Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 10, pp.58684-58700, October, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COMPARATIVE STUDY ON THE INFLUENCE OF DIFFERENT DRUGS AND NUTRIENTS AGAINST ALUMINIUM- INDUCED NEPHROTOXICITY AND HEPATOTOXICITY IN RATS

[1,*]Azza A. Ali, [1]Toqa M. Elnahhas, [2]Abeer I. Abd El-Fattah, [1]Mona M. Kamal and [1]Karema Abu-Elfotuh

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
[2]Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

ARTICLE INFO

Article History:
Received 22nd July, 2017
Received in revised form
13th August, 2017
Accepted 24th September, 2017
Published online 17th October, 2017

Key words:

Aluminum,
Nephrotoxicity,
Hepatotoxicity,
Coenzyme Q10,
Wheatgrass,
Cocoa,
Vinpocetine,
Zinc.

Corresponding author:
Azza A. Ali,
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

ABSTRACT

Background: Environmental pollution with the different aluminium (Al) containing compounds especially those in industrial waste water exposes people to higher than normal levels of Al that represents an environmental risk factor. Cosmetics, Al ware and containers are also sources of Al besides some foods and food additives. In addition to its known neurotoxicity, Al affects other body structures like skeletal system, blood cells, liver and kidney. Accumulation of Al in kidney and liver induces nephrotoxicity and hepatotoxicity. Coenzyme Q10 (CoQ10) is a pseudo-vitamin substance primarily present in the mitochondria. It is a powerful antioxidant and acts as radical scavenger. Wheat grass is a natural product that contains carbohydrates, proteins, vitamins, minerals, enzymes and has antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities. Cocoa is an excellent source of iron, potent antioxidants and can protect against many diseases. Vinpocetine is an antioxidant and anti inflammatory while zinc is an essential trace element involved in cell division and its deficiency is observed in many types of liver disease.
Objective: To evaluate and compare the potency of different drugs and nutrients (CoQ10, wheatgrass, cocoa, vinpocetine and zinc) against nephro- and hepato-toxicity induced by Al in rats.
Methods: Rats were divided to seven groups and received daily for three weeks either saline for control group or $AlCl_3$ (70 mg/kg, IP) for Al-toxicity model groups. Five groups of Al-toxicity model (treated groups) were orally received together with Al each of the following; CoQ10 (200mg/kg), wheat grass (100mg/kg), cocoa powder (24mg/kg), vinpocetine (20mg/kg) or zinc (32mg/kg). Biochemical changes in the serum levels of Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) as well as total bilirubin, cholesterol, triglycerides, glucose, creatinine and urea were measured. Liver and kidney specimens from all groups were also collected for the assessment of hepatic and nephrotic level of inflammatory mediators (TNF-α, IL-6β, nuclear factor kappa B (NF-κB), Caspase-3, oxidative parameters (MDA, SOD, TAC, NO) and DNA fragmentation. Histopathological changes in liver and kidney were also evaluated.
Results: Three weeks of $AlCl_3$ (70 mg/kg, IP) exposure induced nephro- and hepato-toxicity in rats. Treatment by the all used drugs showed protection against hazards of $AlCl_3$. The protective effects were indicated by the significant decrease in ALT, AST, ALP, LDH as well as total bilirubin, cholesterol, triglycerides, glucose, creatinine and urea levels which were increased by Al. Liver and kidney of the treated groups showed decrease in MDA, NO, TNF-α, IL-6β, NF-κB, caspase-3 and DNA fragmentation which were increased by Al, together with significant increase in SOD and TAC which were decreased by Al. The protection against both nephro- and hepato-toxicity was more pronounced especially with CoQ10 and wheat grass than the other used drugs. Histopathological examinations confirmed the biochemical results of toxicity and of protection.
Conclusion: Protection from nephrotoxicity, hepatotoxicity and the consequent degenerations induced by Al can be achieved by using different drugs as CoQ10, wheatgrass, cocoa, vinpocetine and zinc, but CoQ10 as well as wheat grass possesses the most superior protection.

Citation: Azza A. Ali, Toqa M. Elnahhas, Abeer I. Abd El-Fattah, Mona M. Kamal and Karema Abu-Elfotuh, 2017. "Comparative Study on the Influence of Different Drugs against Aluminium- induced Nephrotoxicity and Hepatotoxicity in Rats", *International Journal of Current Research*, 9, (10), 58684-58700.

INTRODUCTION

Aluminum (Al) is regarded as the third most abundant element and the most common metal in the earth's crust (Farina *et al.*, 2002). Exposure to Al is principally through drinking water, food (spices, corn and yellow cheese), pharmaceutical compounds (antacids, deodorants, vaccines and allergen injections), utensils and the environment (Yokel *et al.*, 2008, and Exley, 2011). Aluminum is considered an environmental and industrial pollutant that causes a broad spectrum of toxicity. The kidney is the main organ for Al excretion (Exley

et al., 1996). Early reports revealed that Al was not toxic with regard to kidney function (Tariq *et al.,* 1999). Later reports demonstrated that plasma biochemical alterations, renal atrophy and morphological changes of the Bowman's capsule and several different renal tubules resulted from Al toxicity (Belaïd-Nouira *et al.,* 2013). Impairment of renal function occurred as a consequence of these pathological changes in the kidney structure, as disturbance in the normal anti-oxidative system was induced through the formation of reactive oxygen species (ROS) (Campbell *et al.,* 2004). Lipid peroxidation in liver as well as in kidney was induced by Al toxicity (El-Demerdash, 2004 and Kaneko *et al.,* 2004). Chinoy & Patel (1999) and Moumen *et al.* (2001) reported that hepatotoxicity and changes in the oxidative status have also been reported after Al exposure. Additionally, Neill *et al.* (1996) and Wilhelm *et al.* (1996) reported hepatic Al accumulation immediately after either an intravenous or oral administration both in experimental animals and human beings. As Al binds to DNA, RNA, and inhibits hexokinase, alkaline phosphatases (ALPs) and phospho-oxidase activities (Ochmanski & Barabasz, 2000).

Coenzyme Q10 (CoQ10) is known to be an electron carrier in the electron transport chain. CoQ10 synthesized in the body cells from tyrosine amino acid in the presence of adequate levels of vitamins such as folic acid (VranesiÄ-Bender, 2010). It has been reported that CoQ10 have antioxidant and anti-apoptotic activities by regeneration of other antioxidants, so it has been used as anti-aging and is effective in the treatment of cognitive disorders (Spindler *et al.,* 2009 and Dumont *et al.,* 2011). Vinpocetine is known to be an inhibitor of cyclic GMP phosphodiesterase, a powerful legend of peripheral benzodiazepine binding sites and also can act as a blocker of NaV1.8 sodium channel activity (Ahn *et al.* 1989, Zhou *et al.* 2003 and Gulyás *et al.* 2005). Besides, vinpocetine has an anti-inflammatory action that can inhibit tumor necrosis factor-alpha (TNF-α), inducing nuclear factor-kappa B (NF-κ B) activation and the induction of pro-inflammatory mediators (Jeon *et al.* 2010). Zinc is an essential trace element which required in a number of biological actions and is nontoxic at physiological doses (Betholf, 1988). Several reports have indicated the valuable actions of zinc under the conditions of oxidative damage (Cagen &Klassen, 1979, Cabre *et al.,* 1995, Goel *et al.,* 2007 and Rishi *et al.,* 2008). Zinc stabilizes the cell membrane structure through its antioxidant effects, which may be as a result of its ability to regulate the levels of metallothioneins (Kang, 1999). On the other hand, wheatgrass (*Triticum aestivum L.)* is known to be a broadly used health food, consumed commonly as fresh juice or as capsules, tablets and liquid concentrates. Formulations of wheatgrass possess different pharmacological effects such as antioxidant (Falcioni *et al.,* 2002), tumor suppressor (Arya and Kumar, 2011), hypoglycemic (Mohan *et al.,* 2013) and neuro-protective effects (Jang *et al.,* 2010). Wheat grass is considered an potent source of antioxidant enzymes, antioxidant vitamins such as A, B, C, E and minerals like potassium, sulfur, zinc, calcium, cobalt, iron, phosphorus (Leoncini *et al.,* 2012 and Stevenson *et al.,* 2012). Cocoa is known as a one of the richest flavonoid-containing foods available , it contains antioxidant polyphenols called flavonols that may exert hepato protective effects (Amin *et al.,* 2004, Serafini, 2004 and Cordero-Herrera *et al.,* 2015) and anti malarial effects (Addai, 2010 and Amponsah *et al.,* 2012). It is fortunately that the antioxidant activities of cocoa unchanged after different manufacturing processes (Stahl *et al.,* 2009 and Maleyki & Ismail, 2010).

Since, kidney and liver are of the target tissues of aluminum toxicity as they involved in its elimination or in the metabolism and detoxification (Exley *et al.,* 1996 and Ogueche *et al.,* 2014). So, the essence of the present study was to evaluate the biochemical and histopathological alteration in the kidney and the liver of rats exposed to Al. Additionally, to compare the potency of different drugs and nutrients as CoQ10, vinpocetine, zinc, wheatgrass and cocoa in the protection against nephrotoxicity and hepatotoxicity induced by Al.

MATERIALS AND METHODS

Animals

Seventy male Sprague Dawley rats were used. Rats weighing 260- 280 g were obtained from Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt. They were housed in stainless-steel cages (four per cage) under the same adequate conditions, with alternatively 12 hour light and dark cycles, at a temperature of 25 ± 1°C. Rats were kept under the same adequate conditions and provided with their daily dietary requirements of standard diet pellets (El-Nasr, Abu Zaabal, Cairo, Egypt) contained not less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash and a vitamin mixture, water was given ad-libitum. Rats were taken to test situation one hour before each experiment for adaptation and after removing food and water from the cages. The study was conducted in the period from March to July, 2016 in accordance with ethical guidelines of Al-Azhar University (Faculty of Pharmacy), Egypt.

Drugs and chemicals

From Sigma Chemical Co. (St. Louis, MO, USA); CoQ10 and Aluminum chloride - hydrated ($AlCl_3$.6H2O) were purchased. $AlCl_3$ was freshly dissolved in distilled water while CoQ10 was suspended in 1% aqueous solution of Tween 80; suspensions were freshly prepared every day. All other solvents and chemicals were of the highest grade-commercially available. Vinpocetine was suspended in 1% aqueous solution of Tween 80; suspensions were freshly prepared every day. Wheatgrass was freshly dissolved in distilled water as well as coca and zinc sulphate.

Experimental design

Methods

Rats were randomly assigned to seven groups and received daily for three weeks either saline for control group or injected (I.P) with 70 mg/kg $AlCl_3$.6H2O (Ali *et al.,* 2015) for aluminum toxicity model groups. Five groups of Al-toxicity model (treated groups) were orally received together with Al each of the following; 200mg/kg of CoQ10 (Andreassen *et al.,* 1999), 100mg/kg of wheat grass (Veera *et al.,* 2014), 24mg/kg of cocoa powder (Rozan *et al.,* 2007), 20mg/kg of vinpocetine (Ralf 7 Josef, 1991) or 32mg/kg of zinc sulphate (Agnieszka *et al.,* 2016).

A-Biochemical Investigations

Blood sampling

At the end of the three weeks, blood samples were collected via eye puncture from each rat before scarification into serum separator tubes, allowed to stand (30 min), centrifuged (3000

rpm for 15 min), serum collected and stored at -20°C until the assay of the studied biochemical parameters.

I- Blood biomarkers

i-Estimation of renal functions

Blood urea nitrogen (BUN) and serum creatinine were measured using quantitative colorimetric urea determination (QuantiChrom™ urea assay kit) (Bioassay Systems, Hayward, CA, USA) and quantitative colorimetric creatinine determination (QuantiChrom™ creatinine assay kit).All procedures were performed according to the manufacturers' instructions.

ii-Estimation of hepatic functions

Hepatic function biomarker serum levels namely; Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and total bilirubin were estimated by colorimetric assay kits (Biomed-diagnostics, Cairo, Egypt), according to the methods described by Tietz (1976) and Malloy and Evelyn (1937). Also, alkaline phosphatase (ALP) was estimated using k-assay ELISA kit (Kamiya Biomedical, Seattle, WA, USA). Stanbio Laboratory Kits (Boerne, TX, USA) were utilized for the determination of the serum albumin levels. All procedures were performed according to the manufacturers' instructions.

Estimation of LDH: LDH was determined according to the method of Tietz (1976) (Biosystems S.A, Barcelona, Spain).

iii-Estimation of cholesterol and triglycerides

Colorimetric assay kits for the measurement of serum cholesterol (total and HDL- cholesterol) and triglycerides (Biomed-diagnostics, Cairo, Egypt), were used in this study.

iv-Estimation of glucose levels

Stanbio Laboratory Kits (Boerne, TX, USA) were utilized for the determination of the serum glucose levels.

