ISSN: 0975-833X

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

International Journal of Current Research
Vol. 9, Issue, 10, pp.58649-58669, October, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IMPACT OF STRESS AND PROTEIN MALNUTRITION ON THE POTENTIAL ROLE OF EPIGALLOCATECHIN-3-GALLATE IN PROVIDING PROTECTION FROM NEPHROTOXICITY AND HEPATOTOXICITY INDUCED BY ALUMINUM IN RATS

[1,*]Azza A. Ali, [2]Mona G. Khalil, [1]Hemat A. Elariny and [3]Shereen S. Elshaer

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
[3]Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Modern University for Technology and Information, Cairo, Egypt

ARTICLE INFO

Article History:
Received 22nd July, 2017
Received in revised form
09th August, 2017
Accepted 28th September, 2017
Published online 17th October, 2017

Key words:

Aluminum,
Stress,
Protein malnutrition,
Nephrotoxicity,
Hepatotoxicity,
Epigallocatechin-3-gallate,
Rats.

ABSTRACT

Background: Aluminum (Al) is very abundant metal in the earth's crust. It is a constituent of cooking utensils, medicines, cosmetics, some foods and food additives. Salts of Al are widely used in the treatment of drinking water for purification purposes. Excessive and prolonged exposure to Al causes oxidative stress and impairment of many physiological functions. Its accumulation in liver and kidney causes hepatotoxicity and nephrotoxicity. Social isolation (SI) or Protein malnutrition (PM) also increases oxidative stress and may enhance the toxicity of Al as well as the degeneration in liver and kidney. Epigallocatechin-3-gallate (EGCG) is the most abundant catechin in green tea and has strong antioxidant as well as anti-inflammatory activities and can protect against oxidative stress-induced degenerations.
Objective: To study the influence of stress or PM on Al-induced nephrotoxicity and hepatotoxicity in rats, as well as on the potential role of EGCG in providing protection.
Methods: Rats received daily $AlCl_3$ (70 mg/kg, IP) for three weeks (Al-toxicity groups) except one normal control group received saline. Al-toxicity groups were divided into four treated and four untreated groups; treated rats received EGCG (10 mg/kg, IP) together with $AlCl_3$. One group of both treated and untreated rats served as control for each of them and the others were subjected to either stress (mild using isolation or high using electric shock) or to PM (10% casein diet). Specimens of liver and kidney were used for assessment of inflammatory mediators (TNF- , IL-6 , NF- B), oxidative stress (MDA, SOD, TAC, NO), Caspase-3 and for DNA fragmentation as well as for histopathological examinations. Biochemical changes were also measured in the serum as cholesterol, triglycerides, HDL, albumin, total bilirubin, glucose, creatinine and urea as well as the levels of ALT, AST and ALP.
Results: Nephrotoxicity and hepatotoxicity induced by Al were enhanced in rats exposed to PM as well as to stress, the influence of stress especially high stress was more pronounced as indicated by the increase in liver and kidney MDA, NO, TNF- , IL-6 , NF- B, caspase-3, DNA fragmentation and in serum ALT, AST, ALP, cholesterol, triglycerides, total bilirubin, glucose, creatinine and urea levels, together with the decrease in HDL, albumin, SOD and TAC. EGCG provided protection against hazards of Al as indicated by the decrease in MDA, NO, TNF- , IL-6 , NF- B, caspase-3 and DNA fragmentation as well as in levels of ALT, AST, ALP, cholesterol, triglycerides, total bilirubin, glucose, creatinine and urea, together with the increase in HDL, albumin, SOD and TAC that is confirmed by histopathological examinations. It provided more pronounced protection in high stressful conditions than in mild one than in PM.
Conclusion: Stress have bad impact on Al-induced nephrotoxicity and hepatotoxicity more than PM, thus it can clarify and maximize the role of EGCG in providing protection. Consequently, administration of EGCG is advised with excessive Al-exposure to avoid nephrotoxicity and hepatotoxicity especially in populations more subjected to stress or PM.

Copyright©2017, Azza A. Ali et al. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Citation: Azza A. Ali, Mona G. Khalil, Hemat A. Elariny and Shereen S. Elshaer, 2017. "Impact of stress and protein malnutrition on the potential role of epigallocatechin-3-gallate in providing protection from nephrotoxicity and hepatotoxicity induced by aluminum in rats", *International Journal of Current Research*, 9, (10), 58649-58669.

INTRODUCTION

Aluminum (Al) is classified as one of the trace elements that have a reasonable toxic effect on living organisms (Kowalczyk *et al.*, 2004). It is a non-redox active metal (pro-oxidant) that promotes biological oxidation both in vitro and in vivo (Exley, 2004), which disrupts the pro-oxidant/antioxidant balance of tissues and leads to various biochemical and physiological dysfunctions (Nehru and Bhalla, 2006). The uses of Al are tremendous as compared to any other heavy metal (Neelam *et al.*, 1999). A variety of Al compounds are produced and used

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

for different purposes, such as in water treatment, papermaking, fire retardant, fillers, food additives, colors and pharmaceuticals. Al metal mainly in the form of alloys with other metals has many uses including in consumer appliances, food packaging and cookware (Berihu *et al*., 2015). Different forms of Al are environmental xenobiotics which accumulate in different mammalian tissues such as the kidneys, liver, heart, brain and provoke free radical-mediated hepatotoxicity, nephrotoxicity, cardiotoxicity, and neurotoxicity leading to alterations in antioxidant enzymes, both in vivo and in vitro (Sushma and Rao, 2007; Mahieu *et al*., 2009; Yousef and Salama, 2009; Kan *et al*., 2010; Turkez *et al*., 2010). The toxic effects associated with Al are related to the generation of reactive oxygen species (ROS) that results in the oxidative deterioration of cellular lipids, proteins, and DNA (Sargazi *et al*., 2006). Yousef (2004) and Newairy *et al*. (2009) reported that Al-induced changes in biochemical parameters, increased lipid peroxidation and decreased the activities of the antioxidant enzymes in plasma and different tissues of animals. The liver is a principal organ that contains most of the accumulated metals such as Al as it is the main site for the detoxification process while kidneys are involved in the elimination of Al (Mahieu *et al*., 2003; Kurutas *et al*., 2009). Systemic oxidative stress arising during liver disease can also cause damage to extra-hepatic organs, such as kidney failure while systemic oxidative stress is considered to play a critical role in the pathophysiology of several kidney diseases (Palma *et al*., 2014).

Oxidative stress occurs when ROS are formed in amounts that exceed the capacity of the antioxidant defence system to remove them. Our bodies are able to remove ROS to a certain degree in which these reactive species are not necessarily a threat to the body under physiological conditions (Li *et al*., 2015). ROS such as O_2^-, H_2O_2 and OH are highly toxic to cells. Cellular antioxidant enzymes and the free-radical scavengers normally protect a cell from toxic effects of the ROS. However, when generation of the ROS overtakes the antioxidant defence of the cells, oxidative damage of the cellular macromolecules (lipids, proteins, and nucleic acids) occurs, leading finally to various pathological conditions. ROS-mediated lipid peroxidation (LPO), oxidation of proteins, and DNA damage are well-known outcomes of oxygen-derived free radicals, leading to cellular pathology and ultimately to cell death (Mohale and Chandewar, 2012). Biochemical changes in the tissue can be estimated by biomarkers of oxidative stress. Therefore, LPO indicates oxidative damage via an increase in malondialdehyde (MDA) that contributes to significant toxicity (Devbhuti *et al*., 2009).
Tumor necrosis factor (TNF), a group of cytotoxic pro-inflammatory cytokines, is thought to play a vital role in initiation of liver damage as oxidative stress might act together with endotoxins to increase TNF production. Increased circulating TNF- stimulates TNF- receptors of cell surface, which leads to activation of the stress-related protein kinases resulting in increased production of additional inflammatory cytokines, and reduced insulin sensitivity. Consequently, the inhibition of TNF might be a therapy to relieve liver injury (Coffin *et al*., 2011; Feagins *et al*., 2015). Another factor that may link oxidative stress and liver injury is the redox-sensitive transcriptional factor, nuclear factor kappa B (NF- B) that is considered as an important stress sensor playing crucial role in determining cellular fate during oxidative stress (Martindale and Holbrook, 2002). NF- B is known to be activated by ROS and its activation leads to the transcriptional activation of numerous stress-response genes including antioxidant enzymes such as cyclooxygenase-2 (COX-2), detected in Kupffer cells that are believed to be important factor in liver injury (Nieto *et al*., 2000; Rojo *et al*., 2004).

