The protective role of propolis against multi heavy metals-induced oxidative stress-hepato-renal damage in the male of albino rats

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Abstract

The study was designed to clarify the hepato-renal protective effects of propolis extract against heavy metals-induced toxicity via oral administration to the males of albino rats. Lead (Pb), Nickel (Ni), Cadmium (Cd), and Antimony (Sb) are toxic heavy metals have the ability to produce reactive radicals in the biological systems causing public and animals health hazards through disrupting balances between pro-oxidant and antioxidant defense system, resulting in excessive reactive oxygen species (ROS) production. The most commonly affected organs are liver and kidney. Propolis is a natural product with different shapes and resinous substance collected by honey bees, it attenuates many diseases damage due to its anti-oxidative action and its potentiality to minimize the deleterious effects of free radicals on tissues. The concentrations of Pb, Cd, Ni and Sb as well as the activities of antioxidants endogenous enzymes including; glutathione peroxidase (Gpx), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD) were all determined in the tissues of liver and kidney; while aspartate transaminase (ASAT), alanine transaminase (ALAT), total protein (TP), urea and createnine, were measured in the serum of experimental rats beside histopathologicl examination in the tissues of liver and kidney. The oral administration of propolis provided a significantly therapeutic role against multi-metals-induced hepato-renal toxicity with relative improving to histopathological changes because of its scavenging and chelating properties as concluded from the present investigation.

1- Introduction

Heavy metals are well known for many years to be, toxic, affecting the internal organs of the human body and harm public health even at trace levels (Dessouki et al., 2000). They can disrupt membrane potentials of normal cell and tissue function through binding with proteins and other bio-molecules **(Mudgal** et al., **2010).** Metals cannot be decomposed biologically, instead they transformed from one oxidation state or organic complex to another (Gisbert et al., 2003). Among the various heavy metals, lead (Pb), cadmium (Cd), Nickel (Ni) and Antimony (Sb) are proverbial as the most dangerous metals due to their high toxicity that attributed primarily to oxidative stress which is one of the critical mechanisms implicated in the heavy metals-induced toxicity since, the free radicals in biological systems produced from redox reactions carried out by certain metals, these reactions create oxidative damage to proteins and DNA and inhibit the antioxidant defense systems (Dal-Corso et al., 2010 **and** Fu & Xi, 2019**).** Owing to oxidative damages in various body organs are correlated with toxic metals exposure, great attention was given for the usage of natural products to strengthen the cell antioxidant and to protect it from the heavy metals-induced damage (Dewanjee et al., 2013). Propolis is a natural sticky, resinous mixture produced by honey bees and gathered from the tree buds, leaves, sap, flows, and other botanical sources (Simone-Finstrom and Spivak, 2010**).** It has been characterized variously as an anti-bacterial, anti-viral, anti-inflammatory, anti-oxidant, and anti-carcinogenesis agent (Koya-Miyata, et al., 2009). Propolis consists mainly from flavonoids, phenolics and other various aromatic compounds. Its bio-flavanoids are antioxidant molecules that play very important role in the scavenging of free radicals (Da Silva, et al., 2004 **and** Bankova, 2005**).** Propolis work through various mechanisms and at various sites including chelation or

antagonism of heavy metals in order to, nullifying their capacity to generate free radicals, thereby neutralizing their oxidative effect and interrupting the auto-oxidative chain reaction **(Bolarinwa** et al., **2020).** Liver and kidney are important organs when the effects of toxins are investigated, since these organs play a central role in the metabolism and detoxification of biological substances. Also, most substances absorbed by the intestine passed first through liver or kidney where toxins and heavy metals may accumulate **(**Saidi et al., 2013). Hence, there is little information about the hepato-renal protection of propolis against multi-heavy metals toxicity. Therefore the purpose from this experimental study is to clarify the hepato-renal protective effects of propolis against metals toxicity.

2- Material And Methods

2-1- Chemicals:

All reagents and chemicals were of analytical grade quality with high purity. Lead, cadmium, nickel and antimony (SCP SCIENCE, Canada) were used for mixture preparation of oral administration solutions. Standard solutions (Merck, Germany) were used to create calibration curves for toxic metals analysis, while concentrated HNO_3 (65%, Merck, Germany) and H_2O_2 (30%, Sigma-Aldrich, Germany) were used for tissue digestion. All chemical and reagents for the examination of antioxidant status were purchased from Bio-diagnostic (Egypt).

2-2- Propolis:

Propolis was obtained from hives of royal bee company Cairo, Egypt during summer 2018. It was bulk of glue like brownish material resulted from scrapping off the frames of bee hives. It was initially stored in a freezer in order to kill insect eggs and to facilitate removal of debris and fragmentation. Propolis extraction was performed using Ethanol Extracts of Propolis [EEP] according to Paviani et al., (2010) by using 250g of propolis powder which transferred to 1000 ml volumetric flask, and completed to 1000 ml by 70% ethanol HPLC grade in the absence of bright light, with moderate shaking using a magnet stirrer for 1 day at room temperature. After a week, the extracts were filtered through filtration pump, and the solvent was evaporated off in a vacuum oven at a temperature of 60°C to obtain pure propolis extracts which diluted by saline and given to rats.

2-3- Animals:

Thirty male *Wistar rats* weighing approximately 200 g were purchased from the Egyptian holding company for biological products & vaccines (Cairo, Egypt) and used as experimental animals. Rats were housed and maintained under standard controlled conditions of good ventilation, normal temperatures, and humidity range (temperature 25 ± 4°C, relative humidity of 35–60%, 12-h light-dark cycle) and allowed free access to drinking water (metal-free water) unlike, feeding which was restricted according to times of antioxidant dosing during the experiment period (75 days). All experimental animals were conducted in accordance with criteria of the investigations and ethics committee of the community laws governing the use of experimental animals.