II-Tissue biomarkers

At the end of the three weeks, rats were sacrificed by decapitation then livers and kidneys were dissected and washed with ice-cold saline. Liver and kidney tissues were kept frozen at -80°C till the time of analysis. They were homogenized in saline then the homogenates were used to assess the oxidative stress markers; as superoxide dismutase (SOD), total antioxidant capacity (TAC), lipid peroxides which were expressed as malondialdehyde (MDA) as well as Nitric oxide (NO) was also determined. Anti-inflammatory markers were assessed; by measuring the levels of tumor necrosis factor-α (TNF-α), interleukin -6 β (IL-6β) and natural factor kappa- β (NFκ-B) .Assessment of apoptotic marker; by measuring Caspase-3 activity. Finally, DNA fragmentation was done as well as histopathological examinations; by taking specimens from all kidney and liver areas from different groups.

i-Renal and hepatic oxidative stress estimation

In the kidney and liver homogenates, MDA and SOD as well as TAC were measured. Lipid peroxidation can be determined as MDA, by estimating the level of thiobarbituric acid reactive substances (TBARS), according to the method of Satoh, (1978) using (Biodiagnostic, Cairo, Egypt). SOD content was assessed, relying on the ability of the enzyme to inhibit the phenazine methosulphate mediated reduction of nitroblue tetrazolium dye (Nishikimi *et al.,* 1972), where the increase in absorbance at 560 nm for 5 min was measured. On the other hand, determination of TAC is performed through the reaction with a defined amount of exogenously provide H_2O_2. The residual H_2O_2 is colorimetrically determined by the enzymatic reaction that involves the conversion of 3, 5-dichloro-2-hydroxybenzene sulphonate to a colored product (Koracevic *et al.,* 2001). For NO estimation, vanadium trichloride was used to reduce nitrate to nitrite (Miranda *et al.,* 2001). The method of nitrite estimation is based on Griess reaction that was performed using the kit provided by Biodiagnostic (Cairo, Egypt). All procedures were performed according to the manufacturers' instructions.

ii-Assessment of inflammatory markers.

The involvement of inflammation was assessed by measuring the levels of (TNF-α, IL-6β and NFκ-B) in kidney and liver homogenate of all groups , utilizing the commercially available rat Quantikine®Rat TNF-α ELISA Kits (R&D Systems, MN, USA), RayBio®Rat IL-6β (RayBiotech, Inc., USA) and rat NFκ-B ELISA kit Cusabio Biotech (Cusabio Life Science, Inc., China) respectively.

iii-Assessment of apoptotic markers

Caspase-3 activity was detected in the kidney and liver homogenates using ELISA kit (MyBioSource San Diego, California , USA). The manufacturer's instructions were followed precisely and the developed color was measured spectrophotometrically at 450 nm immediately.

iv-Assessment of DNA fragmentation.

DNA fragmentation% assay was conducted using the procedure supplied by Qiagen kit (Hilden, Germany) .To detect DNA fragmentation. The DNA in the gel was visualized and photographed under UV light(R). In DNA laddering assay, low molecular weight fragments of DNA are extracted selectively from the cells whereas the higher molecular weight DNA stays associated with the nuclei. The isolated DNA is separated by electrophoresis and visualized using ethidium bromide. DNA was electrophoresed using 2% agarose gel and visualized by ultraviolet light following ethidium bromide staining.

B-Histopathological examination of the kidney and liver.

In 10% formalin for 24 h, kidney and liver specimens were fixed then they were washed with tap water, they were prepared and stained for light microscopy (Bancroft and Stevens, 1996). For dehydration; serial dilutions of alcohol were used (methyl, ethyl and absolute ethyl). In hot air oven at 56ºC for 24 h, specimens were cleared in xylene embedded in paraffin. By using microtome at 4 microns thickness, paraffin bees wax tissue blocks were sectioned. Then, sections were collected on glass slides and deparaffinized. They were stained for routine histological examination using Hematoxylin and Eosin stain for routine histopathological examination.

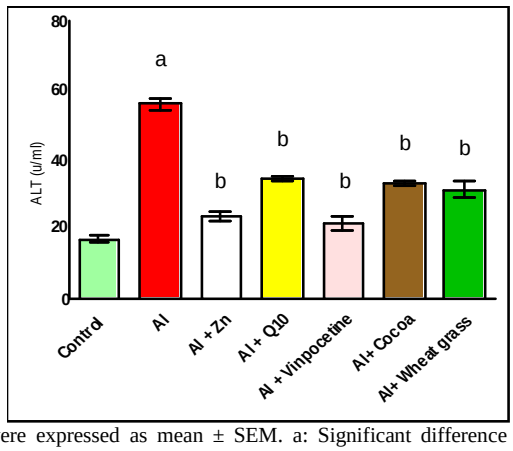
Statistical Analysis

Data are presented as mean ± SEM. Multiple comparisons were performed using one-way ANOVA followed by Tukey Kramer as a post hoc test. Unpaired t-test was used to compare two different treatments. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using Instat (version 3) software package. Graphs were sketched using GraphPad Prism (ISI®, USA) software (version 5).

RESULTS

Serum Enzymes Activities

$AlCl_3$ induced about two-fold marked increase of liver enzymes ALT and AST activities in the blood, compared with control Fig.1 & Fig.2 respectively. Administration of zinc, Q10, vinpocetine, cocoa, and wheat grass, was found effective to reduce that increase significantly by (58%, 38%, 61%, 41% and 44% respectively) of ALT enzyme activity, and by (52%, 350%, 54%, 44% and 56% respectively) of AST enzyme activity as compared to $AlCl_3$ group. With regard to ALP enzyme activity, 76% significant increase was observed with $AlCl_3$ treatment, while (24%, 13%, 33%, 16% and 14%) marked decrease noticed in zinc, Q10, vinpocetine, cocoa, and wheat grass treated groups respectively Fig.3.

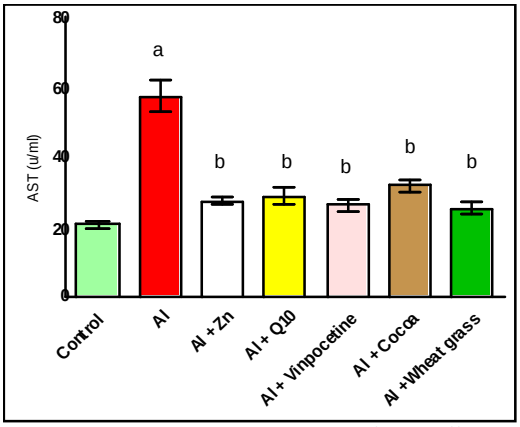


Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

Fig. 1. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on serum ALT activity in rats

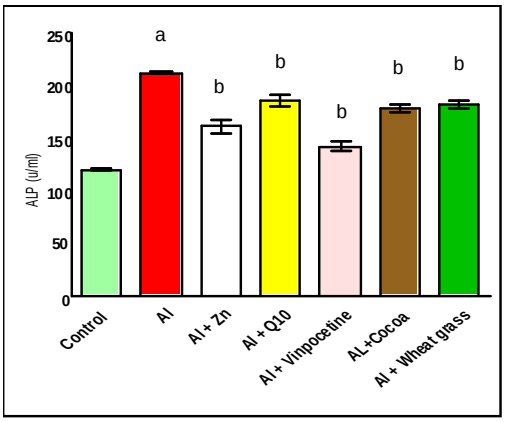
Serum Total Bilirubin

As shown in Fig. 4 $AlCl_3$ increased total bilirubin level significantly by 36%, but zinc, Q10, vinpocetine, cocoa, and wheat grass supplementation ameliorated it registering significant decrease of (54%, 46%, 85%, 53% and 54% respectively). Results revealed (30% and 34%) significant increase in cholesterol, and triglyceride levels respectively in the $AlCl_3$ treated group Fig.5 & 6. A significant decrease (34%) in HDL level was presented in Fig.7 as compared with control group. Co-administration with the protective drugs zinc, Q10, cocoa, or wheat grass ameliorated the previous hyperlipedemic changes significantly with $AlCl_3$ treated group (7%, 15%, 19% and 18% respectively) except vinpocetine, which had no significant change in cholesterol level. Similarly, the significant reduction in triglyceride level (20%, 18%, 41%, 29% and 16%) was presented after treatment with zinc, Q10, vinpocetine, cocoa and wheat grass respectively. On the other hand, (13%, 28%, 17%, 31% and 25%) significant increase in the HDL level with zinc, Q10, vinpocetine, cocoa and wheat grass treatment respectively as compared with Al intoxicated group.



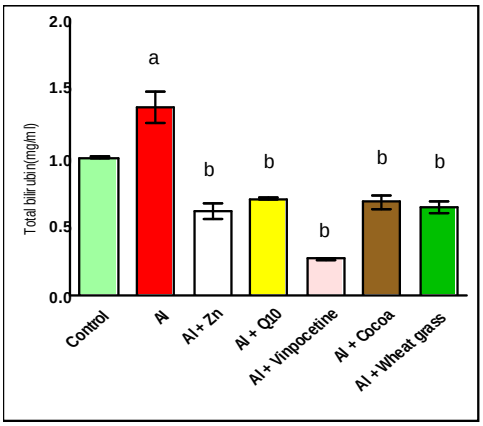
Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

Fig. 2. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on serum AST activity in rats



Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

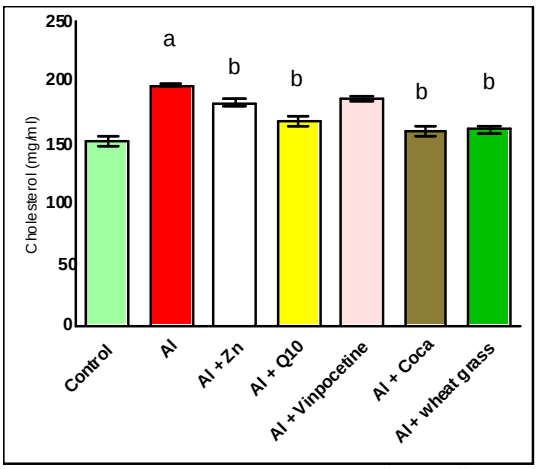
Fig. 3. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on serum ALP activity in rats



Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

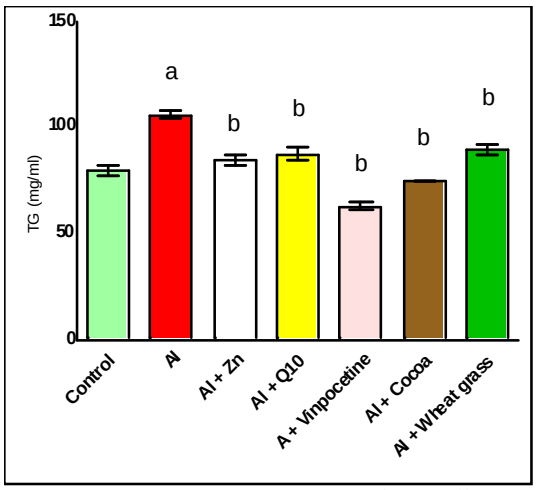
Fig. 4. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on serum Total bilirubin level in rats

Serum Lipid Profile



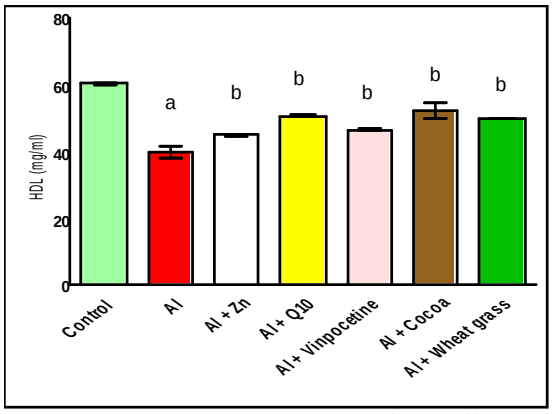
Values were expressed as mean ± SEM. a: Significant difference from the control group at p < 0.05, b: Significant difference from Al- treated group at p < 0.05.

Fig. 5. Effect of both Al alone, and in combination with Zn, CoQ10, Vinpocetine, Cocoa, or Wheat grass on serum Cholesterol level in rat



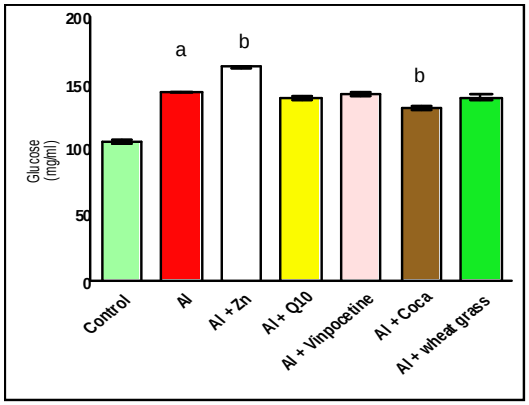
Values were expressed as mean ± SEM. a: Significant difference from the control group at p < 0.05, b: Significant difference from Al- treated group at p < 0.05.

Fig. 6. Effect of both Al alone, and in combination with Zn, CoQ10, Vinpocetine, Cocoa, or Wheat grass on serum Triglycerides level in rats



Values were expressed as mean ± SEM. a: Significant difference from the control group at p < 0.05, b: Significant difference from Al- treated group at p < 0.05.

Fig. 7. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa, or Wheat grass on serum HDL-C level in rats



Values were expressed as mean ± SEM. a: Significant difference from the control group at p < 0.05, b: Significant difference from Al- treated group at p < 0.05.