DNA fragmentation is a hallmark of apoptosis and has been regarded as a critical process in apoptosis.When apoptotic stimuli attack the cells, caspases, specifically caspase-3, cleave the inhibitor of caspase activated DNase (CAD) causes DNA degradation (cleaves chromosomal DNA in a caspase-dependent manner). It is likely that caspase activation is a critical point in the apoptotic pathway, and if the caspases are activated, there are many ways for cells to be killed. Nonetheless, DNA fragmentation is probably the most effective way to kill the cells (Nagata, 2000). Most agents producing ROS induce cell death including apoptosis, by causing LPO and DNA damage (Higuchi, 2003). Several preventive agents such as hydroalcoholic garlic extract (Shrivastava, 2013), vitamin C and E (Yousef *et al*., 2007; Newairy *et al*., 2009; Yousef and Salama, 2009) were studied to minimize Al toxicity but not found significantly effective. Epigalocatechin-3 gallate (EGCG) is the most abundant and active compound responsible for most of green tea's role in promoting good health by acting through different pathways as antioxidant and anti-inflammatory agent, showing gene expression activity, functioning through growth factor-mediated pathways (Shixan *et al*., 2006). The anti-inflammatory effect is due to the COX-2 inhibiting property of EGCG (Ahmed *et al*., 2002) while the antioxidant properties owing to its effective free radical scavenging activity (Kaushik *et al*., 2011). Naidu *et al*. (2002) revealed that EGCG is at least 100 times more effective than vitamin C and 25 times better than vitamin E at protecting cells. As a consequence of these observations, this study firstly aimed to investigate the possible influence of stress or PM on Al-induced nephrotoxicity and hepatotoxicity in rats. Secondly, since there is always need for a successful therapeutic approach that might inhibit the initiation and progression of diseases, the current study also evaluates the potential nephroprotective and hepatoprotective effects of EGCG in ameliorating these possible alterations.

MATERIALS AND METHODS

Animals: The study was conducted in accordance with ethical guidelines of Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. One hundered male Sprague Dawley rats, weighing 250-280 g were used. Rats were obtained from The Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt. They were housed in stainless steel cages, three to four per cage, at a temperature of 25 ± 1°C with alternatively 12 hour light and dark cycles. 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. Experiments were usually carried out at a fixed time around 9 AM: 2 PM.

Drugs and chemicals: Aluminum chloride-hydrated ($AlCl_3.6H_2O$) and EGCG were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All were freshly dissolved in distilled water. All other chemicals and solvents were of highest grade commercially available.

Experimental design: Animals were randomly classified into ten groups (10 rats each) and treated for three weeks as follows; the first group served as control and was given saline daily. Also, there is another group received saline together with EGCG and served as positive control for the control group. The other eight groups were injected daily with $AlCl_3.6H_2O$ (70 mg/kg I.P) (Ali *et al*., 2015). Al-toxicity groups were divided into four treated and four untreated groups; treated rats received EGCG (10 mg/kg, IP) together with $AlCl_3$. One group of both treated and untreated rats served as control for each of them and the others were subjected to either stress (mild using isolation or high using electric shock) or to PM (10% casein diet).

I- Biochemical studies: 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- Estimation of renal and hepatic functions: 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), respectively, as well as alkaline phosphatase (ALP) was estimated using ELISA kit (Kamiya Biomedical, Seattle, WA, USA). Blood urea nitrogen (BUN) and serum creatinine were measured using quantitative colorimetric urea determination (QuantiChrom™ urea assay kit) and quantitative colorimetric creatinine determination (QuantiChrom™ creatinine assay kit). Stanbio Laboratory Kits (Boerne, TX, USA) were utilized for the determination of the serum albumin and glucose levels. All procedures were performed according to the manufacturers' instructions.

ii- Estimation of cholesterol and triglycerides: Using colorimetric assay kits for the measurement of total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG) (Biomed-diagnostics, Cairo, Egypt).

II- Tissue parameters: 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 oxidative stress markers [lipid peroxides expressed as malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), total antioxidant capacity (TAC)] and inflammatory mediators [tumour necrosis factor- (TNF-), interleukin-6 (IL-6), nuclear factor kappa B (NF- B)] as well as Caspase-3 and DNA fragmentation. In addition, specimens from the livers and kidneys from different treated groups were taken for histopathological examination.

i- Renal and hepatic oxidative stress estimation: Lipid peroxidation was determined by estimating the level of thiobarbituric acid reactive substances (TBARS) measured as MDA, according to the method of Satoh (1978) using (Biodiagnostic, Cairo, Egypt). 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). SOD activity was assessed relying on the ability of the enzyme to inhibit the phenazinemethosulphate mediated reduction of nitroblue tetrazolium dye (Nishikimi *et al*., 1972). Finally, determination of TAC was achieved by the reaction of antioxidants with a defined amount of exogenously provided H_2O_2. The residual H_2O_2 was determined colourimetrically by an enzymatic reaction which involves the conversion of 3, 5-dichloro-2-hydroxybenzene sulphonate to a colored product (Koracevic *et al*., 2001).

ii- Assessment of inflammatory markers: The involvement of inflammation was assessed utilizing the commercially available rat NF -B ELISA kit Cusabio Biotech (Cusabio Life Science, Inc., China), RayBio®Rat IL-6 (RayBiotech, Inc., USA) and Quantikine®Rat TNF- ELISA Kit (R&D Systems, MN, USA), respectively.

iii- Assessment of apoptotic marker: Caspase-3 activity was detected in the liver and kidney homogenates using ELISA kit (MyBioSource San Diego, California, USA).

iv- Assessment of DNA fragmentation: DNA fragmentation assay was conducted using the procedure supplied by Qiagen kit (Hilden, Germany). 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.

v- Histopathological examination of liver and kidney: Liver and kidney specimens were fixed in 10% formalin for 24 h then washed with tap water. For light microscopy, the specimens were prepared and stained (Bancroft and Stevens, 1996). Serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56ºC in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by microtome. The obtained tissue sections were collected on glass slides and deparaffinised, then sections were stained with Hematoxylin and Eosin stain for routine histological examination.

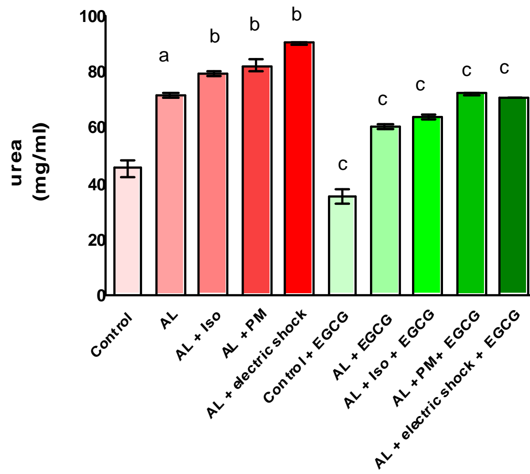
Statistical analysis: Data are presented as mean ± SEM Multiple comparisons were performed using one-way ANOVA followed by Tukey Kramer as a post hoc test. All statistical analysis and graphs were performed using GraphPad Prism (ISI®, USA) software (version 5).

RESULTS

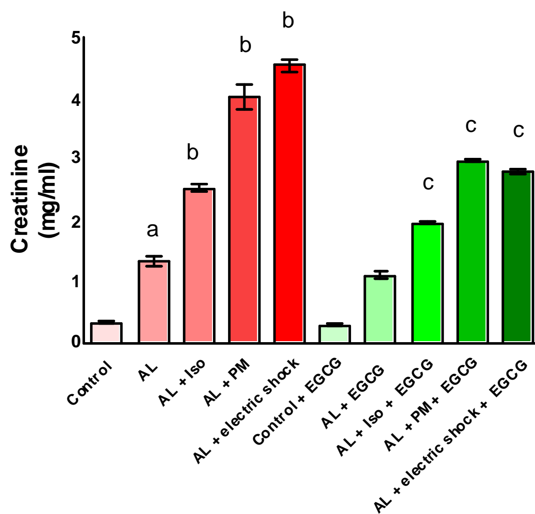
I. Serum parameters

a. Assessment of kidney function parameters: (BUN and serum creatinine): As illustrated in fig 1 (a,b), it was found that administration of $AlCl_3$ (70 mg/kg, IP) for three weeks to rats induced a significant increase in BUN and serum creatinine levels to 157.64% and 415.38% as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to either mild stress using social isolation (SI) or to protein malnutrition (10% casein diet) or high stress using electric shock (ES) showed marked increase in BUN to 111.65%, 115.33% and 126.76% as well as in serum creatinine level to 187.63%, 299.03% and 337.25% respectively as compared to corresponding AD model group. However, administration of EGCG (10 mg/kg, IP), together with the control and $AlCl_3$ treated groups produced a significant decrease in BUN to 77.93% and 84.56% respectively as compared to

corresponding control and Al- treated groups. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI or PMor ES produced a significant decrease in BUN to 80%, 87.72% and 78% as well as in serum creatinine level to 77.3%, 74.02% and 61.8% respectively as compared to corresponding untreated groups.



