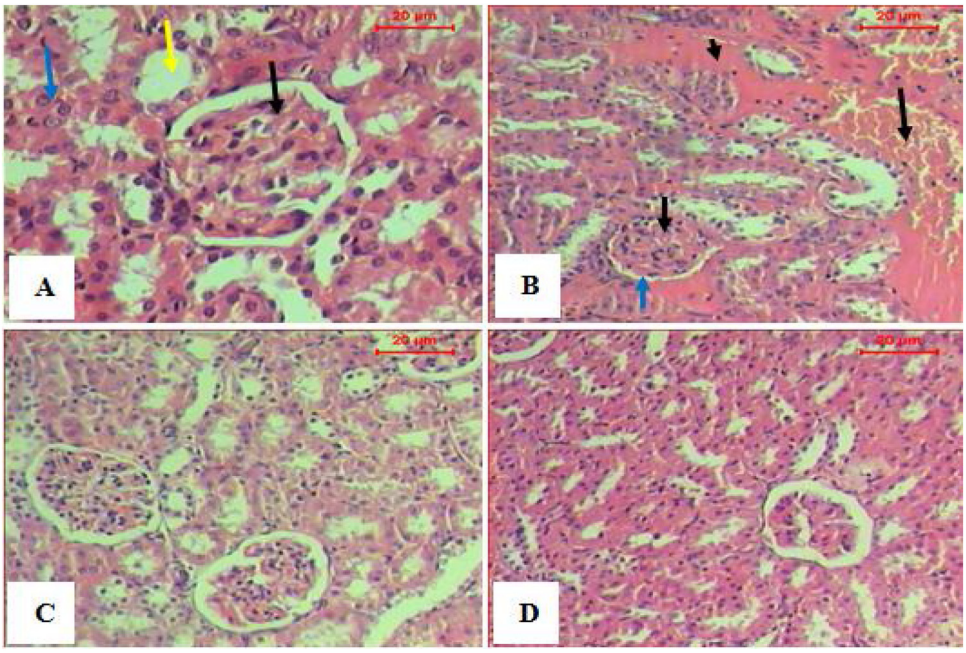

Fig. 4. A photomicrograph of sections of kidney of (A) normal control group, (B) nWKB goup, (C) tWKB-1group, and (D) tWKB-2 group (H & E stain, Scale Bar: 20 μm).

hepatocytes (Fig. 3G). On the other hand, some sections showed the normal hepatic lobules structure. Mild lymphocytic infiltration in the portal and periportal areas with dilated and congested veins was seen (Fig. 3H).

Examination of kidney sections for normal control group showed the normal structure of the nephron, the functional unit of the kidney, consisted of two major components, the renal corpuscle, and the renal tubule. The renal corpuscles were formed of two structures, Bowman's capsule and the glomerulus. The proximal convoluted tubule was lined with simple cuboidal epithelium and its lumen is narrow. The cells of this epithelium had acidophilic cytoplasm and contained only about 3–5 spherical nuclei, usually located in the center of the cells. The distal convoluted tubule was lined with simple cuboidal epithelium. The cytoplasm of these cells appeared somewhat less acidophilic than in the proximal convoluted tubules (Fig. 4A).

Microscopic examination of kidney Sections of rats in nWKB group showed hemorrhagic and edema areas in the interstitial spaces of the cortex. The most characteristic pathologic lesion was the necrosis that hit many of the proximal convoluted tubules. Moreover, the epithelial cell nuclei suffered from different degrees of degeneration (pyknosis and karyolysis). Besides, the renal corpuscles showed a collapse of the glomeruli and wide urinary spaces (Fig. 4B).

Histopathological investigation of the kidney of both groups' tWKB-1 and tWKB-2 showed the normal architecture of the renal corpuscles and the renal tubules (Fig. 4 **C& D**, respectively).

4. Discussion

In recent years, nanotechnology has been widely used in many fields, including ZnO NPs [22]. Zinc is an essential trace element for human health. Zn is either commonly present in food or may be added as nutritional supplement. Also, it is an important component in many metalloenzymes. It also works as a cofactor for RNA and DNA polymerases and it is essential for the biosynthesis of fatty acids, for inflammatory and immune system, rapid cell division and for vitamin A metabolism [44]. Furthermore, zinc antioxidant activity is well documented [45]. To our knowledge, there are little studies on the impact of crops fertilized by ZnO-NPs on human health. Where, using ZnO-NPs in fertilization of crops can affect both consumers (by eating crops) and farmers (during spraying) [14].

In developing countries, animal proteins are so expensive, therefore, plant proteins are the alternative especially that of legumes seeds. Their chemical composition and their biological effects may be varied in consequence of soil, fertilizers, and climate. In our study, we tried to investigate the nutritional, biochemical and histological changes that might result from eating WKB sprayed with different concentration of ZnO-NPs.

The increases of the yield and zinc content of WKB by using 20 ppm and 40 ppm of ZnO-NPs, respectively. This due to using of ZnO nano fertilizer where, zinc plays a critical role in carbohydrate and proteins metabolism as well as it controls plant growth hormone [46]. Similar results were found before by Elizabath et al. [47] they recorded that the foliar application of ZnO-NPs improved the yield of carrot plant (tone/ha) with 150 ppm a concentration of ZnO-NPs. Also, Du et al. [48] observed that the maximum grain yield and biomass of wheat treated with ZnO NPs and ZnSO4 were increased by 56%, 63% and 55%, 72%, respectively when compared with control. Khanm et al. [49] found that the use of ZnO-NPs fertilizers with a concentration (400 ppm) has a large positive effect on physiological and yield of tomato plant compared with zinc sulphate (800 ppm). In addition, Shaviv et al. [50] showed that nano-sizing makes fertilizer nutrients more accessible to nano-scale plant pores.