Fig. 8. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa, or Wheat grass on blood Glucose level in rats

Glucose Level in Blood

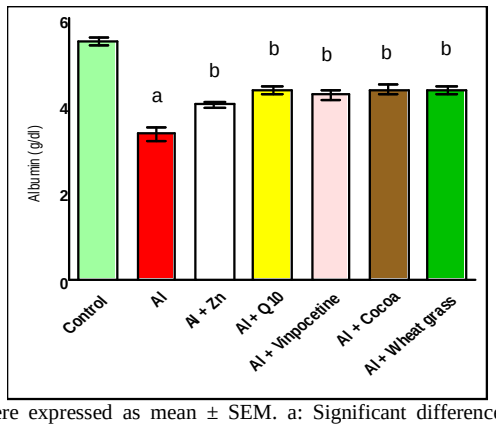
Fig.8 revealed (36%) significant increase of $AlCl_3$ treated group compared with control. Zinc co-administration, increased glucose level significantly (14%).While cocoa, decreased it significantly (8%). Neither significant difference was found with vinpocetine or with wheat grass as compared to $AlCl_3$-intoxicated group.

Albumin level

Albumin level decreased significantly in $AlCl_3$ intoxicated group by 39%. Treatment with zinc, Q10, vinpocetine, cocoa, and wheat grass succeeded to increase it by (21%, 30%, 27%, 30% and 30% respectively) Fig.9.

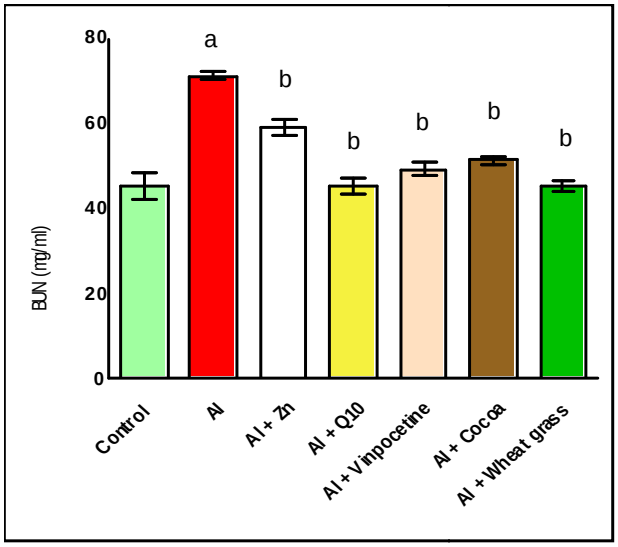
Renal Toxicity Blood Markers

$AlCl_3$ (70 mg/kg, IP) for three weeks in rats, induced significant increase in the blood markers of renal toxicity BUN, and creatinine concentrations (half- fold, and 3 -folds respectively) compared with control. The BUN concentration in Fig.10 was significantly decreased in rats treated with zinc, Q10, vinpocetine, cocoa and wheat grass (17%, 36.5%, 30.6%, 28% and 36% respectively) as compared to $AlCl_3$-intoxicated group. Likewise, the serum creatinine level in Fig.11 was significantly decreased in rats treated with zinc, Q10, vinpocetine, cocoa and wheat grass (30%, 25%, 22%, 38% and 24% respectively) as compared to $AlCl_3$ toxicated group.



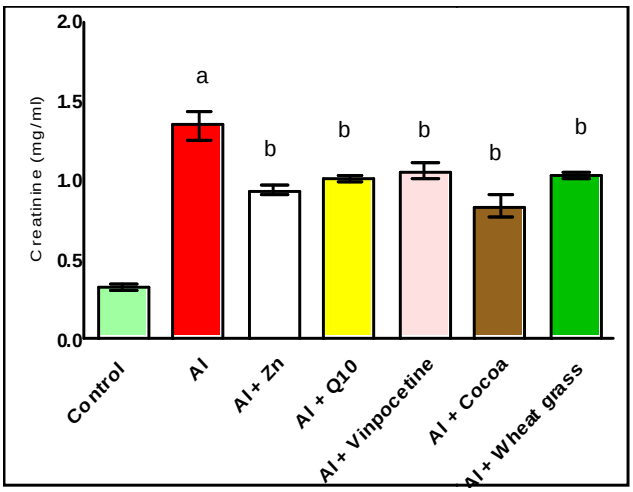
Values were expressed as mean ± SEM. a: Significant difference from the control group at p < 0.05, b: Significant difference from Al- treated group at p < 0.05.

Fig. 9. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on serum Albumin level in rats



Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

Fig. 10. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa, or Wheat grass on BUN level in rats

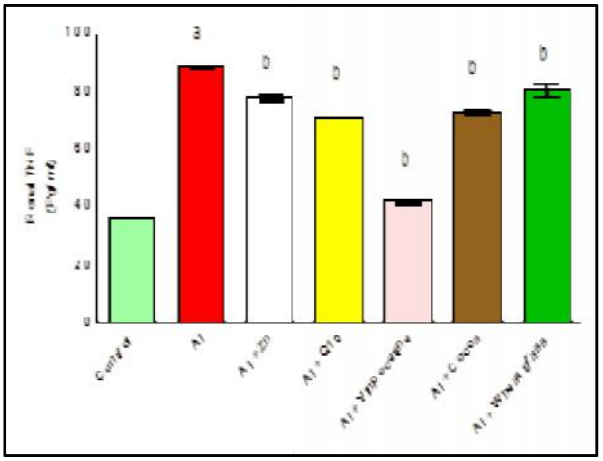


Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

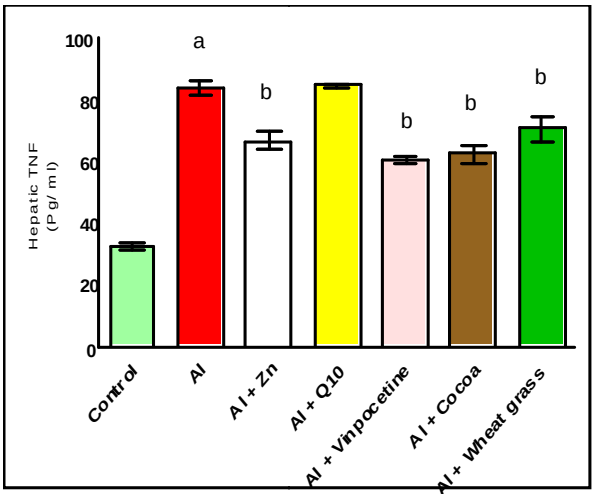
Fig. 11. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa, or Wheat grass on serum Creatinine level in rats

Inflammatory mediators (Tissue specimens, kidney & Liver)

TNF-α level as shown in Fig.12A, 12B presented about one and half-fold significant increase in both kidney and liver tissues after treatment with $AlCl_3$ compared to control., The decrease was significantly in kidney tissue by (12%, 20%, 53%, 17%, and 9%) of Zinc, Q10, vinpocetine, cocoa, and wheat grass treatments respectively. No significant difference with Q10 in liver tissue, while zinc, vinpocetine, cocoa, and wheat grass treatments decreased the cytokine level by (20%, 28%, 25%, and 16%respectively) of liver tissue, compared to $AlCl_3$ treated group. IL-6β level increased relatively one and half- fold in kidney tissue, and increased markedly two and half- fold in liver tissue, after treatment with$AlCl_3$ compared to control as shown in Fig.13A, 13B.



(A)

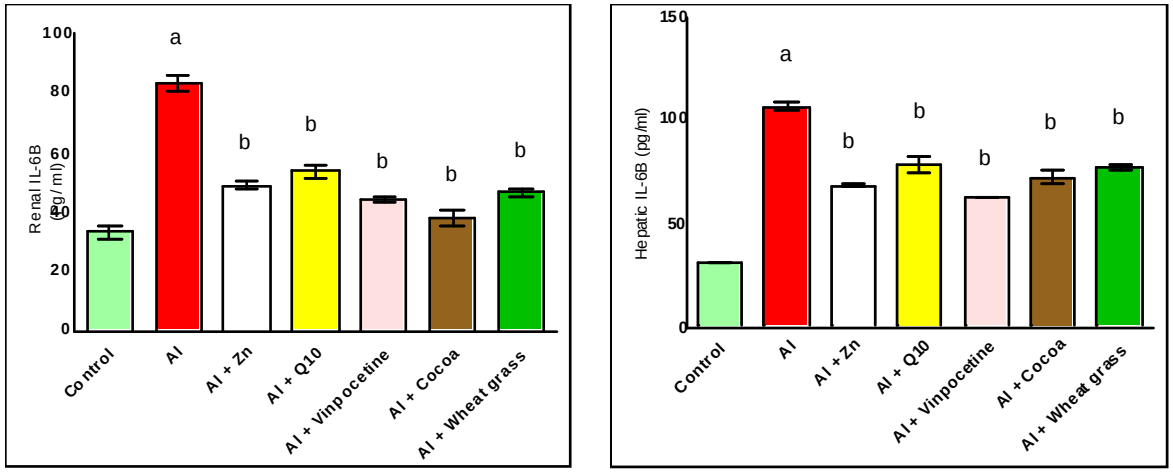


(B)

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$ b: Significant difference from Al- treated group at $p < 0.05$.

Fig. 12. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic TNF-α level (pg/mg protein) in rats

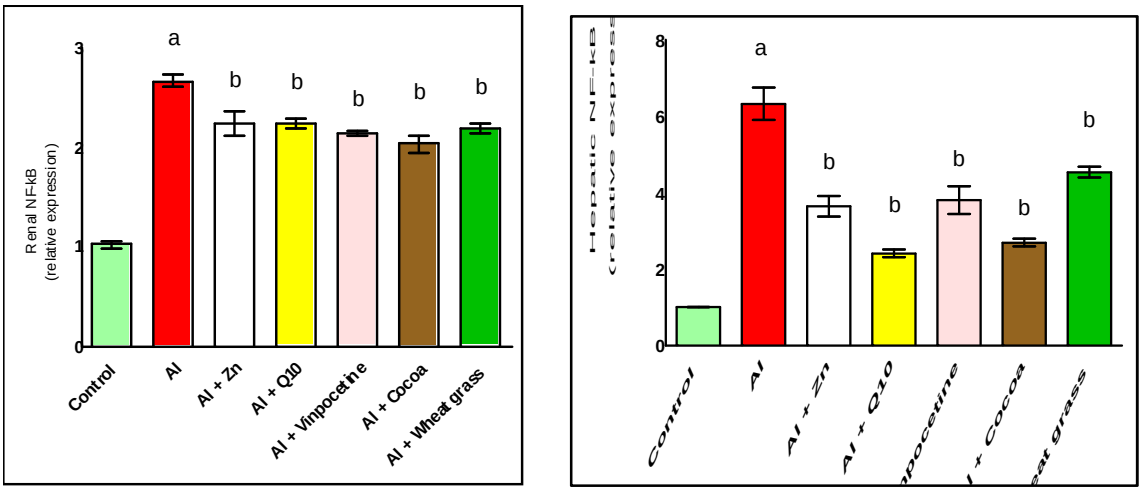
The inflammatory mediator level decreased significantly in both kidney tissues by (41%, 36%, 47%, 55% and 44%), and liver tissues by (36%, 26%, 41%, 32% and 28%) with the co-administration of zinc, Q10, vinpocetine, cocoa and wheat grass respectively as compared to $AlCl_3$ treated group. NF-$_k$B level increased one and half-fold in kidney tissues, while increased to great extent in liver tissues five-fold, versus after $AlCl_3$ treatment compared to control Fig.14A,14B the elevated nuclear factor level reduced markedly by the drugs zinc, Q10, vinpocetine, cocoa, and wheat grass administration(15%, 15%, 19%, 23%, and 15%) in kidney tissues, and (43%, 62%, 40%, 60% and 29%) in liver tissues respectively as compared to $AlCl_3$ treated group. Caspase-3 apoptotic mediator level was enhanced triple-fold significantly in kidney , but quietly significant increase one and half-fold in liver homogenate, under the effect of $AlCl_3$ treatment compared to control Fig.15A, 15B all the drug treatments zinc, Q10, vinpocetine, cocoa, and wheat grass had beneficial effect on the elevated mediator by (57%, 29%, 48%, 45% and 37%) decrease in kidney tissues. However, only zinc, Q10 and vinpocetine, succeeded to reduce the elevated level significantly by (20%, 12% and 24% respectively) in liver tissues as compared to $AlCl_3$ treated group.



(A) **(B)**

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al-treated group at $p < 0.05$.