1a: Blood Urea Nitrogen

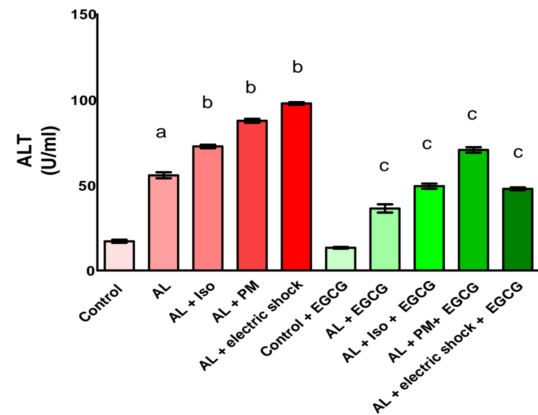


1b: Serum creatinine

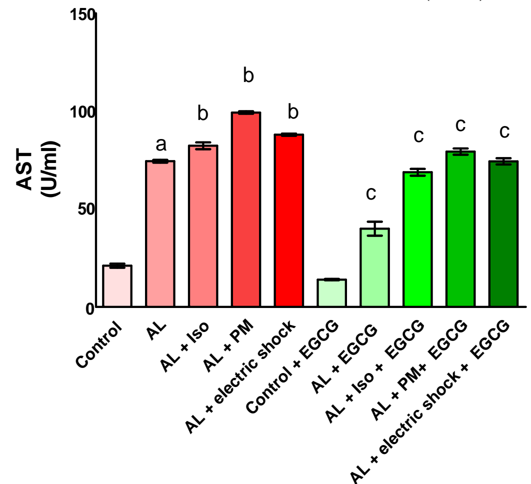
Fig (1a,b): Effect of stress and protein malnutrition on the potential role of Epigallocatechin-3-gallate on BUN and serum creatinine

Data expressed as Mean ± SEM (n = 8).
a: Significant difference from the control group at $p < 0.05$.
b: Significant difference from Al-treated group at $p < 0.05$.
c: Significant difference from corresponding untreated groups at $p < 0.05$.

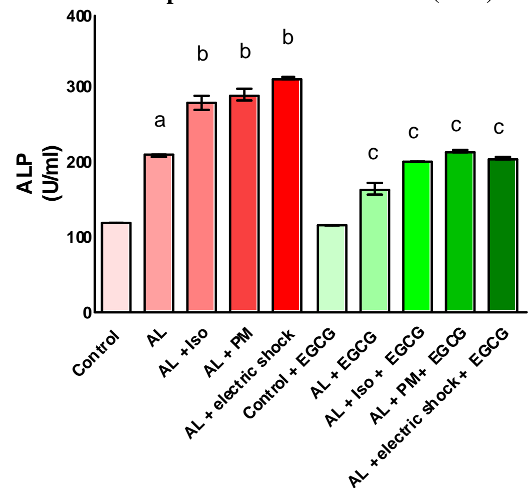
b. Assessment of liver function parameters: (ALT, AST, ALP, total bilirubin, TC, TG, glucose, HDL-C and albumin): As illustrated in fig 2 (a–i), it was found that administration of $AlCl_3$ induced a significant increase in serum ALT, AST, ALP and total bilirubin levels to 325.45%, 352.8%, 175.99% and 135.98% as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI or PM or ES showed marked increase in serum ALT level to 130.34%, 157.26% and 175.5% in serum AST level to 110.6%, 133.4% and 118.2% in serum ALP to133.3%, 138.3% and 149.8% as well as in serum total bilirubin to 133.03%, 152.8% and 146.5% respectively as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$ produced a significant decrease in serum ALT, AST, ALP and total bilirubin levels to 65.2%, 53.73%, 78.33% and 68.74% respectively as compared to Al- treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant decrease in serum ALT to 67.95%, 80.56% and 48.9% in serum AST level to 83.6%, 79.92% and 84.52 in serum ALP to 71.84%, 73.65% and 65.49% as well as in serum total bilirubin to 72.06%, 78.4% and 65.59% respectively as compared to corresponding untreated groups.



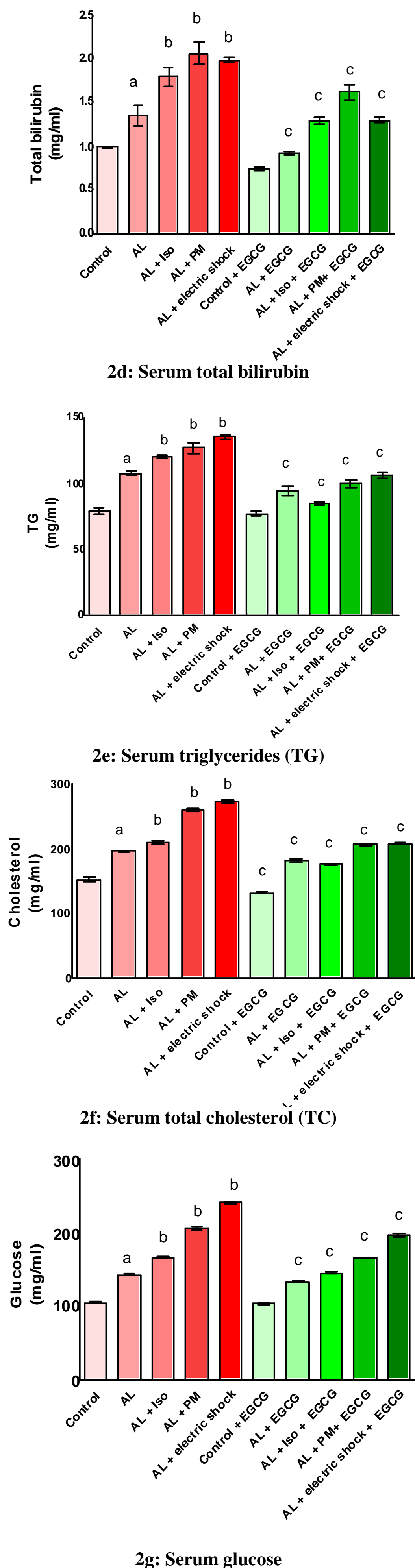
2a. Serum alanine aminotransferase (ALT)



2b. Serum aspartate aminotransferase (AST)



2c: Serum alkaline phosphatase (ALP)

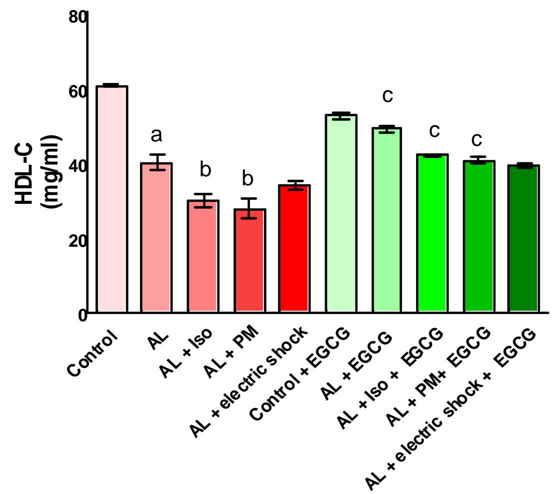


2d: Serum total bilirubin

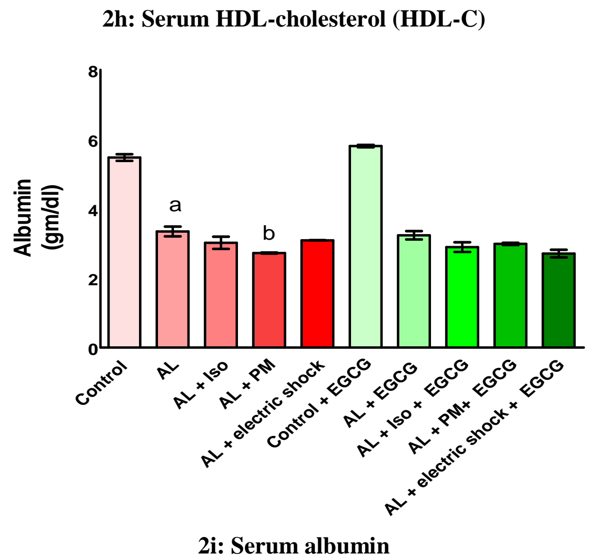
2e: Serum triglycerides (TG)

2f: Serum total cholesterol (TC)

2g: Serum glucose



2h: Serum HDL-cholesterol (HDL-C)



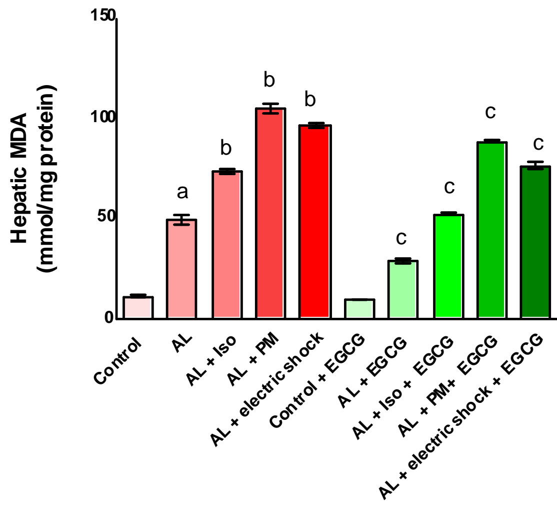
2i: Serum albumin

Fig (2a–i): Effect of stress and protein malnutrition on the potential role of Epigallocatechin-3-gallate on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total cholesterol (TC) triglycerides (TG), glucose, HDL-cholesterol (HDL-C) and albumin

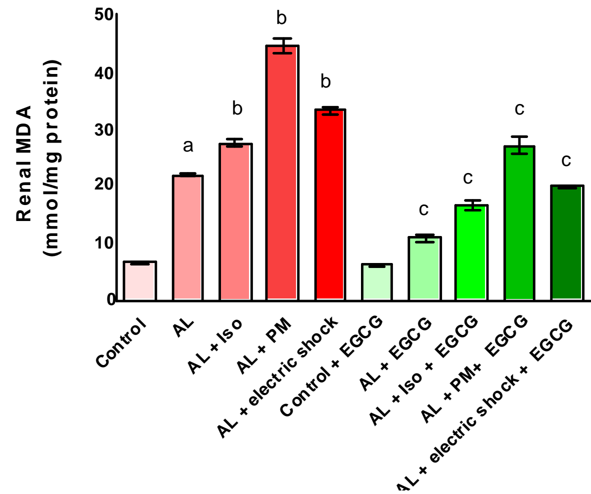
Data expressed as Mean ± SEM (n = 8)
a: Significant difference from the control group at $p < 0.05$.
b: Significant difference from Al-treated group at $p < 0.05$.
c: Significant difference from corresponding untreated groups at $p < 0.05$.