2-4- Study design and experimental procedure:

After ten days of acclimatization, rats were marked then placed into suitable cages by ratio of ten rats per cage and randomly divided into three groups: one negative control group and two experimental groups (heavy metals group and propolis group). A long 75 days experimental groups received a single dose (1 ml/rat/day) of freshly prepared aqueous solution containing heavy metals mixture with concentrations exceeds the maximum limits of both WHO and Egyptian standard regulation by 10 fold which are; (100, 200, 30, and 200) ppb for (lead, nickel, cadmium, and antimony) respectively. The propolis group was post-treated by propolis extraction in a dose 200 mg/kg/day body weight (b.w.) for 75 days while the control group was neither treated nor contaminated. The treatment of all animals was performed by an oral gavage tube directly into stomach.

2-5- Tissue Preparations:

Blood samples were collected where retro-orbital venous plexus exist, after anesthetic administration. Suitable amounts of blood were collected in test tubes without anticoagulant for obtaining serum which was separated and transferred to eppendorf tubes to be frozen for biochemical assays. Organ systems; livers and kidneys, were removed and separated into three parts. One tissue sample (0.5 g) was frozen and stored for the investigation of antioxidant status; a second was frozen and stored for toxic metals analysis and a third tissue sample was preserved in formalin for histopathological examination.

2-6- Toxic Metals Analysis:

After the animals have been sacrificed, wet tissue samples weighing about 0.5 g were placed in Teflon containers with 7 ml conc. HNO_3 and 1 ml H_2O_2 and digested using high performance microwave sample digestion (model Milestone ETHOS UP). Digestion was carried out according to the Milestone's recommendations and Kingston & Walter, (1998) based on **US EPA Method 3052**. After digestion program, the samples were transferred to 50 ml volumetric flasks and the volumes were completed to 50 ml using free-metal water (grade A). The amounts of Pb, Ni, Cd, and Sb in the tissue samples were determined by Inductively Coupled Plasma Optical Emission spectroscopy ICP-OES (I Cap Thermo 7400, Thermo Fisher Scientific, Waltham, Ma, USA) according to **APHA (2012)**.

2-7- Biochemical Assays

The measured biochemical parameters in rat's serum included total protein (TP), alanine aminotransferase enzyme activity (ALAT), aspartate aminotransferase enzyme activity (ASAT), serum urea and serum creatinine. All biochemical assays were performed with commercial reagents and according to good laboratory practices using Auto analyzer Roche Cobas instrument owned to Mabaret El-Asafra Labs. Alexandria, Egypt, awarded by ISO 15189; not only that, but they were confirmed using UV-VIS spectrophotometer (Labomed, Inc; Los Angeles, USA) methods according to Burtis & Ashwood, (1999**); IFCC (1986); Glick**et al., **(1986);** and Bowers and Wong (1980) for TP, (ALAT & ASAT), urea and createnine respectively.

2-8- Antioxidants Analysis:

Tissues samples (livers & kidneys) were rapidly excised, washed in ice-cold 0.9% NaCl, then an exact weight of each organ (0.5 g) was grinded through homogenizer in 4 ml saline solution (NaCl 0.9%). Each sample was centrifuged at 4000 RPM for 15 minutes, the supernatant obtained were transferred into eppendorf tubes, and frozen to be analyzed for antioxidants biomarker. Assessment of the following parameters was performed: glutathione peroxidase (Gpx), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD) according to, **Gross** et al., **(1967); Goldberg and Spooner (1983); Luck, (1974); and Kakkar** et al., **(1984)** respectively.

2-9- Histopathological Analysis

Liver and kidney tissues were subjected to histopathological examination. Microscopic examinations on paraffin embedded 5 µm tissue sections with hematoxylin-eosin were performed. Each section was examined under an optical microscope.

2-10- Statistical Analysis

Statistical analysis was performed using Graphpad prism 6.0 statistics (Graphpad Inc. San Diego, CA, USA) software. One-way and two-way analysis of variance (ANOVA) test were used. p -Values less than 0.05 were considered significant.

3- Results

3-1- Metals concentrations in liver and kidney:

Both experimental groups (heavy metals group and propolis group) receiving a single dose (1 ml/rat/day) of heavy metals mixture, demonstrated significantly higher levels of Pb, Ni, Cd, and Sb in the liver compared to the control group. The measured levels of metals in the liver of experimental groups receiving toxic mixture exhibited a statistically high significant difference when compared to the control *($p < 0.0001$)*. Additionally, within the kidneys tissues heavy metals group showed statistically higher significant *($p < 0.0001$)* in the levels of Ni whereas, Pb, Cd, and Sb were also significant *($p < 0.001$)* when compared to the control group. The amount of toxic metals in the heavy metals group had higher concentrations in comparison with values in the control and propolis groups. Furthermore, treatment with propolis revealed remarkable reduction in the concentrations of Pb, Ni, Cd, and Sb in both, liver and kidney tissues when compared with positive control group (heavy metals group). At the same time, liver exhibited high susceptibility to accumulate Pb and Sb while kidney was more affected by Ni and Cd. The deposition order of metals was Ni > Pb > Sb > Cd in the liver and Ni > Cd > Sb > Pb in the kidney, (Table, 1 and figure, 1).

3-2- Biochemical Assays:

Acute exposure to the investigated toxic metals administered in a mixture form resulted in the altered profile of some biochemical parameters. Both experimental groups had higher concentrations of ALAT, ASAT and urea compared to the control group, while there was a trivial increase in creatinine levels in

heavy metals groups, unlike total protein which showed slight decrease levels in both experimental groups, there were statistically significant differences in the levels of ALAT, ASAT, and urea among heavy metals group, when compared to the controls ($p < 0.001$, $p < 0.01$, $p < 0.01$, respectively), while total protein and createnine levels didn't produced any differences in the same groups when compared to the control. However, urea and createnine reaching significance when compared with heavy metals group ($p < 0.01$, $p < 0.05$). Biochemical parameters are shown in Table, 2; Figures, 2 (a, b, c, d, e).