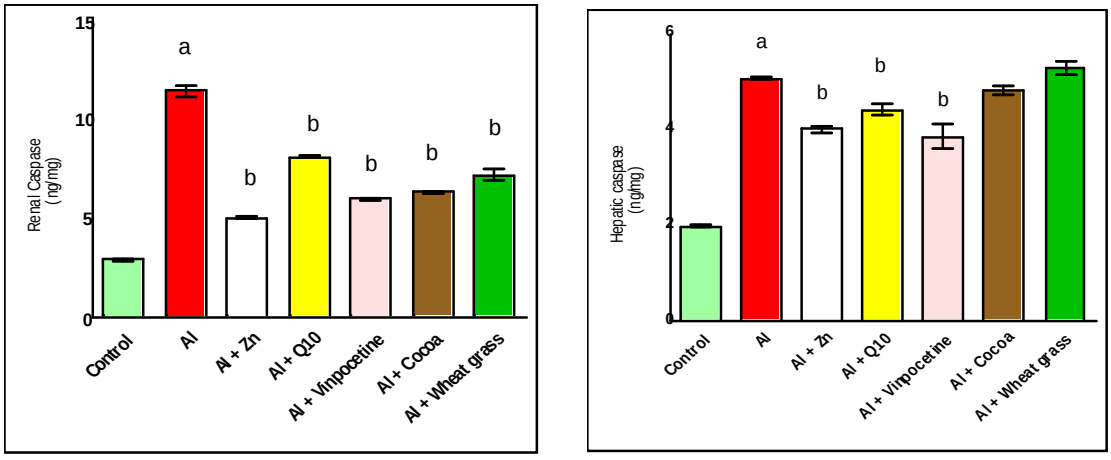
Fig. 13. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic IL-6β level (pg/mg)in rats



(A) **(B)**

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al-treated group at $p < 0.05$.

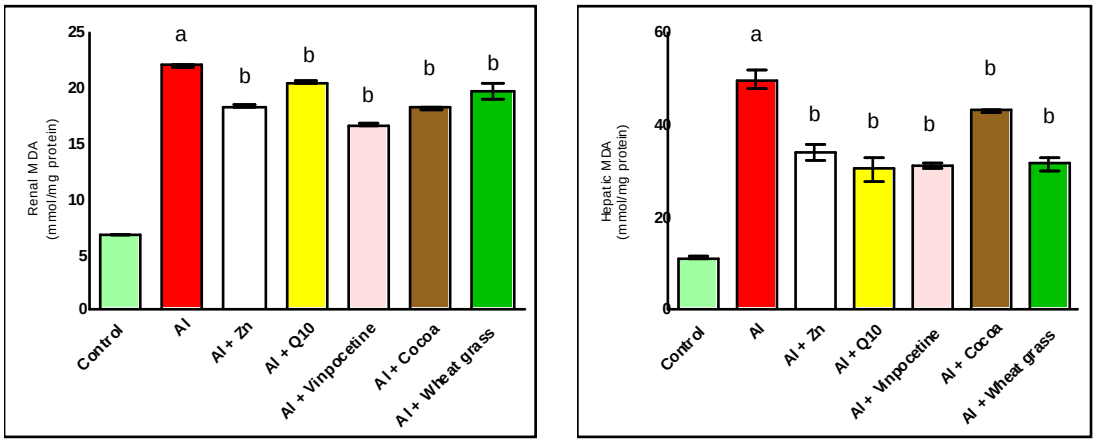
Fig. 14. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic NF-kB level in rats



(A) **(B)**

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al-treated group at $p < 0.05$.

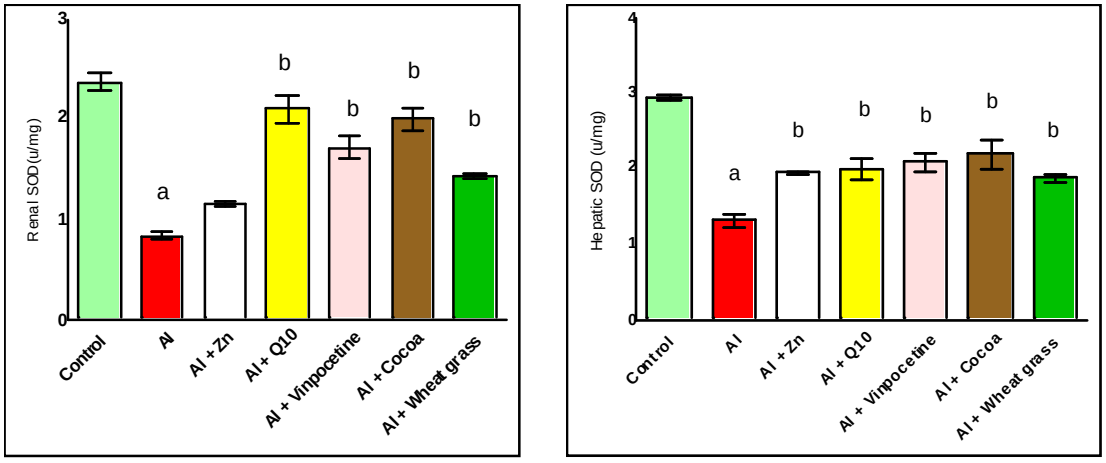
Fig. 15. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic Caspase-3 level in rats



(A) **(B)**

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

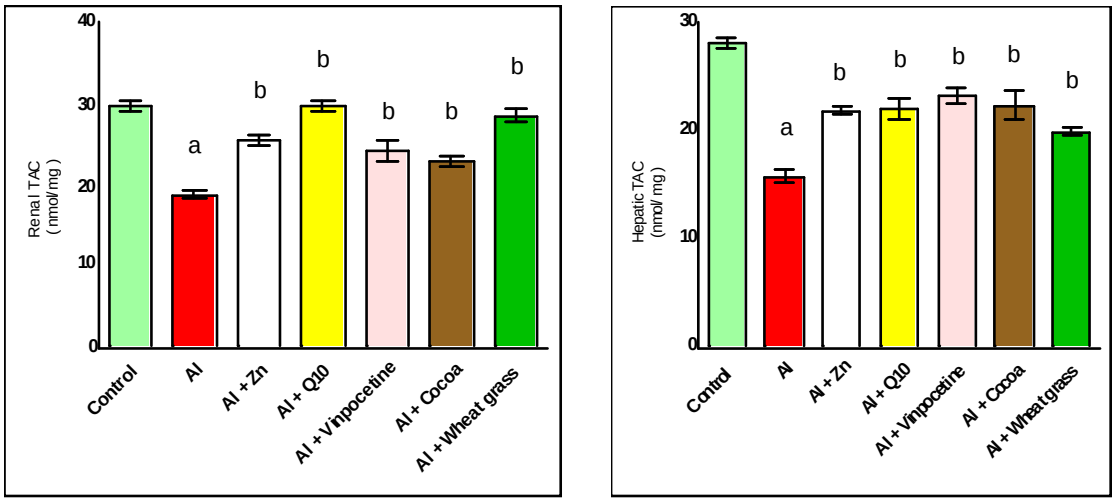
Fig. 16. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic MDA level in rats.



(A) **(B)**

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

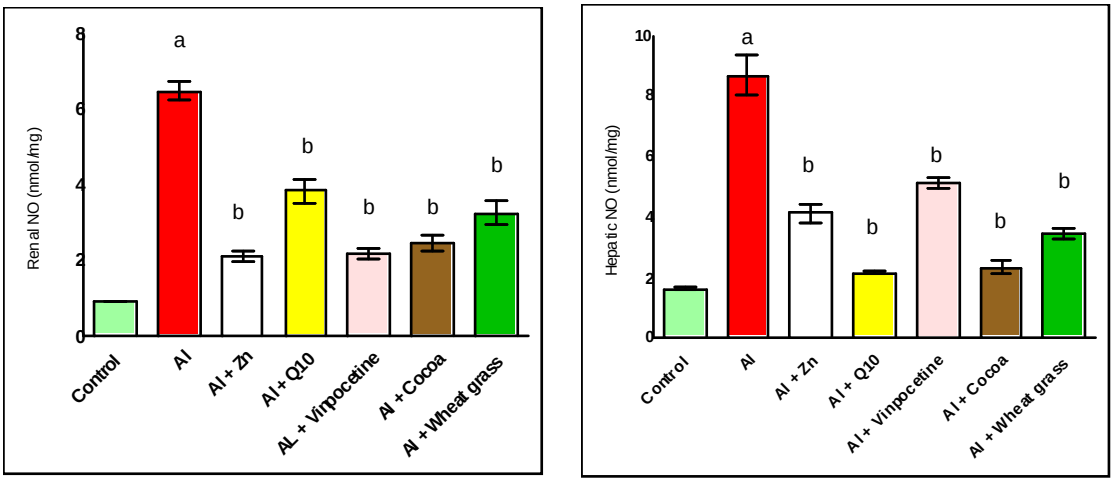
Fig. 17. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic SOD activity in rats.



(A) **(B)**

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

Fig. 18. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic TAC level in rats



(A) **(B)**

Values were expressed as mean ± SEM. a: Significant difference from the control group at $p < 0.05$, b: Significant difference from Al- treated group at $p < 0.05$.

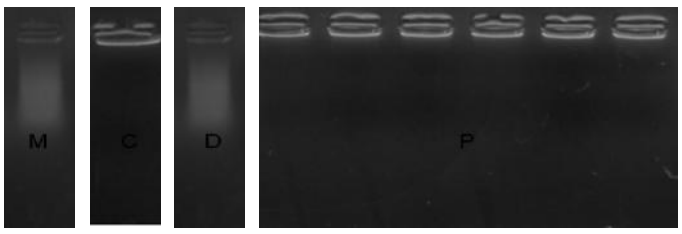
Fig. 19. Effect of both Al alone and in combination with Zn, CoQ10, Vinpocetine, Cocoa or Wheat grass on renal and hepatic NO level in rats

Antioxidant parameters kidney & liver (Tissue specimens)

MDA level in $AlCl_3$ treated group was increased significantly by about two-folds in kidney tissue homogenate and three-folds in liver tissue homogenate Fig.16A, 16B as compared with control. Treatment with zinc, Q10, vinpocetine, cocoa and wheat grass decreased it significantly by (16%, 7%, 24%, 17% and 11%respectively) of kidney tissue and by (32%, 39%, 37%, 14% and 37%respectively) of liver tissue compared to $AlCl_3$ treated group. SOD activity markedly reduced 65% in kidney tissue and 55% in liver tissue Fig. 17A, 17B by the influence of $AlCl_3$ intoxication, compared with control. Reversed results obtained after treatment with zinc, Q10, vinpocetine, cocoa, and wheat grass by significant elevation to (39%, 141%, 105%, 129%, and 68% respectively) of kidney tissue, and to (46%, 54%, 54%, 69% and 38%respectively) of liver tissue compared to $AlCl_3$ treated group. TAC was decreased significantly in both kidney and liver tissue homogenate 37%, and 44% respectively upon exposure to$AlCl_3$, compared to control Fig. 18A, 18B Zinc, Q10, vinpocetine, cocoa and wheat grass treatment came back to within normal range toto (36%, 59%, 29%, 18% and 53%respectively) of kidney tissue and (38%, 39%, 47%, 41% and 27% respectively) of liver tissue compared to $AlCl_3$ treated group. NO produced greatly about six-folds in kidney tissue and four-fold increase in liver tissue as a result of $AlCl_3$ treatment as compared to control Fig. 19A, 19B.

DNA Fragmentation

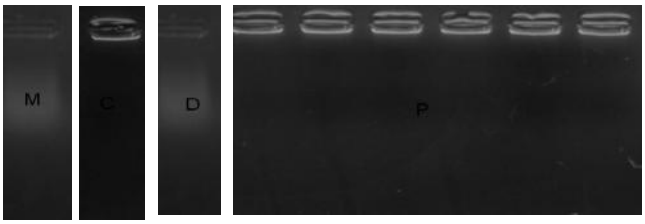
1- Kidney fragment Fig.20 A



An agarose gel electrophoresis show DNA fragmentation
Lane M: DNA marker with 100bp
Group C: Control there are no streaks
Lane D: shows DNA streaks (Groups of AL)
(Induced toxicity which found in the model (M) laddering shape)

Group P: there are no streaks groups of treatment

2- Liver fragment Fig.20 B

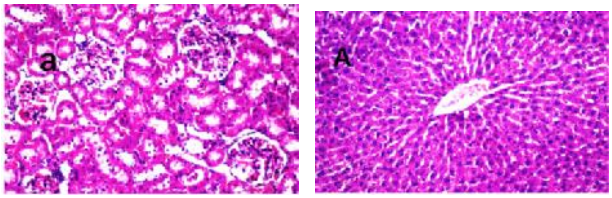


An agarose gel electrophoresis show DNA fragmentation
Lane M: DNA marker with 100bp
Group C: Control there are no streaks
Lane D: shows DNA streaks (Groups of AL)
(Induced toxicity which found in the model (M) laddering shape)
Group P: there are no streaks groups of treatment

On the other hand, Zinc, Q10, vinpocetine, cocoa, and wheat grass treatments were able to reduce the elevated level by (68%, 41%, 66%, 63% and 51%respectively) of kidney tissue, and by (52%, 75%, 42%, 73% and 60% respectively) of liver tissue compared to $AlCl_3$ treated group.

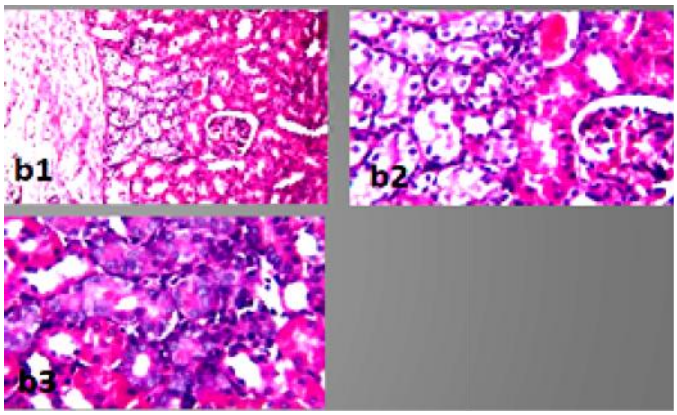
Histopathological changes of kidney & liver

Normal histological structure appeared of the structure of the glomeruli and tubules at the cortexand also normal histological of central vein and surrounding hepatocytes in the parenchyma control rats group Fig.21.