It was found that administration of $AlCl_3$ induced a significant increase in serum TC, TG and glucose levels to 129.6% %, 135.82% and 135.65% respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI or PM or ES showed marked increase in serum TC level to 106.7%, 132.02% and 138.5%, in serum TG level to 111.62%, 118.02% and 125.65 as well as in serum glucose to 116.33%, 143.8% and 168.11% respectively as compared to corresponding AD model group.

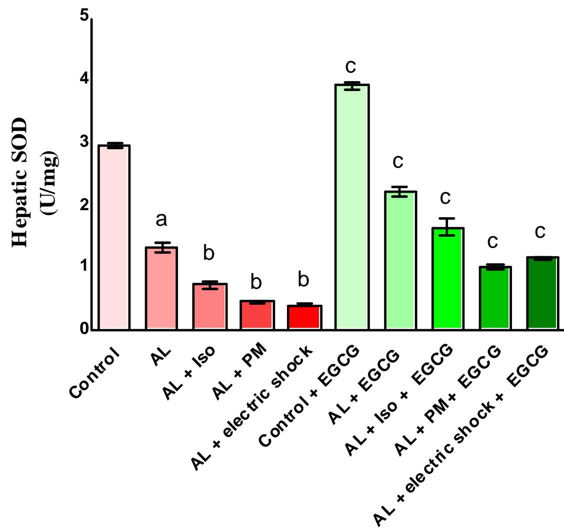
However, administration of EGCG produced a significant decrease in serum TC to 87.42% as compared to corresponding control group. Additionally, administration of EGCG together with $AlCl_3$ produced a significant decrease in serum TC, TG and glucose levels to 92.02%, 87.6 and 93.34 % respectively as compared to Al- treated group.



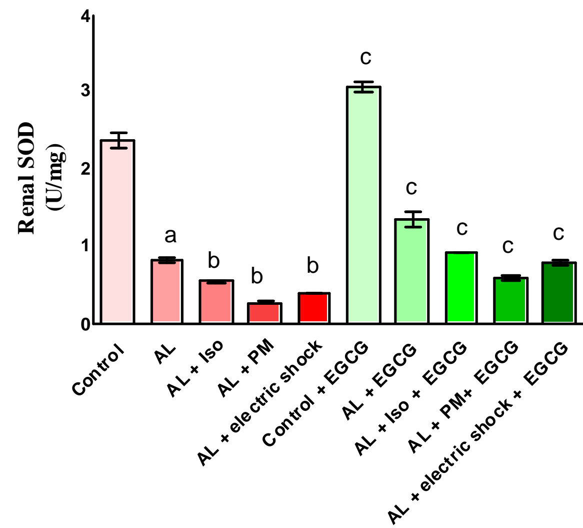
3a: Hepatic malondialdehyde (MDA) content



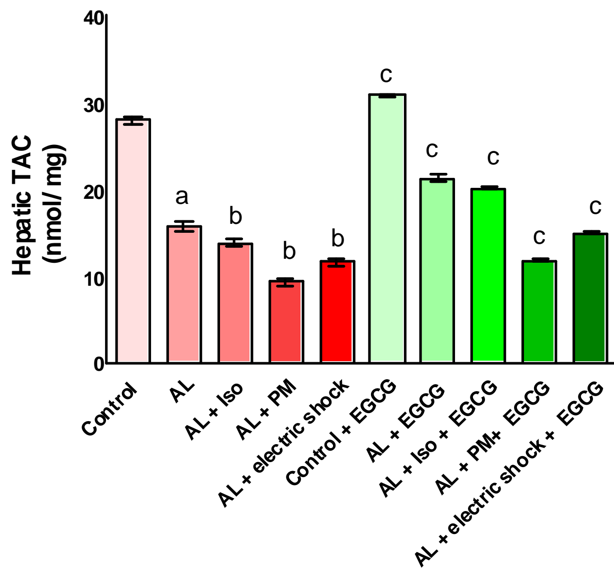
3b: Renal malondialdehyde (MDA) content



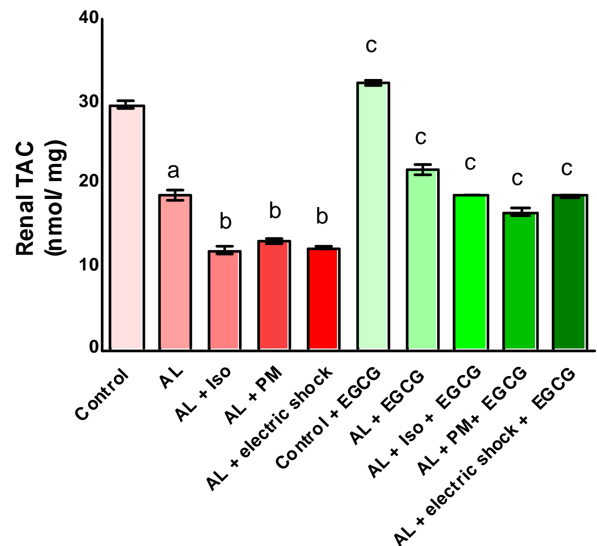
3c: Hepatic superoxide dismutase (SOD) enzyme activity



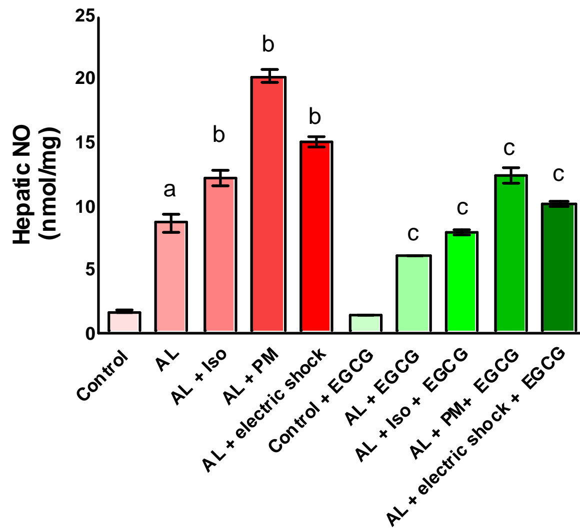
3d: Renal superoxide dismutase (SOD) enzyme activity



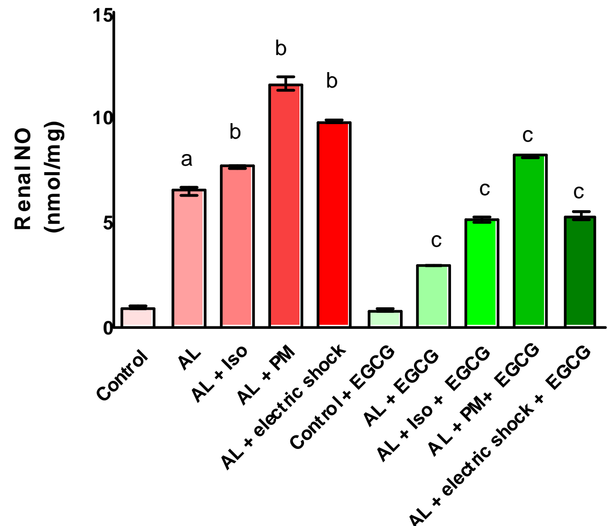
3e: Hepatic total antioxidant capacity (TAC)



3f: Renal total antioxidant capacity (TAC)



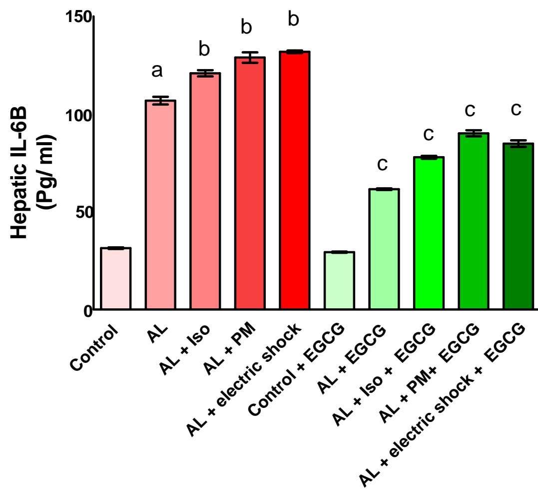
3g: Hepatic nitric oxide (NO)