3-3- Antioxidants Status:

After exposure to the heavy metals mixture, the activities of all investigated antioxidant enzymes (GPx, GR, SOD, CAT) exhibited a downward trend in liver and kidney tissues among both experimental groups (Heavy metals group and propolis group) with statistically significant effects when compared to the control group. Administration of propolis treatment demonstrated remarkable restoring in the activities of GPx, GR, SOD and CAT with statistically significant elevations ($p < 0.001$, $p < 0.0001$, $p < 0.0001$, $p < 0.01$, respectively) in the liver and ($p < 0.01$, $p < 0.01$, $p < 0.001$, $p < 0.01$) in the kidney when compared to heavy metals group. Observed redox parameters in liver, and kidneys of rats are presented in (Table,3 and figure,3).

3-4- Histopathological analysis:

Photomicrograph examination in the liver of control rats (Fig. 4A) showed intact hepatocytes arranged in cord-like pattern (white arrow) separated by blood sinusoids (black arrow) and portal area (P). Treatment with the metal mixture at heavy metal group **(Fig. 4B**), resulted in dilated blood sinusoids (black arrow), congestion and detachment endothelium of central vein (black arrow head), shrinkage and vacuolation of hepatocytes (white arrow) and necrosis of hepatocytes (white arrow head). Furthermore, liver tissues of rats at propolis group (Fig. 4C) showed mild dilation of blood sinusoids (black arrow), mild vacuolation of hepatocytes (white arrow) and intact endothelium of central vein (black arrow head). Additionally, photomicrograph illustration of kidney tissues at control group (Fig. 5A) displayed intact renal corpuscle (white arrow) and renal tubules (black arrow). Meanwhile, metals administrated at heavy metals group **(Fig. 5B**), caused degenerated glomerulus with hyaline cast (white arrow) and degenerated renal tubules (black arrow). Unlike, renal tissues of rats at propolis group (Fig. 5C), which showed normal renal corpuscles (white arrow) and degenerated renal tubules (black arrow).

4- Discussion

4-1- Toxic metals and propolis:

In fact, deposition and accumulation of heavy metals among the soft tissues and internal organs of the body depend upon many factors like, the status of metals, their natures, introducing route, dose

concentration and duration of exposure in addition, their capability to react with cell proteins, consumer sensitivity and susceptibility. Furthermore, livers and kidneys have the highest tendency to accumulate toxic metals after oral administration. In the most cases toxicity transport via blood and undergo intestinal absorption where they able to diffuse through red blood cells and cause severe health hazards. The present study proved that exposure to a mixture of metals in high levels (10x) for 75 days via oral administration caused combined toxicity in male rats such toxicity biomarkers attained significant decreasing after propolis treatment. Rats exposed to metals mixture only, exhibited high significant increasing in the concentrations of these metals inside livers and kidney tissues ($p < 0.0001$) in comparison to unexposed rats; the occurrence of early signs of oxidative stress in our study was observed after mixture treatment this may be due to the capability of investigated metals to stimulate a disturbance in the oxidant and antioxidant balance that is found in cells resulted in excessive formation of free radicals such as singlet oxygen, hydroperoxides ($HO_2^{\check{Z}}$), and hydrogen peroxide (H_2O_2) or overproduction of ROS that cause increase in oxidative damages parameters, as well as decrease in antioxidant defences, like GPx, CAT, SOD and GR, as a clear signs for cellular impairment in accordance to (Mousa et al., 2002; Flora, 2011 **and** Reckziegel et al., 2016). Not only that but, prolonged exposure to Cd may cause renal impairment through a decrease in the glomerular filtration rate and eventually may lead to renal failure rather than the ability of antimony to cause a loss in cell viability and other health effects involve the respiratory and cardiovascular systems likewise, nickel that able to rise structural changes and functional disruptions in various tissues and organs of the body. Similar toxic effects were observed by; (Chen et al., 2003; Xiao et al., 2010 **and** Liang et al., 2012). Other possible reasons for combined toxicity and these observations might be the differences in the metals bioavailability and their competitive affinity to protein transporters following oral administration with possible synergistic involvements; this was notably compatible with Andjelkovic et al. (2019). On the other hand, our data detected that propolis treatment after oral mixture metals exposure, afforded semi-normal recovering from many complications arose by metals effects; where significant reduction in the concentrations of all investigated metals was observed and confirmed statistically among liver tissues ($p < 0.001$) and kidney ($p < 0.01$) except cadmium which reduced without statistically significance. The depletion of lead, nickel, cadmium and antimony in the propolis treated group may be due to the antioxidant property of propolis that able to decrease the oxidative stress, inhibits progressive fluctuations convinced through metals mixture, repaired alterations rising in the liver and kidney close to healthy measurements (Mumtaz et al., 2020). Propolis can scavenge reactive oxygen and nitrogen species, prevents lipid peroxidation, upregulates biosynthesis of various cytoprotective and antioxidant proteins, and has an inhibitory effect on the inflammatory cytokines **(Gupta** et al., **2011;** Alghasham et al., 2013; Hosseini and Hosseinzadeh 2018). Also, propolis has vital role in preventing damage to membranes or proteins and regulating their activity by interacting or regulating specific enzymes and influencing cellular structures in addition to, reactivating the defense characteristics of enzymatic antioxidants to protect hepatic, renal and various tissues from metals induced oxidative damage (Tikare et al., 2013). Reversing the toxic effects of toxic metals is another beneficial property of propolis. The previous suggestions were reported also by many authors including, (Shukla et al., 2003; Rivera-Espinoza and Muriel, 2009 **and** Sethi et al., 2009).