Our nutritional emerged data revealed that diets which supplemented with WKB significantly reduced rat body weights as compared to rats fed normal diet. These results are nearly consistent with those of Tormo et al. [51] and Chokshi [9]. WKB is considered as a rich source of starch blockers which promote the reduction in weight by decreasing complex carbohydrate digestion known as WKB inhibitors of α-amylase activity [51]. Consequently, the reduction of calories derived from carbohydrates was the end result with concomitant weight loss. Besides, there are a variety of antinutritional factors, although they are present in small amounts in cooked bean, they still reduce feed efficiency and impaired the gain of weights. Also, they interfere with protein and carbohydrate

digestibility [29]. Moreover, legumes are considered to be a rich source of fibers. The latter has pronounced effects on weight reduction [52]. Also it was obvious that WKB markedly reduced food intake with a concomitant reduction in food efficiency ratio that is in accordance with Fantini et al. [53]. We can notice that WKB fertilized by ZnO-NPs could modulate the decrease in body weight and food efficiency ratio probably by increasing the food intake. Besides our results demonstrated a reduction in liver weights which attributed to the reduction in body weight. Meanwhile, splenomegaly was found in rats fed WKB which may be explained by the extreme reduction in body weight that may induce anemia with concomitant splenomegaly [54].

Our biochemical data demonstrated that markers of liver functions were not markedly changed in the WKB supplemented groups when compared to the normal control group except for ALT activity in tWKB-1 group, there was a significant increase in ALT level as compared to the normal control group. On the contrary, a significant reduction in AST activities in tWKB-2 group as compared to those of normal control group. The reduction of ALP and AST activities were confirmed by Najafzadeh et al. [55] study. The mechanism involved may be the enhancement of the status of the liver and the biliary duct by the supplement. Afifi et al. [56] and Badkoobeh et al. [19] proved that ZnO-NPs improved the changes in liver induced by diabetics and doxorubicin, respectively via their antioxidant effects. Liver enzymes levels are elevated in cases of liver injury as hepatocytes membranes are ruptured. ALT is present mainly in the cytoplasm of hepatocytes with a minor concentration of AST that mainly present in the mitochondria. Therefore, in mild hepatic injury, serum has the cytoplasmic enzyme (ALT), whereas in severe hepatic disorders the predominant form is AST and the ratio of AST/ ALT is raised [57]. Also, ALP is normally excreted via bile by the liver. In cases of hepatocytes injury, defect in the secretion of bile result in an increase in ALP activity in blood indicating hepato-biliary disorders. Therefore, it is clearly obvious that WKB administration neither affected hepatic or hepato-biliary function. Our histopathologic studies of liver tissues confirm our biological results which showed no change in liver tissues in both tWKB-1and tWKB-2 groups.

In addition, WKB did not alter the hepatic synthetic power as it caused no change in plasma protein level. Besides, there was no apparent renal impairment, where there were not any marked changes in the marker of renal function except for urea level in tWKB-1 group which was significantly increased as compared to the nWKB group. Also, a non-significant reduction of creatinine by WKB and a reduction of creatinine level may be attributed to the increased creatinine clearance and the increased expression of genes that are related to the ammonium elimination [13].

As regards to the effect of both concentrations of ZnO-NPs-WKB treatments on urea, we can deduce that both concentrations increased plasma urea where the increase was significant only in low concentration as compared with nWKB group. This increase may be explained by the changes in protein metabolism not by nephrotoxicity since both uric acid and creatinine levels were not changed. Therefore, uric acid estimation is more accurate than urea determination for the evaluation of kidney function. Our histopathologic studies of renal tissues in both tWKB-1and tWKB-2 groups confirm our biological results which showed no change in renal tissues.

Concerning the influence of WKB on lipid profile and proteins, we could notice that plasma albumin level was elevated in nWKB, tWKB-1 and tWKB-2 groups as compared to that of the control group. The increase in plasma albumin level by WKB, either normal or treated by ZnONPs, may be attributed to the high proteolytic activity of processed seed [58]. Another explanation may be due to the improvement of the liver status with the concomitant increase in albumin synthesis. Albumin level significantly reduced in tWKB-1 group when compared with nWKB group, such effect may be attributed to the detrimental influence of ZnO-NPs on the liver function [24] or may be also explained by consumption of albumin for the defense against oxidative stress induced by nanoparticles [59].

WKB supplementation improved the levels of lipid parameters although they were insignificant. Our results were in agreement with those of Pereira et al. [60] and Olivia et al. [61]. The high fiber content of WKB, saponins, vegetable proteins and polyphenolics may participate in the hypolipidemic effects of WKB [62]. Regarding the changes in lipid parameters by ZnO-NPs-WKB, our data showed that a markedly elevated in LDL-ch and a decrease in HDL-ch levels although it was insignificant in tWKB-1group as compared to those of nWKB group. Also, non-marked increase in total cholesterol, a marked increase in LDL-ch concentrations and slight reduction of the HDL-ch level were observed in tWKB-2 group as compared to those of nWKB group.

5. Conclusion

Our study demonstrated that white kidney bean supplementation had no deleterious effects either on kidney and liver function or on the lipid parameters. Moreover, our findings suggest that WKB sprayed with nanoparticles of ZnO had no obvious toxicity concerning both the hepatic and renal functions. Meanwhile, it slightly affected lipid profile. Therefore, the use of such particles should be with caution and further studies are warranted.

Ethics approval and consent to participate

The study protocol was approved by the Ethical Committee Board of the National Research Centre, Cairo, Egypt, with registration number 16457.

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