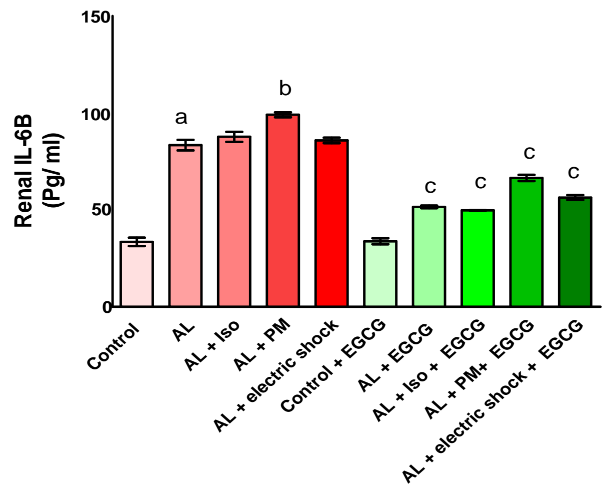
3h: Renal nitric oxide (NO)

Fig (3a-h): Effect of stress and protein malnutrition on the potential role of Epigallocatechin-3-gallate on hepatic and renal malondialdehyde (MDA) content, superoxide dismutase (SOD) enzyme activity, total antioxidant capacity (TAC) and nitric oxide (NO)

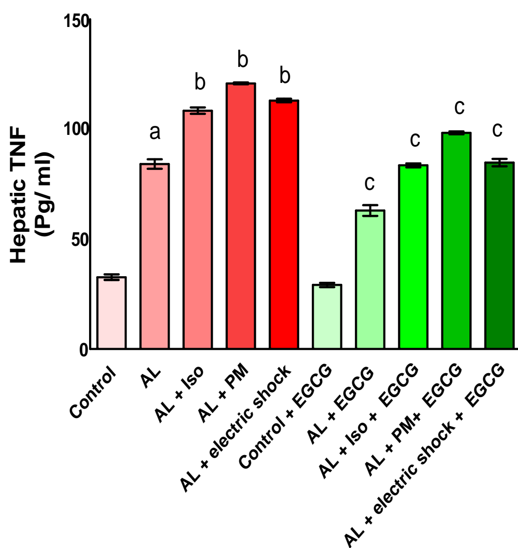
Data expressed as Mean ± SEM (n = 8)
a: Significant difference from the control group at $p < 0.05$.
b: Significant difference from Al- treated group at $p < 0.05$.
c: Significant difference from corresponding untreated groups at $p < 0.05$.



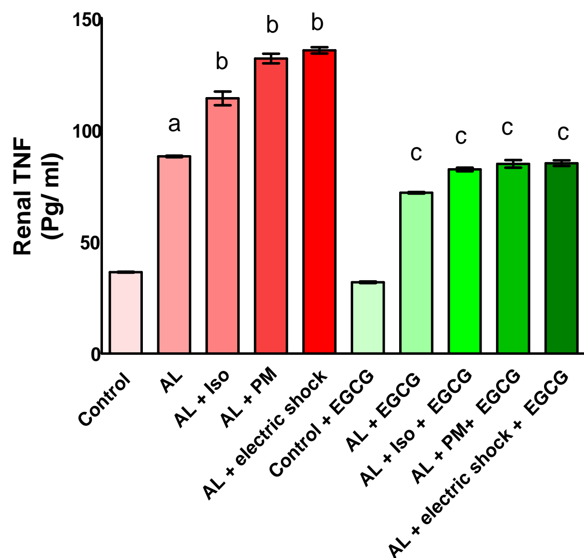
4a: Hepatic interleukin IL-6 (IL-6)



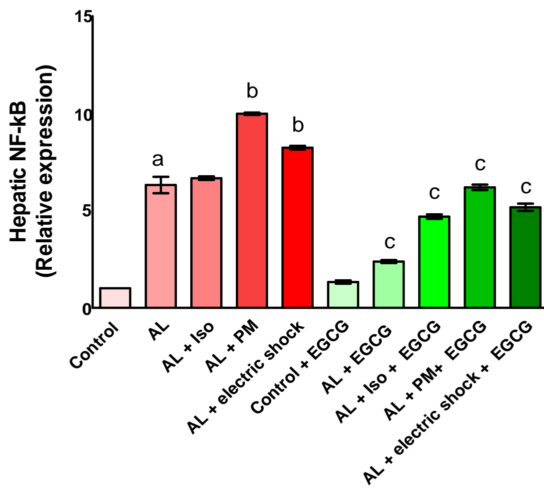
4b: Renal interleukin 6 (IL-6)



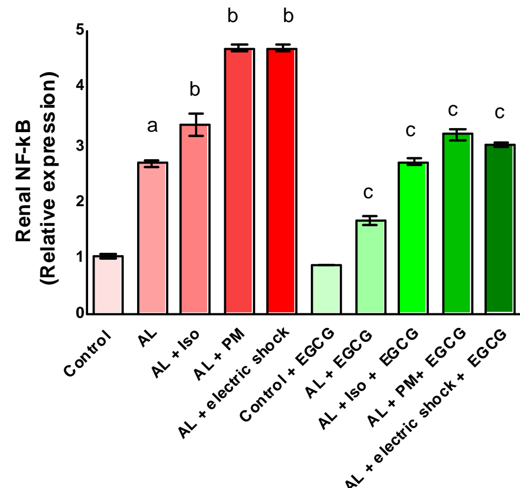
4c: Hepatic tumor necrosis factor-alpha (TNF-)



4d: Renal tumor necrosis factor-alpha (TNF-)



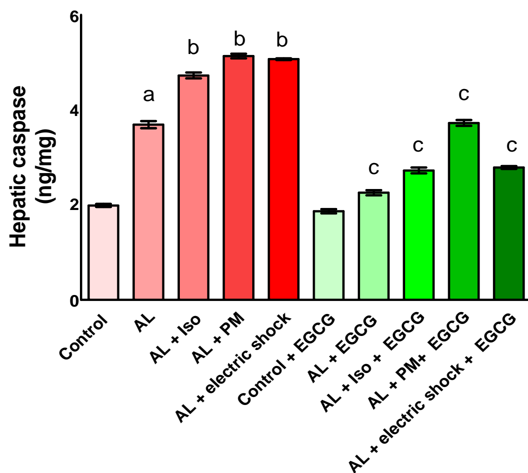
4e: Hepatic nuclear factor kappa B (NF- B)



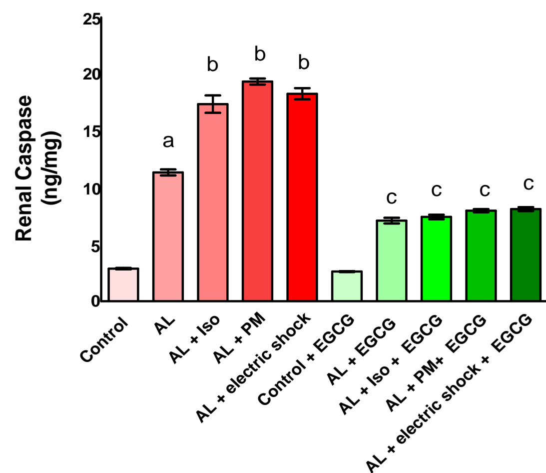
4f: Renal nuclear factor kappa B (NF- B)

Fig (4a-f): Effect of stress and protein malnutrition on the potential role of Epigallocatechin-3-gallate on hepatic and renal inflammatory mediators [interleukin IL-6 (IL-6), tumer necrosis factor-alpha (TNF-), nuclear factor kappa B (NF- B)]

Data expressed as Mean ± SEM (n = 8)
a: Significant difference from the control group at $p < 0.05$.
b: Significant difference from Al- treated group at $p < 0.05$.
c: Significant difference from corresponding untreated groups at $p < 0.05$.



5a: Hepatic caspase-3



5b. Renal caspase-3

Fig (5a-b): Effect of stress and protein malnutrition on the potential role of Epigallocatechin-3-gallate on hepatic and renal apoptotic marker (Caspase-3)

Data expressed as Mean ± SEM. (n = 8)
a: Significant difference from the control group at $p < 0.05$.
b: Significant difference from AL- treated group at $p < 0.05$.
c: Significant difference from corresponding untreated groups at $p < 0.05$.
Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer as a post hoc test.

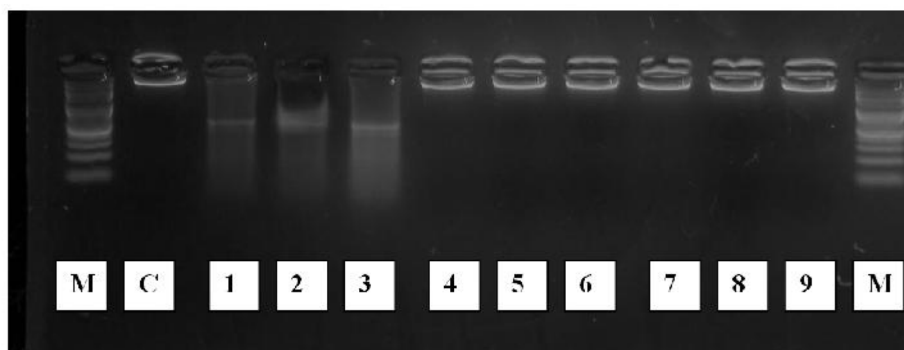


Fig (6a): Effect of stress and protein malnutrition on the potential role of Epigallocatechin-3-gallate on hepatic DNA fragmentation

An agarose gel electrophoresis show DNA fragmentation
Lane M: DNA marker with 100bp; Lane C: Control, there is no streaks
Lane 1, 2, 3 show DNA streaks were as groups of Al, Al + PM, Al + Stress induced toxicity which found in the model (M) laddering shape while in groups 4, 5, 6, 7, 8, 9, there is no streaks (groups of treatment).