4-2- Effect on Biochemical attributes:

The present study declared that heavy metals able to cause alterations in the blood biochemical attributes. Administration of oral mixture metals to rats resulted in significant differences in the investigated biochemical (decreased level of total protein; increased levels of urea nitrogen and creatinine, activities of ALAT and ASAT). We found no statistically changes in the level of total protein in the serum of rats from both experimental groups when compared to control although that, slight depletion was observed in TP; this is thought to be related to energy production during metals toxicity through metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; similar observations were reported by, Karmakar et al., (2000); Jadhav et al., (2007) and El-Amawy, (2016**)**. Seif et al., (2019) revealed significant elevation in serum activities of TP levels in comparison to control rats. We obtained significant elevation in urea level ($p < 0.01$) in heavy metals group and higher significant level ($p < 0.0001$) after propolis treatment; this may be caused when heavy metals inhibit the activities of antioxidant enzymes and reaches the soft tissues like kidney in the form of metal-metallothionein which filtrated in the glomerulus, and subsequently reabsorbed in the proximal tubules. It then, remains in the tubule cells and results in tubular damage. This interpretation is consistent with Godt et al., (2006); Jadhav et al., (2007) and Obianime & Roberts (2009**)**. Moreover, creatinine showed slight elevation after metal exposure, such elevation is a reflection of the degree of damage to glomerular filtration and is a sensitive biomarker for predicting kidney dysfunction Al-Attar, (2011**);** Athmouni et al., (2018) and AL-Megrin et al., (2020); however; createnine seemed normal after propolis treatment. Furthermore, statistically significant elevation in the activity of ALAT enzyme was observed in both experimental group among liver ($p < 0.0001$) and kidney ($p < 0.001$) which may attributed to subsequently releasing of mitochondrial enzymes into the blood as a result of tissues damage under toxic metals stress Gao et al., (2013) and Cobbina et al., (2015). Similarly, high activity of ASAT in both groups probably occurred under toxicity of heavy metals that caused different degrees of injuries in the liver leading to the enzyme releasing into the blood as biomarker of liver cell damage, this is line with other authors; Reckziegel et al. (2016); Okesola et al. (2018).

4-3- Histopathological effects:

Histological investigations showed that the present concentrations of heavy metals mixture caused relative damages to the tissues of rats at heavy metal group. Only liver and kidneys of animals from the control area almost showed no histopathological changes. alterations in the liver tissue of rats from metals group could attributed to the toxic effect of heavy metals mixture that affected liver functions causing loss of hepatocytes membrane integrity, liver enzyme elevations and reduction in the serum total protein in addition to, formation of highly reactive radicals and subsequent lipid peroxidation, which might caused cytotoxicity in accordance with El-Refaiy and Eissa, (2013**);** Aksu et al., (2017) and Abdelhamid et al., (2020). At the same time, there was relative damage reduction in the propolis group which displayed reduction in liver damages markers that converted to; mild dilation of blood sinusoids, mild vacuolation of hepatocytes and intact endothelium of central vein. Similar observations were

reported by; García-Niño & Pedraza-Chaverrí, (2014) and Okail et al., (2020). Additionally, there were renal damages in the contaminated heavy metals group appeared in, degenerated glomerulus with hyaline cast and degenerated renal. These changes may be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by free metal ions in the renal tissues which binds to metallothionein forming a complex released into the blood stream. This complex causes injury, mainly in the cortical region, reaching the proximal tubule and causing a gradual loss of the organ's function. These observations were in the same way of Thijssen et al., (2007) and Renugadevi & Prabu, (2009). Nonetheless, these histopathological changes were reduced in the kidney of rats treated with propolis consequently, mild degeneration and less necrosis in renal tubules appeared with propolis as well as, normal renal corpuscles. Our findings were in agreement with Garoui et al., (2012); Soliman et al., (2015); Okail et al., (2020).

4-4- Antioxidant enzymes and propolis:

Dosing of heavy metals mixture produced lower level of glutathione peroxidase (GPx) in liver ($p < 0.0001$) and kidney ($p < 0.001$) tissues at heavy metals group in comparison with control group, this could probably due to the utilization of its defense mechanism against toxic metals within these organs as a result of ROS generation; such suggestion is matched with Ercal et al., (2001); Saxena et al., (2005) **and** Bas & Kalender (2016). However, rats that exposed to toxic metals and treated with propolis exhibited significant improving in the activities of GPx in livers ($p < 0.001$) as well kidney ($p < 0.01$) when compared with heavy metals group; that issue clarify the protective role of propolis in recovering the affected tissues of liver and kidney as healthy rats through reducing combined toxicity of investigated metals; these findings were corroborated very well with Salama & El-Bahr, (2007); Bhadauria, (2012) **and** Mohamed et al., (2017). Additionally, after propolis treatment, both livers and kidneys of rats exhibited statistical improving ($p < 0.0001$ and $p < 0.01$, respectively) in the activities of glutathione reductase (GR) which previously showed significant decrease among the livers ($p < 0.0001$) and kidneys ($p < 0.001$) of rats at heavy metals group comparing to control, this may attributed to the ability of investigated metals to overcome the vital role of GR in order to interfere with the disulfide bond of glutathione enzyme and inhibits its activity therefore, prevent the optimal balance and make cells more susceptible to oxidative damage; our observations are in the same way of those obtained by; Ercal et al., (2001) and Jomova & Valko, (2011). In line with this, activities of superoxide dismutase (SOD) enzyme attained significance decreasing in liver ($p < 0.0001$) and kidney ($p < 0.001$) tissues at heavy metals group which might interpreted to copper depletion which leads to decreased capability of cells to produce SOD, thus increasing their propensity to oxidative damage and disrupt SOD pivotal role on producing H_2O_2 in cells by a dismutation of superoxide radicals generated in the oxidative process, the present findings are nearly in accordance with Dixit et al., (2012) and Emediong et al., (2019); however, the antioxidant property of propolis led to statistically significant increasing at propolis group by $p < 0.0001$ in liver and $p < 0.001$ in kidneys comparing to heavy metal group. Finally, catalase activity in the heavy metals group was almost beneath the half of its activity in control group within both liver ($p < 0.01$) and kidney ($p < 0.001$) tissues; this observation reflects how far the toxic metals affect catalase activity which relatively restored in the investigated organs from propolis groups ($p < 0.01$) by the action of antioxidant property; the depletion of

catalase is probably because of its able to prevent the toxic metal-induced consumption of O_2 inside cells, thus, capturing H_2O_2 before it can escape the cell then converting it to water and molecular oxygen. In this way, catalase can maintain the concentration of O_2 either for repeated rounds of chemical reduction or for direct interaction with the toxin. Similar interpretations were published by; Matés, (2000**)** and **HO** et al., **(2004)**.