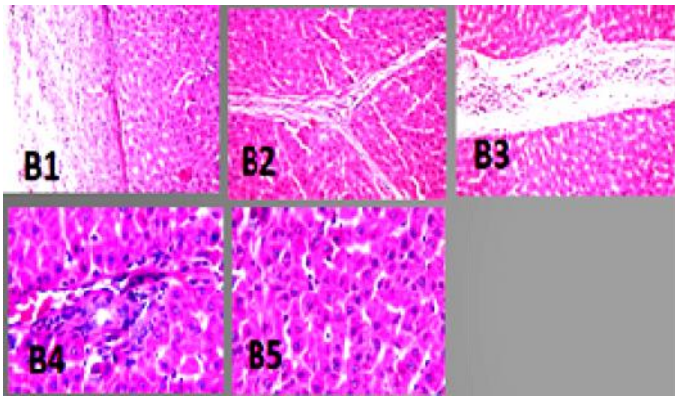


a: Kidney tissue **A**: liver tissue

Fig. 21. Histopathological changes in rats tissues of control group



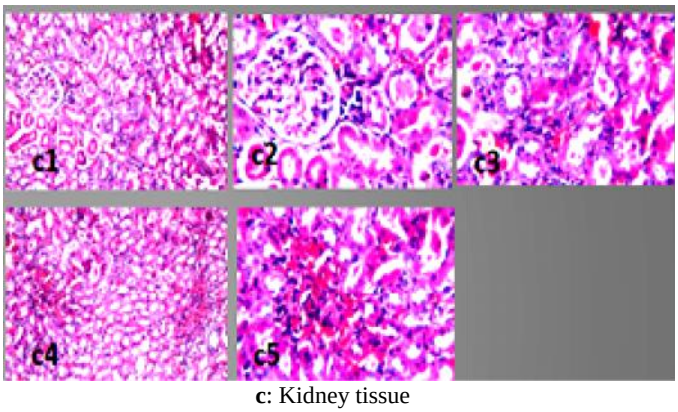
b: Kidney tissue



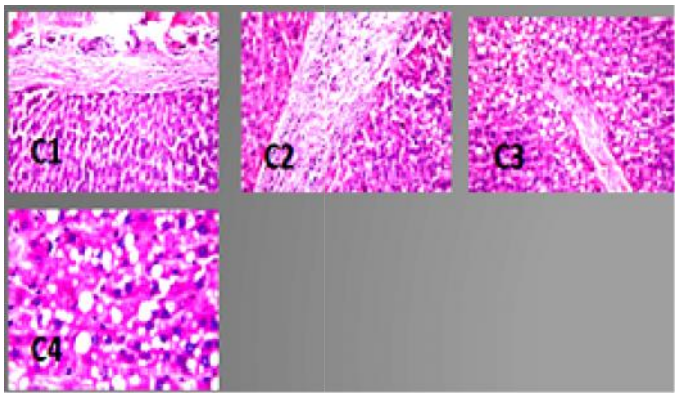
B: liver tissue

Fig. 22. Effect of Al on histopathological changes of rats tissues

Fig.22b showed oedema with fibrosis. Detected in the thick renal capsule (b1), degeneration in the lining tubular epithelium (b2), Hyperplasia & dysplasia were noticed in the lining epithelium of some focal tubules (b3) in the Al treated groups. While Fig.22B Showed thickening in the hepatic capsule.By inflammatory cells infiltration and fibroblastic cells proliferation (B1) between the hepatocytes in the hepatic parenchyma (B2, B3). Inflammatory cells infiltration (B4) in the portal area, diffusekupffer cells, proliferation between the hepatocytes (B5). Fig.23c showed renal tubules showed degeneration. Chang and coagulative necrosis (c1,c2), focal area of the tubules showed hyperplasia and dysplasia in the lining epithelium (c3), focal haemorrhage and inflammatory cells infiltration in cortico-medullary junction (c4,5) of Al treated group plus zinc administration. While Fig.23C Showed Capsule was thick by fibrous Connective tissue proliferation (C1), fibrosis with inflammatory cells infiltration between the hepatocytes in the parenchyma (C2) & Fatty changes in hepatocytes.

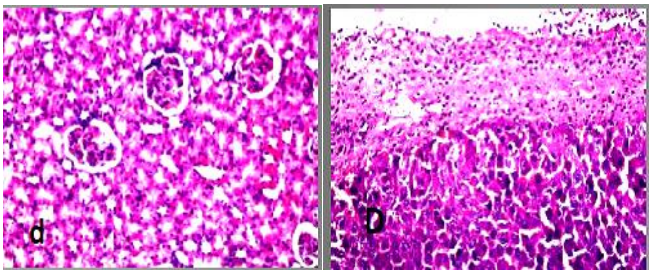


c: Kidney tissue



C: liver tissue

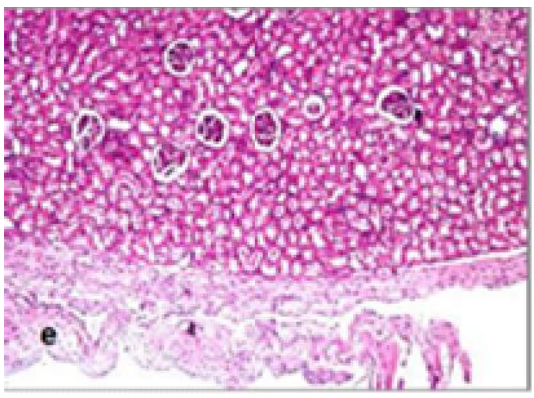
Fig. 23. Effect of Al and Zinc on histopathological changes of rats tissues



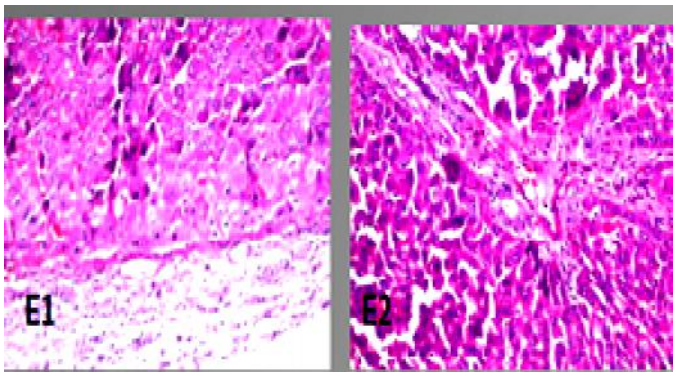
d: Kidney tissue **D**: liver tissue

Fig. 24. Effect of Al and COQ10 on histopathological changes of rats tissues

No histopathological alteration in kidney tissues in Al group treated with COQ10 as shown in Fig.24d. While Fig.24D Showed thickening in the hepatic capsule by oedema and inflammatory cells infiltration. Thickening in the renal capsule by oedema and inflammatory cells infiltration are shown in Fig.25e of Al treated group administered with vinpocetine. Fig.25E Showed thickening of hepatic capsule, by oedema and inflammatory cells infiltration, hepatocytes showed apoptosis (E1), inflammatory cells infiltration and fibrosis were detected in the portal area (E2). In Fig.26f kidney tissues showed, thickening in the capsule by inflammatory cells infiltration and fibroblastic cells proliferation. Fig.26F Showed inflammatory cells infiltration and fibroblastic cells proliferation in the thick capsule (F1), collagen fibers extended between hepatocytes in the parenchyma (F2), Apoptosis in some individual hepatocytes surrounding the central vein (F3, F4), congestion in the portal areas, periductal inflammatory cells infiltration and oedema (F5).

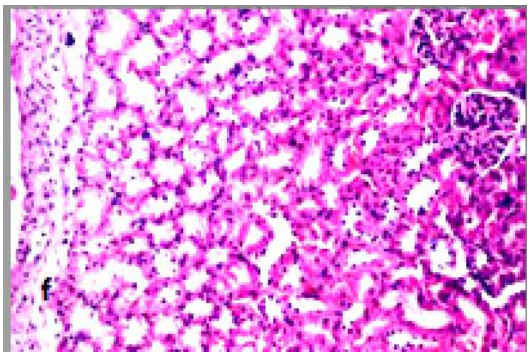


e: Kidney tissue

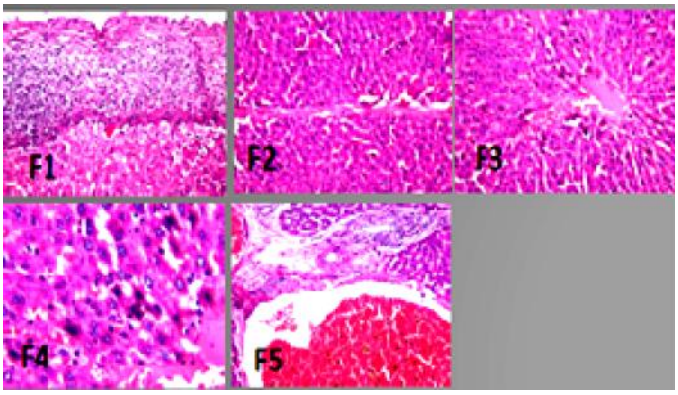


E: liver tissue

Fig. 25. Effect of Al and vinpocetine on histopathological changes of rats tissues



f: Kidney tissue



F: liver tissue

Fig. 26. Effect of Al and cocoa on histopathological changes of rats tissues

Kidney tissues in Fig.27 g Showed tubules degeneration and coagulative necrosis (g1,g2). While Fig.27 G Showed thickening in the hepatic capsule by oedema and inflammatory cells infiltration (G1) extended in the parenchyma between the hepatocytes (G2). Congestion in the portal vein (G3) was present.

DISCUSSION

Aluminum accumulation is common in all tissues of mammals, such as the kidneys, liver, blood, bones, heart, and brain (Al-Kahtani, 2010) and it was reported that the toxic actions of Al were prominent in the kidney as well as the liver (Abubakar *et al.*, 2004 and Jianyu *et al.*, 2016). In the present study, injection of rates by AlCl3 (70 mg/kg, IP) daily for consecutive three weeks induced a significant increase in blood urea nitrogen (BUN) and serum creatinine concentrations in the AlCl3-intoxicated group when compared to control. These findings were in accordance with El-Demerdash, (2004) reported that Al exposure induces changes in kidney function. Recently, Jianyu *et al.* (2016) also reported that BUN alterations indicate that suppression of glomerular filtration function occurred as a result of AlCl3 exposure. On the other hand, the current study revealed a significant increase in the serum levels of ALT, AST, ALP, LDH, total bilirubin and significant decrease in serum albumin in the AlCl3-intoxicated group when compared to control. As a matter of fact, the increase in transaminases levels are encountered in conditions inducing hepatocellular damage, cell membrane functional integrity loss and necrosis (Ninh *et al.*, 2003). Cell necrosis induces an increase in LDH enzyme concentrations in serum and tissue.

The LDH released into the medium considered an index of cell death and membrane permeability to LDH as a result of cell membrane disintegration and enzyme leakage (Shen *et al.*, 1995 and Lindell *et al.*, 1996). Gonzalez *et al.* (2007) and Tripathi *et al.* (2008) demonstrated also that interactions between oxidative stress and hepatic damage may enhance the progression of chronic hepato-degenerative diseases. Additionally, the present work revealed a significant elevation in serum lipid profile (manifested by a significant increase in serum TG and TC with a significant decrease in HDL-C) in $AlCl_3$-intoxicated group compared to the control group. This came in accordance with other studies reported that Al-triggered dyslipidemia may lead to a variety of hepatic abnormalities. Li *et al.* (2006) and Mailloux *et al.* (2007) reported an accumulation of lipids in the liver in chronic kidney disease resulting from Al overload. There is a balance between the oxidants (reactive oxygen species [ROS]) and antioxidants in healthy individuals. If this balance is altered as a result of over production of ROS, oxidative stress may occur, which affects oxidative damage to organs (Joshi *et al.*, 2013). In parallel to these results, the oxidative markers of the current study in renal and hepatic tissues, showed a significant elevation in renal and hepatic MDA and NO while a significant decline in antioxidant activity of renal and hepatic SOD and TAC were also reported. These results are in accordance with Ferretti *et al.* (2003), Candan & Tuzmen (2008) and Mailloux *et al.* (2011), demonstrated that altered redox status and raised lipid peroxidation are considered hallmarks of an oxidative environment, and these dysfunctions are all linked to the Al toxicity. Therefore, the suggested mechanism by which Al can inflict its toxic effects is; creating free radicals in the body. It causes a toxic action due to its ability to transfer electrons which can affect cell integrity, producing lipid peroxidation in the intracellular membranes, affecting also its permeability of subcellular organelles, the structure and functions of proteins and nucleic acids (Taus *et al.*, 2013). Additionally, Abubakar *et al.* (2003) ascertained that even minute quantities of aluminum in hepatocytes associated with ROS increase and peroxidation. Newairy *et al.* (2009) supported this suggestion also as an increase in the level of thiobarbituric acid reactive substance (TBARS) and a decline in the activities of GST, SOD and CAT in liver, kidney and brain of rats treated with (34 mg/kg body weight AlCl3 daily for 70 days) were reported. Garrel *et al.* (1994) and Bondy *et al.* (1998) have reported that aluminum-enhanced peroxidation may be related to aluminum-induced nitric oxide synthase (NOS) activity and raised NO products in rat brain tissue and microglial cells. NO is generated from L-arginine by the aid of NOS enzyme which formed in a variety of tissues and included in various physiological and pathological processes (Moncada *et al.*,

1991). Ward *et al.* (2001) suggest another mechanism for Al toxicity, as Al exposure could enhance disruptions in the mineral balance, leading to Al ions replacing iron and magnesium, which would then result in a decline in Fe^{+2} binding to ferritin. Hence, Al-induced free iron ions releasing from biological complexes can catalyze hydroperoxides decomposition to hydroxyl radicals through Fenton's reaction. The initiation of lipid peroxidation, causing membrane damage occurred as a result of this high hydroxyl radical reactivity.