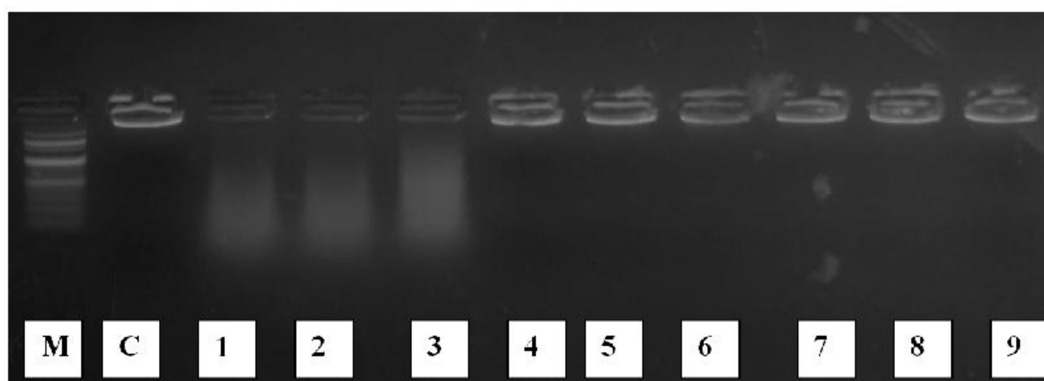


Fig (6b): Effect of stress and protein malnutrition on the potential role of Epigallocatechin-3-gallate on renal DNA fragmentation

An agarose gel electrophoresis show DNA fragmentation
Lane M: DNA marker with 100bp; Lane C: Control, there is no streaks
Lane 1, 2, 3 show DNA streaks were as groups of Al, Al + PM, Al + Stress induced toxicity which found in the model (M) laddering shape while in group 4, 5, 6, 7, 8, 9 there is no streaks (groups of treatment).

On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant decrease in serum TC to 83.7%, 79.32% and 76.4%, in serum TG level to 70.75%, 78.9% and 78.85% as well as in serum glucose to 87.65%, 80.7% and 81.8% respectively as compared to corresponding untreated groups. Finally, it was found that administration of $AlCl_3$ induced a significant decrease in serum HDL-C to 65.84% as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI or PM showed marked decrease in serum HDL-C to 74.8% and 69.11% respectively as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$ produced a significant increase in serum HDL-C to 122.7 as compared to Al treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI or PM produced a significant increase in serum HDL-C to 140.4% and 147.09% respectively as compared to corresponding untreated groups. Also, it was found that administration of $AlCl_3$ induced a significant decrease in albumin to 61.13% as compared to normal control group. Additionally, rats treated by $AlCl_3$ and subjected to PM showed marked decrease in albumin to 81.36% as compared to corresponding AD model group.

II-Tissue parameters

i. Assessment of oxidative stress markers (MDA, SOD, TAC, NO)

As shown in fig 3 (a–h), it was found that administration of $AlCl_3$ induced a significant increase in hepatic and renal MDA content to 442.04% and 328.6 respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI or PM or ES showed marked increase in hepatic MDA content to 147.96%, 210.94% and 193.77% as well as in renal MDA content to 125.69%, 203.5% and 151.71% respectively as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$ produced a significant decrease in hepatic and renal MDA content to 58.19 and 50.4% respectively as compared to Al-treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant decrease in hepatic MDA content to 71.32%, 84.44% and 79.31% as well as in renal MDA content to 60.57%, 60.97% and 60.45%, respectively as compared to corresponding untreated groups. It was found that administration of $AlCl_3$ induced a significant decrease in hepatic and renal SOD activity to 44.5% and 35.28% respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI, PM or ES showed marked decrease in hepatic SOD activity to 54.92%, 34.42% and 30% as well as in renal SOD activity to 66.07%, 33.73% and 47.66% respectively as compared to corresponding AD model group. However, administration of EGCG together with the control and $AlCl_3$ treated groups produced a significant increase in hepatic SOD activity to 132.5% and 168.6% as well as in renal SOD activity to 129.91% and 162.3% respectively as compared to corresponding control and Al treated groups. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI or PM or ES produced a significant increase in hepatic SOD activity to 228.12%, 224.8% and 293.7% as well as in renal SOD activity to 168.26%, 211.82% and 198.06% respectively as compared to corresponding untreated groups.

It was found that administration of $AlCl_3$ induced a significant decrease in hepatic and renal TAC to 56.15% and 63.14% respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI, PM or ES showed marked decrease in hepatic TAC to 88.3%, 59.34% and 74.03% as well as in renal TAC to 64.1%, 70.95% and 65.86% respectively as compared to corresponding AD model group. However, administration of EGCG together with the control and $AlCl_3$ treated groups produced a significant increase in hepatic TAC to 110.15% and 135.46% as well as in renal TAC to 108.92% and 116.8% respectively as compared to corresponding control and Al treated groups. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant increase in hepatic TAC to 145.5%, 127.43% and 128.63% as well as in renal TAC to 156.3%, 125.94% and 151.2% respectively as compared to corresponding untreated groups. It was found that administration of $AlCl_3$ induced a significant increase in hepatic and renal NO to 531.23% and 685.8% respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI, PM or ES showed marked increase in hepatic NO to 140.3%, 234.0% and 173.6% as well as in renal NO to 117.8%, 179.23% and 151.46% respectively as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$ produced a

significant decrease in hepatic and renal NO to 70.31 and 46%, respectively, as compared to Al treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant decrease in hepatic NO to 65.3%, 61.23% and 67.93% as well as in renal NO to 66.95%, 70.15% and 53.8% respectively as compared to corresponding untreated groups.

ii. Assessment of inflammatory mediators (IL-6 , TNF- , NF- B)

As illustrated in fig 4 (a-f), it was found that administration of $AlCl_3$ induced a significant increase in hepatic and renal IL-6 to 339.9% and 249.04% respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI, PM or ES showed marked increase in hepatic IL-6 to 113.1%, 120.7% and 123.4% respectively as compared to corresponding AD model group, while rats treated by $AlCl_3$ and subjected to PM showed marked increase in renal IL-6 to 117.73% as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$ produced a significant decrease in hepatic and renal IL-6 to 57.64% and 61.82%, respectively, as compared to Al treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant decrease in hepatic IL-6 to 64.45%, 69.94% and 64.4% as well as in renal IL-6 to 56.73%, 67.12% and 65.63% respectively as compared to corresponding untreated groups. It was found that administration of $AlCl_3$ induced a significant increase in hepatic and renal TNF- to 257.66% and 242.05%, respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI, PM or ES showed marked increase in hepatic TNF- to 128.9%, 143.62% and 134.35% as well as in renal TNF- to 129.4%, 149.6% and 153.8% respectively as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$ produced a significant decrease in hepatic and renal TNF- to 74.88% and 81.55% respectively as compared to Al treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant decrease in hepatic TNF- to 77.01%, 81.34% and 75.0% as well as in renal TNF- to 72.17%, 64.28% and 62.8% respectively as compared to corresponding untreated groups.

It was found that administration of $AlCl_3$ induced a significant increase in hepatic and renal NF- B to 626.7% and 258.93% respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to PM or ES showed marked increase in hepatic NF- B to 157.9%, and 130.28%, respectively, as compared to corresponding AD model group, while rats treated by $AlCl_3$ and subjected to SI, PM or ES showed marked increase in renal NF- B to 125.9%, 175.92% and 176.23% respectively as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$produced a significant decrease in hepatic and renal NF- B to 37.7% and 62.35% respectively as compared to Al treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PMor ES produced a significant decrease in hepatic NF- B to 70.37%, 62.09% and 62.81% as well as in renal NF- B to 80.17%, 67.67% and 63.76% respectively as compared to corresponding untreated groups.

iii. Assessment of apoptotic marker

As illustrated in fig 5 (a, b), it was found that administration of $AlCl_3$ induced a significant increase in hepatic and renal caspase-3to 185.51% and 394.8% respectively as compared to normal control group. Also, rats treated by $AlCl_3$ and subjected to SI, PM or ES showed marked increase in hepatic caspase-3 to 128.12%, 139.23% and 137.42% as well as in renal caspase-3 to 152.95%, 170.57% and 161.06% respectively as compared to corresponding AD model group. However, administration of EGCG together with $AlCl_3$ produced a significant decrease in hepatic and renal caspase-3 to 61.2% and 62.53% respectively as compared to Al treated group. On the other hand, rats received EGCG together with $AlCl_3$ and subjected to either SI, PM or ES produced a significant decrease in hepatic caspase-3 to 57.67%, 72.54% and 55.01% as well as in renal caspase-3 to 42.8%, 41.23% and 44.45% respectively as compared to corresponding untreated groups.