Although several studies regarding propolis efficacy versus metals induced ROS toxicity have been conducted, there is an obvious lack of data on mechanisms underlying the propolis antioxidant property against toxicity of some metals.

5 - Conclusion

Our results showed a more profound toxicity of metal mixtures (Pb, Ni, Cd, Sb) via oral administration that induced toxic effects in the liver, and kidneys of adult *Wistar rats*. The main toxicity mechanism of combined metals is oxidative stress which confirmed by a disturbed redox status and histopathological changes in the investigated tissues of experimental rats besides clear biochemical alterations. The present study also demonstrated that propolis is capable to reduce metal deposition inside liver and kidney and improve biochemical alterations as well as histopathological alterations in addition to augment the activities of enzymatic antioxidants under investigation through manysuggested mechanisms including lipid peroxidation inhibition, peroxidative prevention and neutralizing reactive species.

Declarations

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Authors' contributions: All authors contributed significantly to this work; Ayman El-Amawy and Dr. Samir Zaahkouk are the two first authors responsible for the Study conception, design, performing the experimental analysis and overall data presented here, Dr. Hesham Abdel-Rashid helped with the organization, references, tables, and data presentation; Dr. Bassem Elaraby was responsible for technical and scientific support during the experiment in addition to statistical analysis.

Data availability: The datasets from which the current study was created are available from the corresponding author on reasonable request.

Compliance with ethical standards:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The experimental protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Science, Al-Azhar University, Egypt.

Consent to participate: Not applicable

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Tables

Table, (1): Concentrations of toxic metals (µg/g wet wt. ± SE) in livers and kidneys of different groups.

Groups	Organs \ Metals	Lead (Pb)	Nickel (Ni)	Cadmium (Cd)	Antimony (Sb)
Control	Liver	0.23 ± 0.05	20.77 ± 0.22	1.48 ± 0.09	0.5 ± 0.13
	Kidney	1.43 ± 0.1	105.1 ± 9.27	6.41 ± 0.67	5.17 ± 1.17
Heavy metals	Liver	179.07 ± 8.92 ****	1176.16 ± 24.52 ****	56.74 ± 4.19 ****	144.34 ± 12.04 ****
	Kidney	47.4 ± 3.92 ***	1829.59 ± 41.55 ****	103.05 ± 5.54 ***	53.06 ± 7.51 ***
Heavy metals +Propolis	Liver	†††† 33.56 ± 6.64 **	††† 532.28 ± 12.73 ***	†††† 49.36 ± 1.39 **	†††† 29.07 ± 7.63 **
	Kidney	†† 17.36 ± 2.34	†††† 1014.54 ± 132.45 ****	39.04 ± 7.08 ***	††† 20.5 ± 2.55 *

Observed metals are expressed on wet tissues. Statistically significant differences *($p < 0.05$)* compared to control group are indicated by *, while those compared to heavy metals group are indicated by †. Statistical evaluation was performed using one-way ANOVA followed by Tukey's multiple comparisons test. * † *$p < 0.05$;* ** †† *$p < 0.01$;* *** ††† *$p < 0.001$;* **** †††† *$p < 0.0001$.*

Table, (2): Levels of some Biochemical parameters in the serum of rats at the experimental groups.

Groups \ Parameters	ALAT	ASAT	Total Protein	Urea	Creatinine
Control	**28.0** ± 1.52	**169.50** ± 4.63	**8.43** ± 0.24	**29.75** ± 2.42	**0.48** ± 0.02
Heavy metals	**45.80** ± 2.27 ***	**236.0** ± 16.98 **	**8.28** ± 0.25	**41.80** ± 1.98 **	**0.53** ± 0.02
Heavy metals +Propolis	**50.80** ± 2.92 ****	**229.0** ± 13.55 *	**8.04** ± 0.17	†† **51.80** ± 0.86 ****	† **0.45** ± 0.01

Values are presented as means ± standard error. Statistically significant differences ($p < 0.05$) compared to control group are indicated by *, while those compared to heavy metals group are indicated by †. Statistical evaluation was performed using one-way ANOVA followed by Tukey's multiple comparisons test. * † *$p < 0.05$;* ** †† *$p < 0.01$;* *** ††† *$p < 0.001$;* **** †††† *$p < 0.0001$.*

Table, (3): Activities of investigated antioxidant enzymes U/g wet weight ± SE in liver and kidney of rats at experimental groups.