Regarding the relationship between AlCl3 and immune function in rats, the current results exhibited significant elevations in the levels of pro-inflammatory cytokines; including TNF-α and IL-6β and NFκ-B in kidney and liver tissues of AlCl3- intoxicated group, which fits with the results of Mannaa *et al.* (2013) who mentioned that chronic inflammatory process might contribute to AlCl3 toxicity. Kuo *et al.* (2011) illustrated the role of Nuclear factor kappa beta (NF-κ B) in the inflammation as it controls the expression of different genes encoding pro-inflammatory cytokines, and inducible enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) leading to NO and TNF-α production.

The elevated inflammatory state in our emerging data confirmed with histopathological examination of the kidney and liver sections of the AlCl3 - intoxicated group. Firstly, the kidney homogenate showed oedema with fibrosis and thickening of renal capsule, degeneration in the lining tubular epithelium, hyperplasia and dysplasia were noticed in the lining epithelium of some focal tubules.These findings were in agreement with previous results of in vivo and in vitro studies demonstrating that AlCl3 increases the thickness of Bowman's capsule basement membrane, resulted in atrophy of glomerulus capillaries and proximal and distal tubules damage (Stacchiotti *et al.*, 2006 and Sargazi *et al.*, 2006). Secondly, the histopathological examination of liver tissues in the present study confirmed the previous results, as it revealed a thickening in the hepatic capsule by inflammatory cells infiltration and fibroblastic cells proliferation between the hepatocytes in the hepatic parenchyma. Besides, inflammatory cells infiltrations in the portal area, kupffer cells diffusion, proliferation between the hepatocytes were also reported.These findings were in accordance with Bogdanovic *et al.* (2008) reported a congestion of central vein, sinusoidal dilatation and lipid accumulation in liver. To explore the mechanism by which AlCl3-induce apoptosis, caspase-3 protein level was measured in kidney and liver homogenates. The current work showed a significant elevation in the level of renal and hepatic caspase 3 in AlCl3- intoxicated group. These results potentiated by observing DNA fragmentation in liver and kidneyin AlCl3- intoxicated group (Lane D: shows DNA streaks, Fig. 20A & 20B). The hallmark of apoptosis is DNA fragmentation (Nagata, 2000). Zou *et al.* (1997) suggested the molecular mechanisms underlying apoptosis including cytochrome release, caspases activation, condensation of chromatin, DNA fragmentation as well as dead cells phagocytosis, and debris by scavenger cells. Therefore, $AlCl_3$ induced apoptosis through the activation of caspase-3, by the following consequences; reactive oxygen species (ROS) increase the mitochondrial membrane permeability leading to mitochondrial failure (Huang *et al.* 2008). The mitochondrial membrane permeability is dependent upon the mitochondria permeability transition pore that results in cytochrome c release from the mitochondria and into the cytosol (Yang *et al.* 2014). Once released, cytochrome cbinds A paf-1 in the cytoplasm forming a complex that can activate caspase-9 with subsequent activation of death-inducingcaspase-3. (Ghribi *et al.* 2002). Due to the previous nephrotoxic effects of injection of $AlCl_3$ (70 mg/kg, IP) for successive three weeks, the present study was designed to evaluate and compare the potency of different drugs and nutrients(CoQ10, vinpocetine, zinc, wheatgrass and cocoa) against nephrotoxicity induced by Al in rats.

The nephro-protective potency of the studied drugs and nutrients against Al toxicity revealed a significant improvement in the kidney function activity in Al-treated groups with different drugs and nutrients as CoQ10 (200mg/kg), wheat grass (100mg/kg), cocoa powder (24mg/kg), vinpocetine (20mg/kg) or zinc (32mg/kg) when compared with Al-toxicity model. There was a significant decline in the serum levels of BUN and creatinine. Additionally, CoQ10 treated group showed the more significant decline in the serum level of BUN when compared to the other treated groups. These findings came in agreement with several studies have demonstrated that some of the renal protective effect of CoQ10 in rats with renal dysfunction probably related to its antioxidant effect (Manning *et al.*, 2005 and Liu *et al.*, 2006). CoQ10 is known to be a member of the mitochondrial electron transport chain, which can accept either one or two electrons. It acts as a powerful natural antioxidant, oxygen-derived free radical scavenger and as membrane stabilizer (Ostrowski, 1999).

Regarding the oxidative stress markers in kidney homogenate, the current study demonstrated a significant decline in renal MDA and NO, while a significant increase in renal SOD and TAC was also reported in Al-treated groups with different drugs and nutrients when compared with Al-toxicity model. Interestingly, there was a predominant significant increase in level of renal SOD in CoQ10 treated group when compared to other treated ones. Additionally, there was a predominant significant increase in level of renal TAC in CoQ10 followed by wheat grass treated group when compared to other treated ones. These results are in parallel with McCarthy *et al.* (2004) reported that in animal models, CoQ10 have protective activities against toxin-induced oxidative stress. The recent study of Sayed *et al.* (2015) also proposed that CoQ10 might be valuable as a potent cellular defense against oxidative damage after aluminum toxicity. He believed that in the presence of Al induced cell toxicity, CoQ10 can increase cytochrome c oxidasea activity; consequently, should help to restore mitochondrial activity and ATP production. To interpret the present nephro-protective results of wheat grass treated group, Khan *et al.* (2013) suggested that the antioxidant enzymes present in wheat grass helps rid of free radicals via the regulation of cellular homeostasis and augmentation of self-defense to oxidative stress. For the evaluation of the anti-inflammatory and anti-apoptotic properties of the studied drugs and nutrients, the present work revealed a significant decrease in renal TNFα, IL-6β, NFκ-B and caspase 3 in Al-treated groups with different drugs and nutrients when compared with Al-toxicity model. These findings were confirmed by DNA fragmentation in the kidney as, Lane D: shows DNA streaks (Groups of AL-induced toxicity which found in the model (M) laddering shape) Group P: there are no streaks groups of treatment (Fig. 20A).

Additionally, the current results revealed a more significant decrease in renal TNFα level in vinpocetine followed by CoQ10treated groups when compared with other treated groups. These results came in accordance with Jeon *et al.* (2010) who reported that Vinpocetine exerts an anti-inflammatory action inhibiting (TNF-α)-induced nuclear factor-kappa B (NF-κB) activation, and the induction of pro-inflammatory mediators. In accordance with these findings also, Schmelzer *et al.* (2007) suggests that CoQ10 has a number of independent anti-inflammatory activities. The novelty of the present results of nephroprotective evaluation that the histopathological examination of kidney revealed that the most nephroprotective drug was CoQ10 as, no histopathological alteration was observed which suggest that CoQ10of prominent protection against the nephrotoxic effects of Al toxicity. In contrast with other drugs which showed thickening in the renal capsule by oedema and inflammatory cells infiltration. These results came in accordance with several studies which suggest the anti-inflammatory mechanism of CoQ10 by reduction of pro-inflammatory cytokines secretion in monocytes and lymphocytes after an inflammatory stimulus through an influence on the expression of NFκ-B -dependent genes(Sohet *et al.,* 2009, Bentinger *et al.,* 2010 and Mohseni *et al.,* 2014). Furthermore, in a series of reports in patients with coronary artery disease, CoQ10 used as a treatment(60–300 mg CoQ10/10day for 12 weeks), to reduce oxidative stress and improve the antioxidant enzyme activity as well as lowering inflammation as assessed by plasma levels of inflammatory markers such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 (Lee *et al.,* 2012a, 2012b and 2013). Similarly, dietary CoQ10 (diet supplemented with 0.07%–0.7% (w/w) CoQ10 for26 weeks) was accompanied with a decline in plasma oxidative stress and inflammatory markers in a rat model of the metabolic syndrome (Kunitomo *et al.,* 2008). There are also, a lot of animal studies which suggest that antioxidant activities of CoQ10have beneficial effects on kidney disease (Manning *et al.,* 2005, Nagase *et al.,* 2006 and Liu *et al.,* 2009).

Secondly, the hepatoprotective potency of the studied drugs and nutrients against Al toxicity revealed a significant improvement in the liver function activity in Al-treated groups with different drugs and nutrients [CoQ10 (200mg/kg), wheat grass (100mg/kg), cocoa powder (24mg/kg), vinpocetine (20mg/kg) or zinc (32mg/kg)] when compared with Al-toxicity model. As there was a significant decline in the serum levels of ALT, AST, ALP, LDH as well as total bilirubin and significant increase in hepatic synthetic function in treated groups (measured by serum albumin). These results were in parallel with other studies of Bhasin *et al.* (2014) and Lamuela-Ravent´ *et al.* (2005) suggested that zinc and cocoa have antioxidant activity, so be useful in preventing the toxic effects of Al on the liver. These results are in continuation of earlier studies observed a remarkable improvement in the levels of transaminases following zinc administration (Dhawan *et al.,* 1992; Dhawan and Goel, 1994). The protective effect is related to the role of zinc in the protein metabolism regulation, which in turn regulates the levels of trans aminases. The current study demonstrated a significant decline in hepatic MDA and NO, while a significant increase in antioxidant hepatic SOD and TAC was also reported in Al-treated groups with different drugs and nutrients when compared with Al-toxicity model. In accordance with these results Adaramoye *et al.* (2008) reported that Al increases oxidative stress via increasing the level of superoxides (O2−) and peroxides (H2O2). Since these oxides were not measured in this work, but it is believed that ROS can bind and react with hepatic cellular components inducing hepatic injury, and sodeteriorating liver function (Adekunle *et al.,* 2009). Taking these mechanisms into consideration. Adekunle *et al.* (2009) also stated that drugs that have antioxidant effect or have the ability to decrease oxidative stress can be of valuable role in inhibiting the bad effects of Al on the liver. Similar observations have also been reported by Esparza *et al.* (2005) in experimental animals treated with zinc after Al exposure. This could be related to the antioxidant character of zinc, which was able to normalize the activity of SOD.

The present results also showed the more prominent hepatoprotective effect of CoQ10 (200mg/kg), to Al toxicity model rather than other treated groups, as a significant decrease in hepatic MDA and NO levels was reported when compared to other treated groups. These results came in accordance with Ostrowski, (1999) stated that CoQ10 acts as a potent natural antioxidant, oxygen-derived free radical scavenger and as membrane stabilizer (Ostrowski, 1999). In addition, CoQ10 exerts inhibiting character on mitochondrial ROS generation and inner mitochondrial depolarization (Kwong *et al.,* 2002). Moreover, CoQ10 supplementation protects plasma membrane against oxidative stress (Gómez-Díaz *et al.,* 2003).The above mentioned effects of CoQ10 permit this coenzyme to exhibit an improvement in each of the oxidative stress markers as investigated in the present study. The present work revealed a significant decrease in hepatic TNFα, IL-6β, NFκ-B and caspase 3 in Al-treated groups with different drugs and nutrients when compared with Al-toxicity model. These findings were confirmed by DNA fragmentation in the liver as, Lane D: shows DNA streaks (Groups of Al) (induced toxicity which found in the model (M) laddering shape) while group P: there is no streaks in the groups of treatment (Fig.20B).

Additionally, Co enzyme Q10 (200mg/kg), treated group showed the more significant decline in the level of hepatic NFκ-B when compared to the other treated groups. Besides, the histopathological examination of Co enzyme Q10 treated group showed also slight thickening in the hepatic capsule by oedema and inflammatory cells infiltration when compared to Al toxicity model which can indicate possible hepato-protective role of Co enzyme Q10 supplementation against Al toxicity in rats. These results were in agreement with several studies demonstrated that supplementation of the diet with CoQ10 has been found to affect plasma antioxidant and inflammatory markers in healthy individuals(Gökbel *et al.,* 2010) and animal studies have demonstrated effects on liver oxidative stress and lipid metabolism (Cano *et al.,* 2009, Sohet *et al.,* 2009, Safwat *et al.,* 2009). In conclusion, this study showed a protective effect of using different drugs and nutrients as CoQ10, wheatgrass, cocoa, vinpocetine and zinc against AlCl3 induced renal and hepatic damage. Interestingly, CoQ10 as well as wheat grass possess the most superior protection. It is our belief that CoQ10 should be able to not only alleviate oxidative stress at the level of the mitochondria and consequently increase cell survival, but is also likely to help to resolve liver and kidney dysfunction present in patients with aluminum toxicity. Reduction of oxidative stress and inflammation may be considered as the main pathways of action. However, further experiments at the molecular levels are required to illustrate clearly the mechanism by which these drugs and nutrients reverse $AlCl_3$ induced nephrotoxicity and hepatotoxicity.