iv- DNA Fragmentation

As illustrated in fig 6 (a,b) by agarose gel electrophoresis, it was found that DNA isolated from control liver tissue did not show any DNA fragmentation (Figure 6a, Lane c). However, groups of Al as well as Al + PM and Al + Stress (Figure 6a, Lanes 1-3) showed characteristic DNA fragmentation as that was found in the model (M) laddering shape. However, DNA isolated from rats received EGCG together with $AlCl_3$ and subjected to different stressors (protective groups) did not show any DNA fragmentation (Figure 6a, Lane 4-9). Additionally, it was found that DNA isolated from control kidney tissue did not show any DNA fragmentation (Figure 6b, Lane c). However, groups of Al as well as Al + PM and Al + Stress (Figure 6b, Lanes 1-3) showed characteristic DNA fragmentation as that was found in the model (M) laddering shape. However, DNA isolated from rats received EGCG together with $AlCl_3$ and subjected to different stressors (protective groups) did not show any DNA fragmentation (Figure 6b, Lane 4-9).

v- Histopathological alterations in liver and kidney

A- Liver

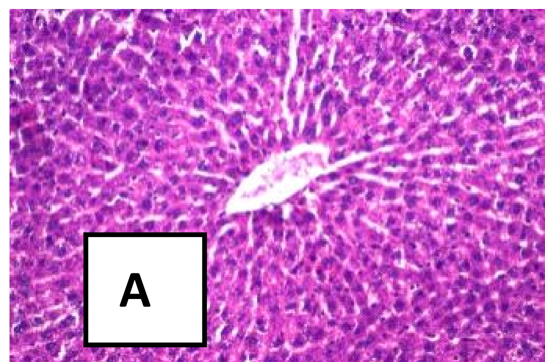


Figure (A): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of control group showing normal histological structures of the central vein and surrounding hepatocytes in the parenchyma.

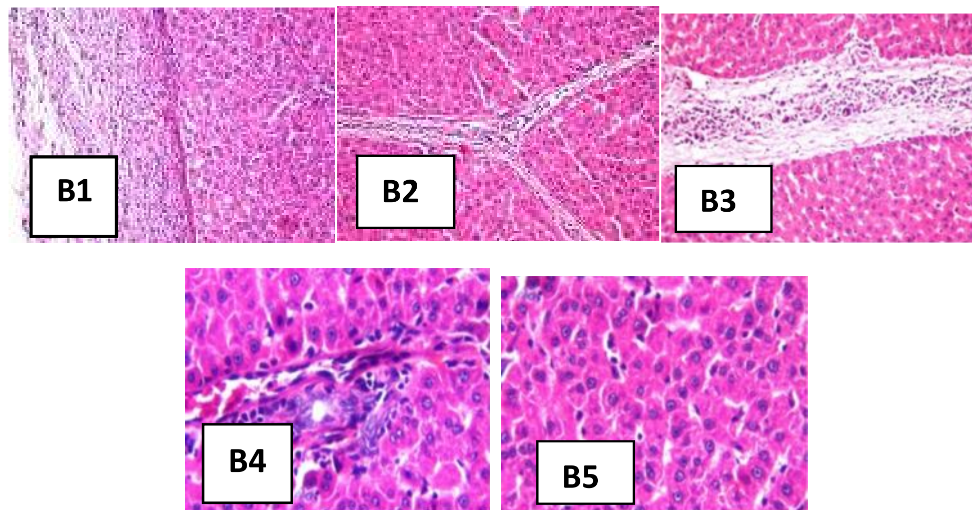


Figure (B): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of AL- induced toxicity showing thickening in the hepatic capsule by inflammatory cells infiltration and fibroblastic cells proliferation (B1) which were extended in between the hepatocytes in the hepatic parenchyma (B2, B3). The portal area showed inflammatory cells infiltration (B4). Diffuse kupffer cells proliferation was detected in between the hepatocytes (B5)

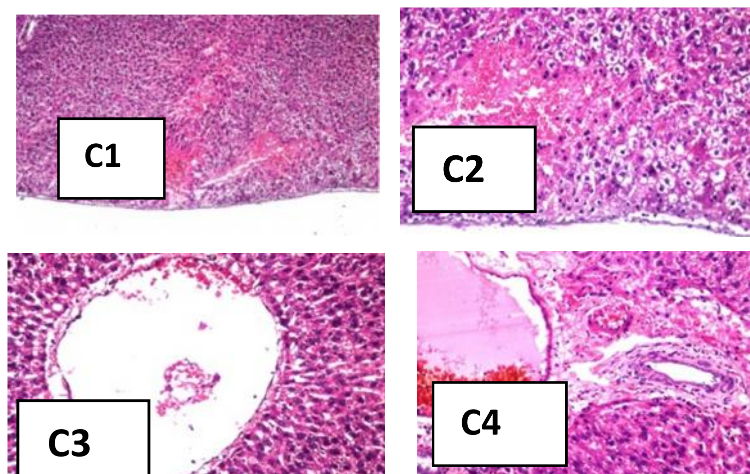


Figure (C): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of AL- induced toxicity with social isolation showing focal haemorrhage in between the degenerated hepatocytes in the parenchyma (C1, C2) associated with dilatation of the central and portal veins with oedema and periductal fibrosis in the portal area (C3, C4).

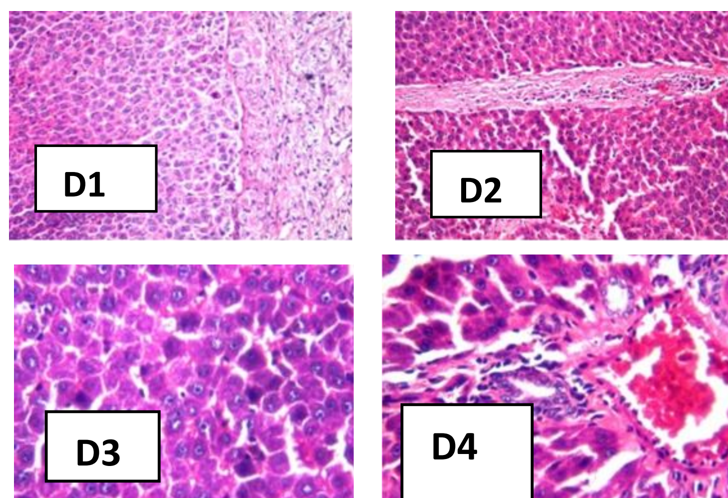


Figure (D): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of AL- induced toxicity with protein malnutrition showing thickening with oedema in the hepatic capsule, inflammatory cells infiltration and fibroblastic cells proliferation (D1), in which the fibrosis and collagen were extended in between the hepatocytes in the parenchyma (D2). There was apoptosis in some of the hepatocytes (D3), associated with inflammatory cells infiltration and congestion in the portal vein (D4)

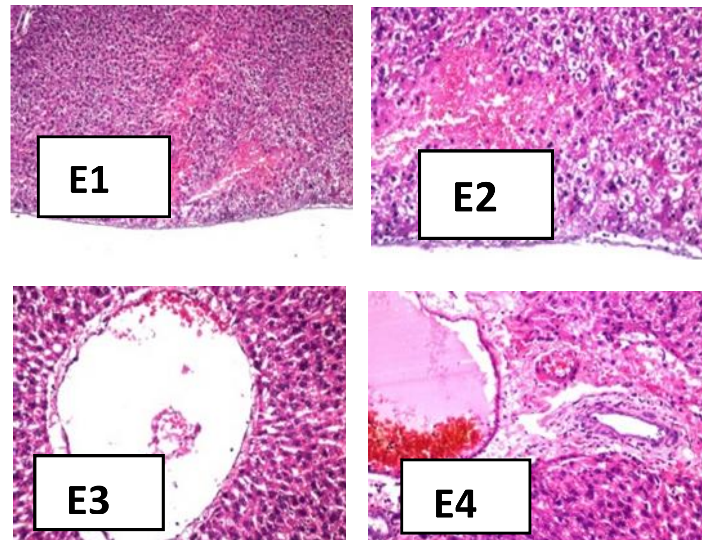


Figure (E): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of AL- induced toxicity with electric shock showing focal haemorrhage in between the degenerated hepatocytes in the parenchyma (E1, E2) associated with dilatation of the central and portal veins with oedema and periductal fibrosis in the portal area (E3, E4).

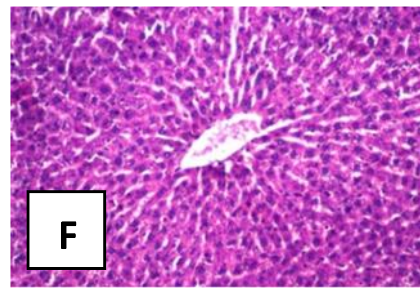


Figure (F): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of control group with EGCG showing normal histological structures of the central vein and surrounding hepatocytes in the parenchyma.