Groups	Enzymes / Organs	GPx	GR	SOD	CAT
Control	Liver	**196.94** ± 1.41	**61.13** ± 0.97	**34.50** ± 0.67	**227.50** ± 4.23
	Kidney	**55.74** ± 0.92	**28.89** ± 0.38	**13.82** ± 0.48	**356.30** ± 6.93
Heavy metals	Liver	**123.24** ± 1.72 ****	**27.23** ± 1.21 ****	**17.08** ± 0.70 ****	**98.75** ± 6.19 **
	Kidney	**26.90** ± 0.98 ***	**18.75** ± 0.51 ***	**5.68** ± 0.29 ***	**188.95** ± 9.73 ***
Heavy metals +Propolis	Liver	†††† **175.85** ± 1.41 **	†††† **49.91** ± 0.90 ***	†††† **28.67** ± 0.77 ***	†† **176.55** ± 5.35 *
	Kidney	†† **45.38** ± 0.90 *	†† **25.23** ± 0.32 *	††† **10.99** ± 0.36 **	†† **299.55** ± 5.71 *

Observed Antioxidants enzymes are expressed on wet tissues as means ± standard error.. Statistically significant differences *($p < 0.05$)* compared to control group are indicated by *, while those compared to heavy metals group are indicated by †. Statistical evaluation was performed using one-way ANOVA followed by Tukey's multiple comparisons test. * † $p < 0.05$; ** †† $p < 0.01$; *** ††† $p < 0.001$; **** †††† $p < 0.0001$.

Figures

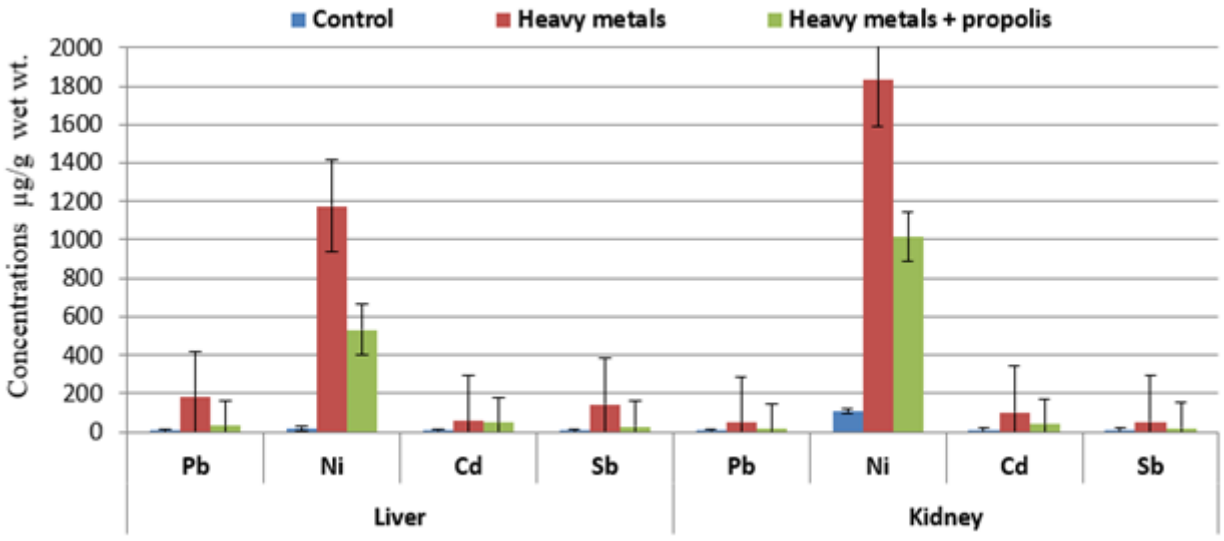

Figure 1

Toxic metals concentrations in livers and kidneys of rats at experimental groups.