REFERENCES

Adaramoye A, D O Osaimoje, A M Akinsanya, C MNneji, M A Fafunso, O G Ademowo. 2008. Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether-lumefantrine and halofantrine in rats, *Basic and Clinical Pharmacology and Toxicology*, vol. 102, no. 4, pp. 412–418.

Adekunle A S, C O Falade, E O Agbedana, A Egbe,. 2009. Assessment of side-effects of administration of artemether in humans, Biology and Medicine, vol. 1, no. 3, pp. 15–19.

Abubakar MG, Taylor A, Ferns GA. 2003. The effects of aluminium and selenium supplementation on brain and liver antioxidant status in the rat. *Afr J Biotechnol*. 3(1):88-93.

Abubakar MG, Taylor A, Ferns GA. 2004. The effects of aluminium and selenium supplementation on brain and liver antioxidant status in the rat. *African Journal of Biotechnology* 3: 88–93.

Addai F K. 2010. Natural cocoa as diet-mediated anti malarial prophylaxis. *Medical Hypotheses*, vol. 74, no. 5, pp. 825–830.

Agnieszka P, Kamilla B-K, Justyna P, Ewa W-T(2016. Influence of Long-Term Zinc Administration on Spatial Learning and Exploratory Activity in Rats. *Biol Trace Elem Res*, 172:408–418

Ahn HS, Crim W, Romano M, Sybertz E, Pitts B. 1989. Effects of selective inhibitors on cyclic nucleotide phosphodiesterases of rabbit aorta. *Biochem Pharmacol* 38:3331–3339.

Al Kahtani MA. 2010. Renal damage mediated by oxidative stress in mice treated with aluminium chloride: Protective effects of taurine. *Journal of Biological Sciences*; 10: 584-595.

Ali AA, Ahmed HI, Abu-Elfotuh K. 2015. Modeling stages of Alzheimer's disease induced by different doses of aluminum in rats: Focus on progression of the disease in response to time. *J Alzheimers Dis Parkinsonism* 5: 94.

Amin I, Koh B, and Asmah R. 2004. Effect of cacao liquor on tumor marker enzymes 492 during chemical hepatocarcinogenesis in rats. *Journal of Medicinal Food*. vol. 7, no. 1, pp. 7–12.

Amponsah S K, Bugyei K A, Osei-Safo D *et al.* 2012. In vitroactivity of extract and fractions of natural cocoa powder on Plasmodium falciparum. *Journal of Medicinal Food*, vol. 15, no.5, pp. 476–482.

Andreassen OA, Weber C, Jørgensen HA. 1999. Coenzyme Q10 does not prevent oral dyskinesias induced by long-term haloperidol treatment of rats. *Pharmacol Biochem Behav* 64: 637-642.

Arya, P. and Kumar, M.. 2011. Chemoprevention by *Triticum aestivum* of mouse skin carcinogenesis induced by DMBA and croton oilassociationwith oxidative status. *Asian Pac. J. Cancer Prev.* 12, 143–148

Bancroft J and Stevens A. 1996. Theory and practice of histological techniques. 4th ed. Edinburgh: Churchill Livingstone.

Belaïd-Nouira Y, Bakhta H, Haouas Z, Flehi-Slim I, Ben Cheikh H. 2013. Fenugreek seeds reduce aluminum toxicity associated with renal failure in rats. *Nutr Res Pract* 7:466–474.

Bentinger, M, Tekle, M, Dallner, G. 2010. Coenzyme Q-biosynthesis and functions. *Biochem.Biophys. Res. Commun*. 396, 74–79.

Betholf RL. 1988. Zinc. In: Seiler HG, editor. Handbook Toxicology of Inorganic Compounds. New York: Siegel Dekker. p 788.

Bhasin Punita, Singla Neha, Dhawan D K. 2014. Protective Role of Zinc During Aluminum-Induced Hepatotoxicity. *Environ Toxicol* 29: 320–327.

Bogdanovic M, Janeva AB, Bulat P. 2008. Histopathological changes in rat liver after a single high dose of aluminium. *Archives of Industrial Hygiene and Toxicology* 59: 97–101.

Bondy, SC, Liu D, Guo-Ross. 1998. Aluminum treatment induces nitric oxide synthase in the rat brain. *Neurochem Int*, 33: 51-54.

Cabre M, Ferre N, Folch J, Paternain JL, Hernandez M, CastilloD, Joven G, Camps. J. 1995. Inhibition of hepatic cell nuclear DNA fragmentation by zinc in carbon tetrachloride-treated rats. *J Hepatol* 31:228–234.

Cagen SZ, Klassen CD. 1979. Protection of carbon tetrachlorideinduced hepatotoxicity by Zinc: Role of metallothionein. Toxicol App Pharmacol 51:107–116.

Campbell A, Becaria A, Lahiri DK, Sharman K, Bondy SC. 2004. Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. *J Neurosci Res* 75:565–572.

Candan N and Tuzmen N.. 2008. Very rapid quantification of malondialdehyde. MDA. in rat brain exposed to lead, aluminium and phenolic antioxidants by high-performance liquid chromatography fluorescence detection. *Neurotoxicology*. 29:708–13.

Cano, A, Ciaffoni, F Safwat, GM, Aspichueta, P, Ochoa, B, Bravo, E, Botham, KM. 2009. Hepatic VLDL assembly is disturbed in a rat model of nonalcoholic fatty liver disease: Is there a role for dietary coenzyme Q? J Appl. *Physiol* 107, 707–717.

Chinoy NJ, Patel TN. 1999. Reversible toxicity of fluoride and aluminium in liver and gastrocnemius muscle of female mice. *Fluoride* 32:215–229.

Cordero-Herrera I, Mart´ın M A, Goya L, Ramos S. 2015. Cocoa flavonoids protect hepatic cells against high-glucoseinducedoxidative stress: relevance of MAPKs. *Molecular Nutrition and Food Research*, vol. 59, no. 4, pp. 597–609.

Dhawan DK, Goel A, Gautam CS.. 1992. Effects of zinc intake on liver enzymes in toxicity carbontetrachloride induced liver injury. *Med Sci Res* 20:55–60.

Dhawan DK, Goel A. 1994. Protective role of zinc on rat liver function in long-term toxicity induced by carbon tetrachloride. *J Trace Elem Exp Med* 7:1–9.

Dumont M, Kipiani K, Yu F, Wille E, Katz M, *et al.*. 2011. Coenzyme Q10 decreases amyloid pathology and improves behavior in a transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis* 27: 211-223.

El-Demerdash FM. 2004. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. *J Trace ElemMed Biol* 18:113–121.

Esparza JL, Go´mez M, Rosa Nogue´s M, Paternain JL, Mallol J, Domingo JL.. 2005. Melatonin reduces oxidative stress and increases gene expression in the cerebral cortex and cerebellum of aluminum-exposed rats. *J Pineal Res* 39:129–136.

Exley C, Burgess E, Day JP, Jeffery EH, Melethil S, Yokel RA(1996. Aluminum toxicokinetics. *J Toxicol Env Heal* 48:569–584.

Exley C, Burgess E, Day JP, Jeffery EH, Melethil S, Yokel RA(1996. Aluminum toxicokinetics. *J Toxicol Env Heal* 48:569–584.

Exley C. 2011. Aluminium-based adjuvants should not be used as placebos in clinical trials. *Vaccine* 29:9289.

Falcioni G, Fedeli D, Tiano L, Calzuola I, Mancinelli L, Marsili V, Gianfranceschi G. 2002. Antioxidant activity of wheat sprouts extract *in vitro*: Inhibition of DNA oxidative damage. *J. Food Sci*. 67, 2918–2922.

Farina M, Lara FS, Brandão R, Jacques R, Rocha JB. 2002. Effects of aluminum sulfate on erythropoiesis in rats. *Toxicol Lett* 132: 131-139.

Ferretti G, Marchionni C, Bacchetti T, Galeazzi T, Dousset N. 2003. Effect of aluminium on lipid peroxidation of human high density lipoproteins. *Free Radic Res*. 37:515–21.

Garrel C, JL Lafond, P Guiraud, P Faure, A Favier. 1994. Induction of production of nitric oxide in microglial cells by insoluble form of aluminum. *Ann New York Acad Sci*, 738: 455.

Ghribi O, Herman MM, Savory J. 2002). The endoplasmic reticulumis the main site for caspase-3 activation following aluminum induced neurotoxicity in rabbit hippocampus. *Neurosci. Lett*. 324, 217–221.

Goel A, Dani V, Dhawan DK. 2007. Zinc mediates normalization of hepatic drug metabolizing enzymes in chlorpyrifos-induced toxicity. *Toxicol Lett* 169:26–33.

Gökbel, H, Gergerlio ˘ glu, HS, Okudan, N, Gül, I, Büyükba¸s, S Belviranli, M. 2010. Effects of coenzyme Q10 supplementation on plasma adiponectin, interleukin-6, and tumor necrosis factor-_ levels in men. *J. Med. Food*. 13, 216–218.

Gómez-Díaz C, Burón MI, Alcaín FJ, González-Ojeda R, González-Reyes JA, *et al.*. 2003. Effect of dietary coenzyme Q and fatty acids on the antioxidant status of rat tissues. *Protoplasma* 221: 11-17.

Gonzalez MA, Alvarez ML, Pisani GB. 2007. Involvement of oxidative stress in the impairment in biliary secretory function induced by intraperitoneal administration of aluminum to rats. *Biological Trace Element Research* 116: 329–348.

Gulyás B, Halldin C, Vas A, Banati RB, Shchukin E, Finnema S, Tarkainen J, Tihanyi K, Szilágyi G, Farde L. 2005. Vinpocetine: a prospective peripheral benzodiazepine receptor ligand for primate PET studies. *J Neurol Sci* 229–230:219–223.

Huang SH, Lin CM, Chiang BH. 2008. Protective effects of Angelica sinensis extract on amyloid beta-peptide-induced neurotoxicity. *Phytomedicine* 15, 710–721.

Jang JH, Kim CY, Lim SH, Yang CH, Song KS, Han HS, Lee HK. 2010. Neuroprotective effects of *Triticum aestivum* L. against β -amyloid-induced cell death and memory impairments. *Phytother. Res*. 24**,** 76–84.

Jeon KI, Xu X, Aizawa T, Lim JH, Jono H,Kwon DS, Abe J, Berk BC, Li JD, Yan C. 2010. Vinpocetine inhibits NF-kappaB-dependent inflammation via an IKK-dependent but PDE-independent mechanism. *Proc Natl Acad Sci U S A* 107:9795–9800.

Jianyu Liu, Qin Wang, Xudong Sun, Xu Yang, Cuicui Zhuang, Feibo Xu, Zheng Cao, Yanfei Li. 2016. The Toxicity of Aluminum Chloride on Kidney of Rats. *Biol Trace Elem Res*; 173:339–344.

Joshi DK, Choudhary M, Tripathi S, Singh Negi MP, Mahdi AA. 2013. Age dependent relative risk of aluminum toxicity: levels of metals and enzymic and non enzymic antioxidants status in liver, kidney and brain of aluminum treated young and old rats. *Int J Biol Pharm Res*. 4(3):176-185.

Kaneko N, Yasui H, Takada J, Suzuki K, Sakurai H. 2004. Orallya dministrated aluminum-maltolate complex enhances oxidative stress in the organs of mice. *J Inorg Biochem* 98:2022–2031.

Kang YJ. 1999. The antioxidant functions of metallothionein in the heart. *Proc Soc Exp Biol Med* 222:263–273.

Khan GM, Ansari SH, Ahmad F. 2013. Pharmacognistic standardization, antioxidant and free radical scavenging activity of the seeds of Triticum aestivum L-a dietary staple. *J Young Pharm* ; 5(2): 54-59.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. 2001. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 54: 356-361.

Kunitomo, M, Yamaguchi, Y, Kagota, S, Otsubo, K. 2008. Beneficial effect of coenzyme Q10 on increased oxidative and nitrative stress and inflammation and individual metabolic components developing in a rat model of metabolic syndrome. *J. Pharmacol. Sci*. 107, 128–137.

Kuo, CF, JD Su, CH Chiu, CC Peng, CH Chang, and TY Sung. 2011. Anti-inflammatory effects of supercritical carbon dioxide extract and its isolated carnosic acid from *Rosmarinus officinalis* leaves. *Journal of Agricultural and Food Chemistry*, 59: 3674-3685.

Kwong LK, Kamzalov S, Rebrin I, Bayne ACV, Jana CK, *et al.*. 2002. Effects of coenzyme Q. 10. administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat. *Free Radical Biol Med* 33: 627- 638.