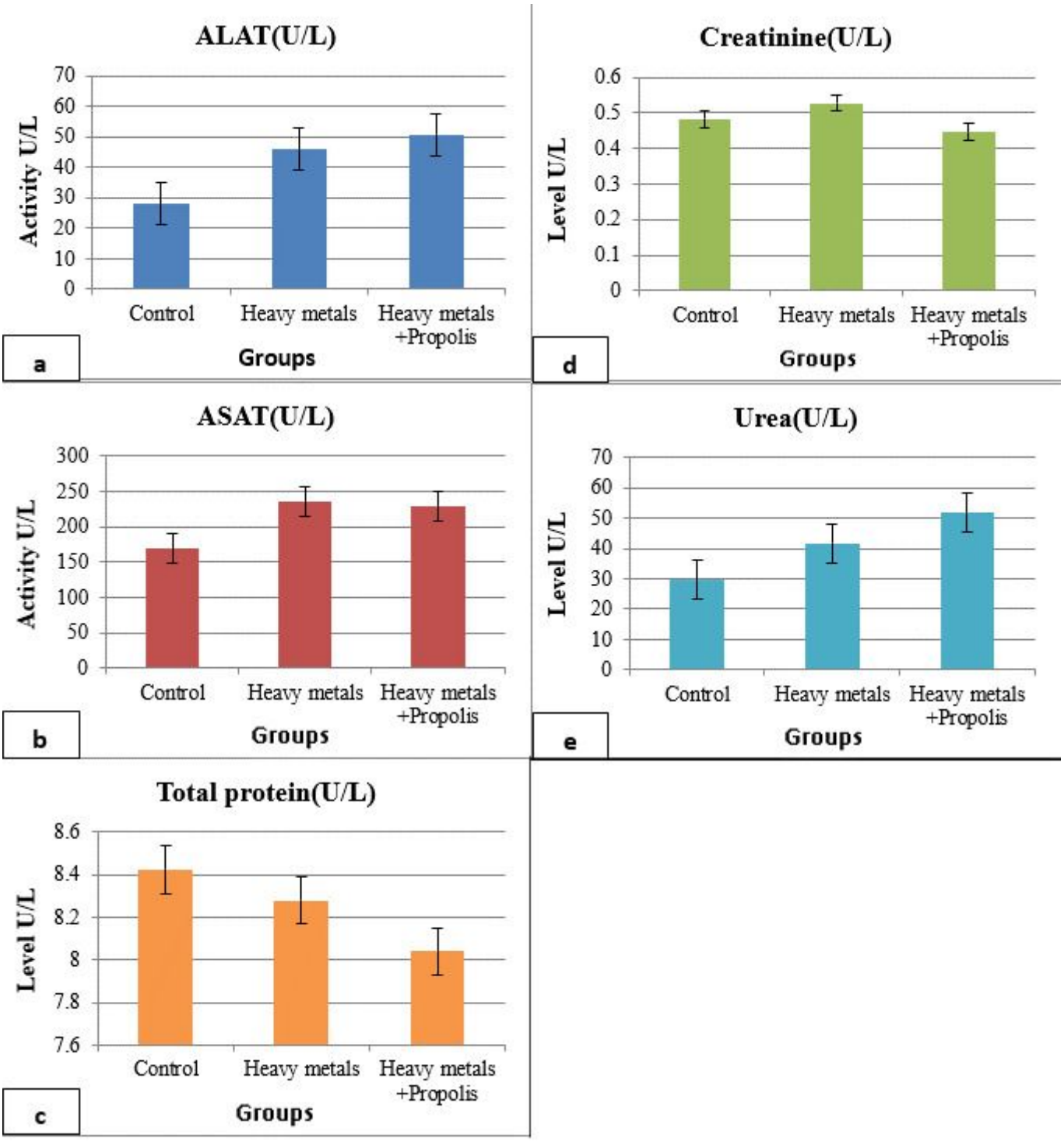

Figure 2

Effect of metals administration and propolis role on some Biochemical parameters in rats. Serum alanine aminotransferase (ALAT), serum aspartate aminotransferase (ASAT), serum total protein (TP), serum createnine and serum urea were all represented on panel (a), (b), (c), (d), (e), respectively.

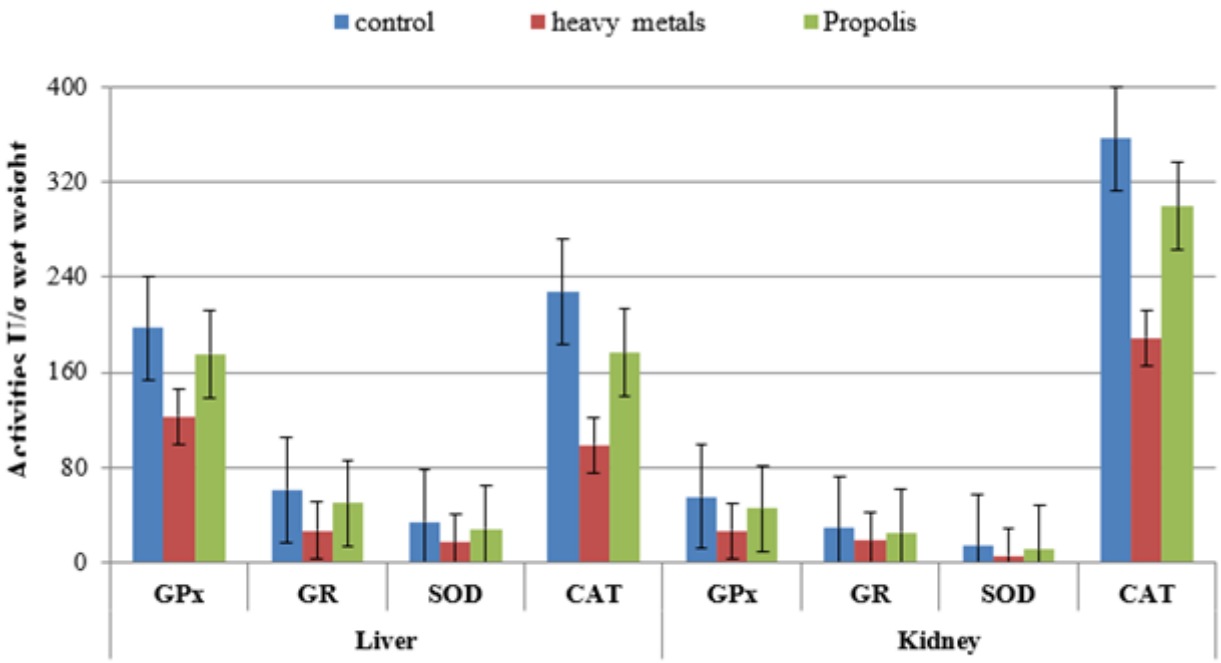

Figure 3

Levels of antioxidant enzymes in livers and kidneys of rats at experimental groups

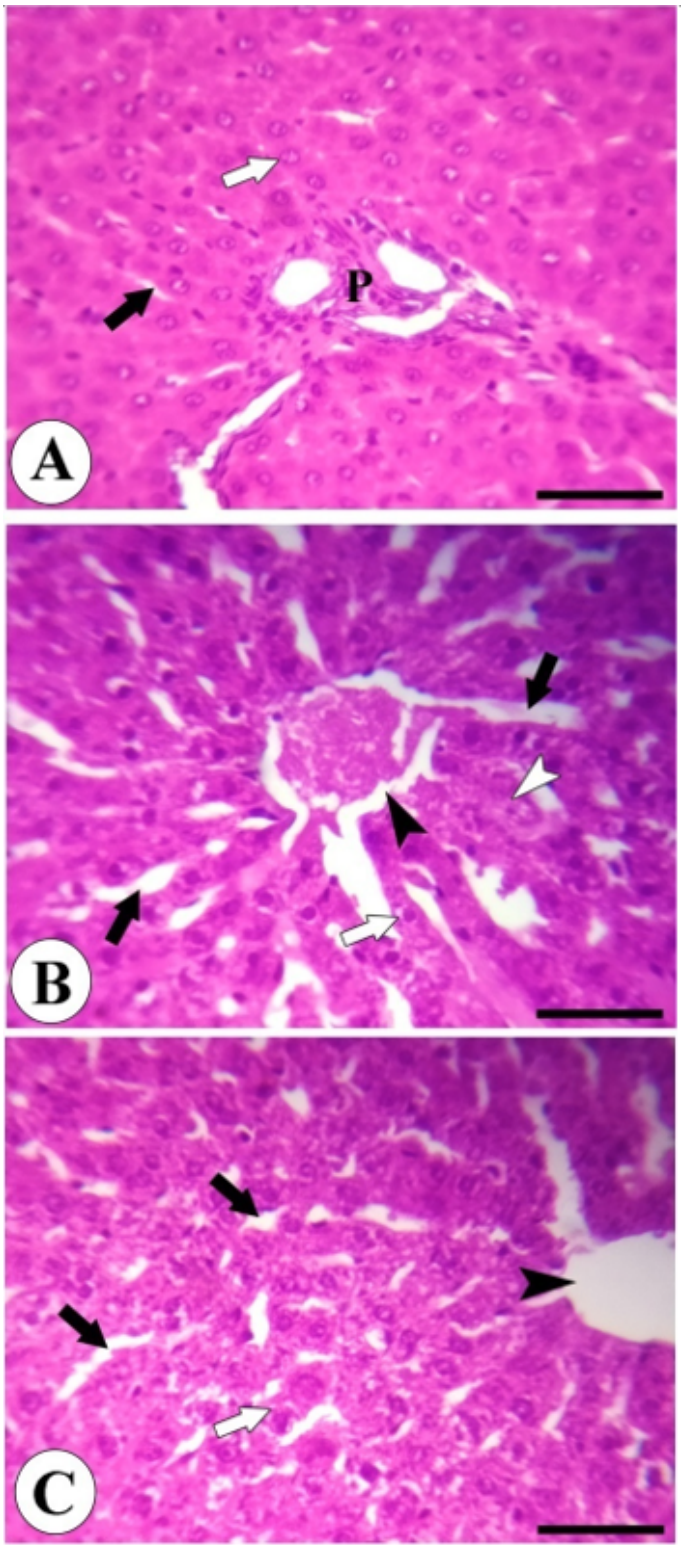

Figure 4

Effect of heavy metals mixture (Pb, Ni, Cd, Sb) on microstructures of rats liver after acute oral exposure. Panel (A): control group; panel (B): Heavy metals group; panel (C): Propolis group.

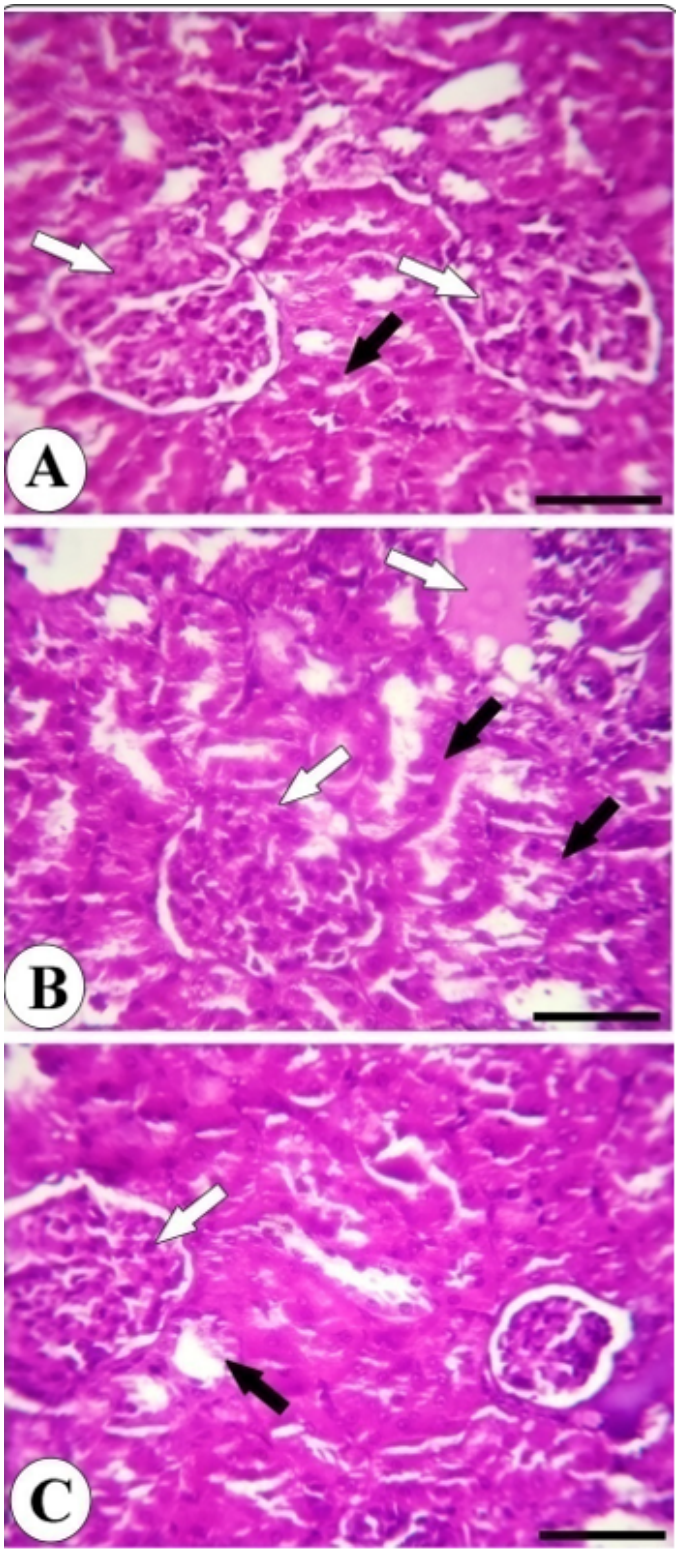

Figure 5

Effect of heavy metals mixture (Pb, Ni, Cd, Sb) on microstructures of rats kidney after acute oral exposure. Panel (A): control group; panel (B): Heavy metals group; panel (C): Propolis group.