Lee, BJ, Huang, YC, Chen, SJ, Lin, PT. 2012a. Effects of coenzyme Q10 supplementation on inflammatory markers. high-sensitivity C-reactive protein, interleukin-6, and homocysteine. in patients with coronary artery disease. *Nutrition*, 28, 767–772.

Lee, BJ, Lin, YC, Huang, YC, Ko, YW, Hsia, S, Lin, PT. 2012b. The relationship between coenzyme Q10, oxidative stress, and antioxidant enzymes activities and coronary artery disease. Scientific World Journal. 792756.

Lee, BJ, Tseng, YF, Yen, CH, Lin, PT. 2013. Effects of coenzyme Q10 supplementation. 300 mg/day. on antioxidation and anti -inflammation in coronary artery disease patients during statins therapy: A randomized, placebo-controlled trial. *Nutr. J*. 12, 142.

Leoncini E, Prata C, Malaguti M, Marotti I, Segura-Carretero A, Catizone P, *et al.*. 2012. Phytochemical profile and nutraceutical value of old and modern common wheat cultivars. PLoS ONE; 7(9): e45997.

Li J, Bosch-Marce M, Nanayakkara A, Savransky V, Fried SK, Semenza GL, *et al.*. 2006. Altered metabolic responses to intermittent hypoxia in mice with partial deficiency of hypoxia inducible factor-1α. *Physiol Genomics*. 25:450–7.

Lindell SL, Hansen T, Rankin M. 1996. Donor nutritional status—a determinant of liver preservation injury. Transplant 61: 239–247.

Liu F, Wei CC, Wu SJ, Chenier I, Zhang SL, Filep JG, *et al.* 2009. Apocynin attenuates tubular apoptosis and tubule interstitial fibrosis in transgenic mice independent of hypertension. *Kidney Int*. 75:156–66.

Mailloux R, Lemire J, Appanna V. 2007. Aluminum-induced mitochondrial dysfunction leads to lipid accumulation in human hepatocytes: a link to obesity. Cell Physiol Biochem 20:627–38.

Maleyki J A and Ismail A. 2010. Antioxidant properties of cocoa Powder. *Journal of Food Biochemistry*, vol. 34, no. 1, pp. 111–128.

Malloy HT and Evelyn KA. 1937. The determination of bilirubin with the photometric colorimeter. *J Biol Chem* 119: 481-490.

Mannaa FA, Abdalla MS, Abdel-Wahhab KG, El-Kassaby MI. 2013. Effect of some nutraceutical agents on aluminum-induced functional neurotoxicity in senile rats: Effect of rosemary aqueous extract and docosahexaenoic acid. *Journal of Applied Sciences Research* 9: 2322-2334.

Manning RD Jr, Tian N, Meng S. 2005. Oxidative stress and antioxidant treatment in hypertension and the associated renal damage. Am J Nephrol. 25:311–7.

McCarthy S, Somayajulu M, Sikorska M, Borowy-Borowski H, Pandey S. 2004. Paraquat induces oxidative stress and neuronal cell death; neuroprotection by water-soluble coenzyme Q10. *Toxicol Appl Pharmacol*; 201:21e31.

Miranda KM, Espey MG, Wink DA. 2001. A rapid simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*; 5. (1): 62-71.

Mohan Y, Jesuthankaraj G N, Ramasamy Thangavelu N. 2013. Antidiabetic and antioxidant properties of *Triticum aestivum* instreptozotocin-induced diabetic rats. *Adv. Pharmacol. Sci*., 1–9.

Mohseni, M, Vafa, MR, Hajimiresmail, SJ, Zarrati, M, Rahimi Forushani, A, Bitarafan, V, Shidfar, F. 2014. Effects of coenzyme Q10 supplementation on serum lipoproteins, plasma fibrinogen, and blood pressure in patients with hyperlipidemia and myocardial infarction. *Iran Red Crescent Med. J*. 16, e16433.

Moncada, S, RM Palmer, Higgs, EA. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*, 43: 109-142

Moumen R, Ait-Oukhatar N, Bureau F, Fleury C, Bougle´ D,Arhan P, Neuville D, Viader F. 2001. Aluminium increasesxanthine oxidase activity and disturbs antioxidant status in therat. *J Trace Elem Med Biol* 15:89–93.

Nagase M, Yoshida S, Shibata S, Nagase T, Gotoda T, Ando K, *et al*.. 2006. Enhanced aldosterone signaling in the early nephropathy of rats with metabolic syndrome: possible contribution of fat derived factors. *J Am Soc Nephrol*. 17:3438–46.

Nagata S. 2000. Apoptotic DNA Fragmentation. *Experimental Cell Research*. 256, 12–18.

Neill D, Leake A, Hughes D, Keith AB, Taylor GA, Allsop D, Rima BK, Morris C, Candy JM, Edwardson JA. 1996. Effect of aluminium on expression and processing of amyloid precurs or protein. *J Neurosci Res* 46:395–403.

Newairy AS, Salama AF, Hussien HM, Yousef MI. 2009. Propolis alleviates aluminum-induced lipid peroxidaion and biochemical parameters in male rats. *Food Chem. Toxicol*. 47(6):1093-1098.

NinhT, Nguyen MD, Scott Braley MD. 2003. Comparison of postoperative hepatic function after laparoscopic versus open gastric bypass. *The American Journal of Surgery* 186: 40–44.

Nishikimi M, Rao NA, Yagi K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophys Res Comm* 46: 849-864.

Ochmanski W, Barabasz W. 2000. Aluminium-occurrence andtoxicity for organisms. *Przegl Lek* 57:665–668.

Ogueche P N, Ugwu C E,Ezejindu D N,Omeje M, Dike1C C, Okonkwo C O and Maduka1 Obidoa H C. 2014. Aluminum Intoxication Induced Biochemical and Histopathological Alterations in Male Wistar Albino Rats Hepotocytes. *Journal of Natural Sciences Research* ISSN 2224-3186. Paper. ISSN 2225-0921. Online)Vol.4, No.24.

Ostrowski RP. 1999. Effect of coenzyme Q10. CoQ10. on superoxide dismutase activity in ET-1 and ET-3 experimental models of cerebral ischemia in the rat. *Folia Neuropathol* 37: 247-251.

Ostrowski RP. 1999. Effect of coenzyme Q10. CoQ10. on superoxide dismutase activity in ET-1 and ET-3 experimental models of cerebral ischemia in the rat. Folia Neuropathol 37: 247-251.

Lamuela-Ravent´os, R M., A I Romero-P´erez, C Andr´es-Lacueva, A. Tornero,. 2005. Review: health effects of cocoa flavonoids, *Food Science and Technology International*, vol. 11, no. 3, pp. 159–176.

Ralf R and Josef K. 1991. Protective Effect of Vinpocetine against Brain Damage Caused by Ischemia. *Japan J Pharmacol*. 56,349-356.

Rishi P, Kaur P, Virdi JS, Shukla G, Koul A. 2008. Amelioratory effects of zinc supplementation on Salmonella-induced hepatic damage in the murine model. *Dig Dis Sci* 53:1063–1070.

Rozan P, Hidalgo S, Nejdi A, Bisson J-F, Lalonde R, Messaoudi M. 2007. Preventive Antioxidant Effects of Cocoa Polyphenolic Extract on Free Radical Production and Cognitive Performances after Heat Exposure in Wistar Rats. *Journal of food science*. Vol. 72, Nr. 3, S203-S206.

Safwat, GM, Pisanò, S, D'Amore, E, Borioni, G, Napolitano, M, Kamal, AA, Ballanti, P, Botham, KM, Bravo, E. 2009. Induction of non-alcoholic fatty liver disease and insulin resistance by feeding a high-fat diet in rats: Does coenzyme Q monomethyl ether have a modulatory effect? *Nutrition*, 25, 1157–1168.

Sargazi M, Shenkin A, Roberts NB. 2006. Aluminium-induced injury to kidney proximal tubular cells: Effects on markers of oxidative damage. *J Trace Elem Med Biol*. 19: 267-273.

Satoh K. 1978. Serum lipid peroxides in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta*; 90(1):37-43.

Sayed Mahdi Marashi, Mohammad Majidi, Mehran Sadeghian, Mostafa Jafarzadeh, Sogand Mohammadi , Zeynab Nasri-Nasrabadi. 2015. Protective role of coenzyme Q10 as a means of alleviating the toxicity of aluminum phosphide: An evidence-based review *Tzu Chi Medical Journal* 27:7- 9.

Schmelzer, C, Lorenz, G, Rimbach, G, Döring, F. 2007. Influence of Coenzyme Q_{10} on release of pro-inflammatory chemokines in the human monocytic cell line THP-1. *Biofactors*, 31, 211–217.

Serafini M. 2004. Polyphenols from tea and cocoa: ancient ingredients for human health? *Agro food Industry Hi-tech* 15:13–5.

Shen HM, Ong CN, Shi CY. 1995. Involvement of reactive oxygen species in aflatoxin b-1-induced cell injury in cultured rat hepatocytes. *Toxicology* 99: 115–123.

Sohet, FM, Neyrinck, AM, Pachikian, BD, de Backer, FC, Bindels, LB, Niklowitz, P, Menke, T, Cani, PD, Delzenne, NM,. 2009. Coenzyme Q10 supplementation lowers hepatic oxidative stress and inflammation associated with diet-induced obesity in mice. *Biochem. Pharmacol*. 78, 1391–1400.

Sohet, FM, Neyrinck, AM, Pachikian, BD, de Backer, FC, Bindels, LB, Niklowitz, P, Menke, T, Cani, PD, Delzenne, NM. 2009. Coenzyme Q10 supplementation lowers hepatic

oxidative stress and inflammation associated with diet-induced obesity in mice. *Biochem. Pharmacol*. 78, 1391–1400.

Spindler M, Beal MF, Henchcliffe C. 2009. Coenzyme Q10 effects in neuro degenerative disease. *Neuropsychiatr Dis Treat* 5:597–610.

Stacchiotti A, Rodella L, Ricci F, Rezzani R, Lavazza A, Bianchi R. 2006. Stress proteins expression in rat kidney and liver chronically exposed to aluminium sulphate. *Histol Histopathol* 21: 131-140.

Stahl L, Miller K B, Apgar J *et al.* 2009. Preservation of cocoa antioxidant activity, total polyphenols, flavan-3-ols, and procyanidin content in foods prepared with cocoa powder. *Journal of Food Science*, vol. 74, no. 6, pp. C456–C461.

Stevenson L, Phillips F, O'Sullivan K, Walton J. 2012. Wheat bran: its composition and benefits to health, a European perspective. *IntJ Food Sci Nutr*; 63(8): 1001-1013.

Tariq M, Morais C, Sujata B, Sobki S, Sulaiman MA, Khader AA. 1999. Aluminum exacerbates cyclosporin induced nephrotoxicity in rats. *Ren Fail* 21:35–48.

Taus N, Farraj M, Tănase S, Mironescu A, Boicu M, Necula V, Taus L. 2013. Aluminum - a chemical neurotoxic agent. Bulletin of the Transilvania University of Braşov Series VI Med Sci, 2(55):1-8.

Tietz Textbook of Clinical Chemistry. 2nd ed. Edited by CA Burtis, ER Ashwood. Philadelphia, WB Saunders Company, 1976.

Tripathi S, Somashekar BS, Mahdi AA, Gupta A, Mahdi F, Hasan M, *et al.*. 2008. Aluminium-mediated metabolic changes in rat serum and urine: a proton nuclear magnetic resonance study. *Journal of Biochemical and Molecular Toxicology* 22: 119–127.

Veera RB, Deepthi R, Nalini M, Annapurna A. 2014. Effect of wheat grass powder on aluminum induced Alzheimer's disease in Wistar rats. *Asian Pac J Trop Med* ; 7(Suppl 1): S278-S281

VranesiÄ-Bender D. 2010. The role of nutraceuticals in anti-aging medicine. *Acta Clin Croat* 49: 537-544.

Ward RJ, Zhang Y, Crichton RR. 2001. Aluminium toxicity and iron homeostasis. *J Inorg Biochem*, 87: 9-14.

Wilhelm M, Jaeger DE, Schu¨ll-Cablitz H, Hafner D, Idel H. 1996)Hepatic clearance and retention of aluminium: Studies in theisolated perfused rat liver. *Toxicol Lett* 89:257–263.

Yang W, Shi L, Chen L *et al.* 2014. Protective effects of perindoprilon d-galactose and aluminum trichloride induced neurotoxicity via the apoptosis of mitochondria-mediated intrinsic pathway in the hippocampus of mice. *Brain Res. Bull*. 109, 46–53.

Yokel RA, Hicks CL, Florence RL. 2008. Aluminium bioavailability from basic sodium aluminium phosphate, an approved food additive emulsifying agent, incorporated in cheese. *Food ChemToxicol;* 46:2261–2266.

Zhou X, Dong XW, Crona J, Maguire M, Priestley T. 2003. Vinpocetine is a potent blocker of rat NaV1.8 tetrodotoxin-resistant sodium channels. *J Pharmacol Exp Ther* 306:498–504.

Zou, H., Henzel, W. J., Liu, X., Lutschg, A, Wang, X. 1997. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 90, 405–413.

****