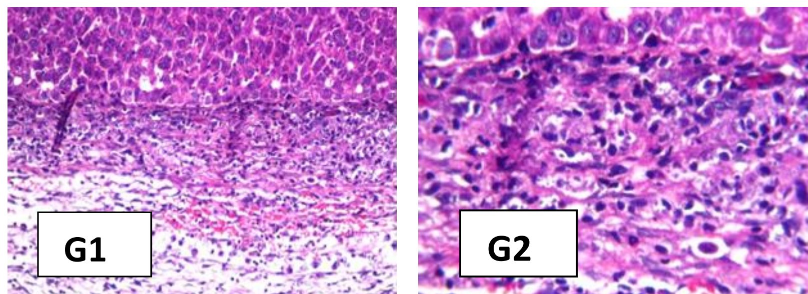


Figure (G): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of Al-induced toxicity with EGCG showing oedema with inflammatory cells infiltration in the thick capsule (G1, G2)

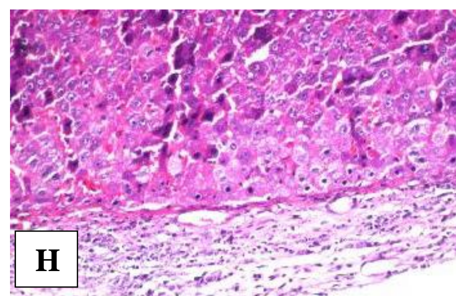


Figure (H): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of Al- induced toxicity with social isolation and EGCG showing that the hepatic capsule was thick by oedema and inflammatory cells infiltration while the underlying hepatocytes showed apoptosis

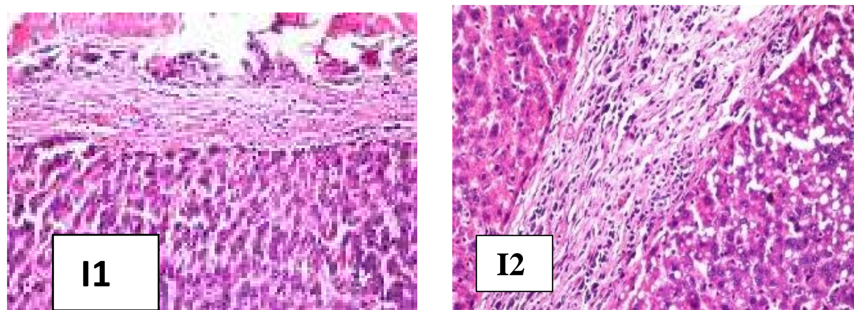


Figure (I): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of AL- induced toxicity with protein malnutrition and EGCG showing that the hepatic capsule was thick by fibrous connective tissue proliferation (I1), while the fibrosis with inflammatory cells infiltration was extended in between the hepatocytes in the parenchyma (I2)

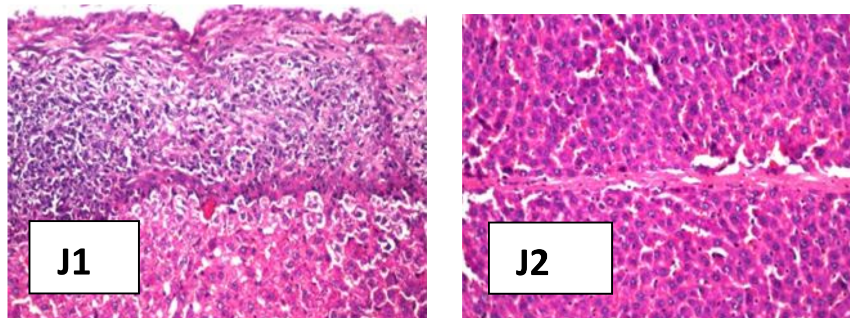


Figure (J): Representative photomicrographs of liver sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from liver of AL- induced toxicity with electric shock and EGCG showing inflammatory cells infiltration and fibroblastic cells proliferation in the thick capsule (J1). The collagen fibers were extended in between the hepatocytes in the parenchyma (J2)

B. Kidney

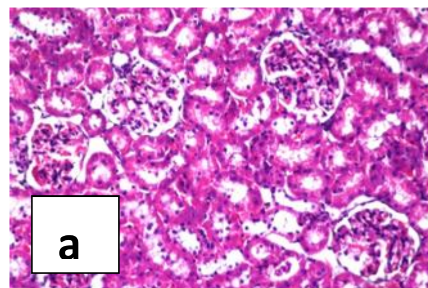


Figure (a): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of control group showing normal histological structures of the glomeruli and tubules at the cortex

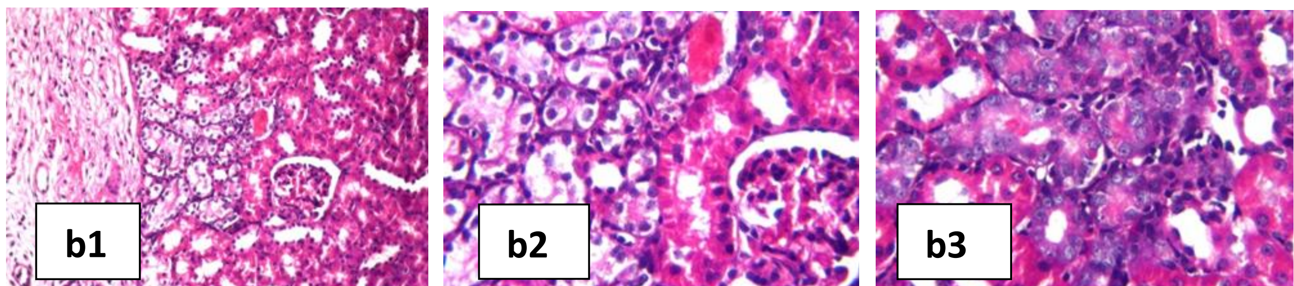


Figure (b): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of Al- induced toxicity showing oedema with fibrosis in the thick renal capsule (b1), associated with degeneration in the lining tubular epithelium (b2). Hyperplasia and dysplasia were noticed in the lining epithelium of some focal tubules (b3)

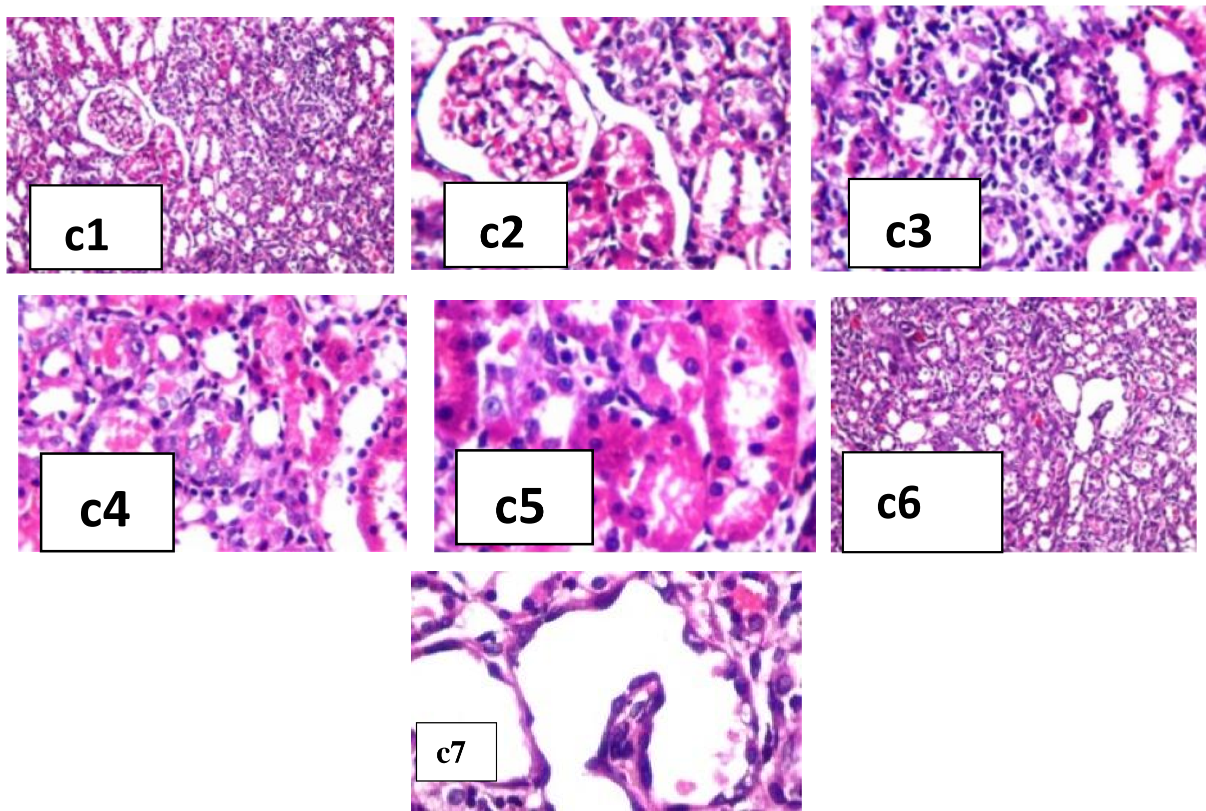


Figure (c): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of Al-induced toxicity with social isolation showing vaculization and swelling in the endothelial cells lining the glomerular tufts associated with focal inflammatory cells infiltration in between the degenerated and necrosed renal tubules (c1, c2, c3). Coagulative necrosis was detected in the tubular lining epithelium (c4, c5). The corticomedullary portion showed proliferation in the tubular lining epithelium with polyps formation and cystic dilatation (c6, c7)

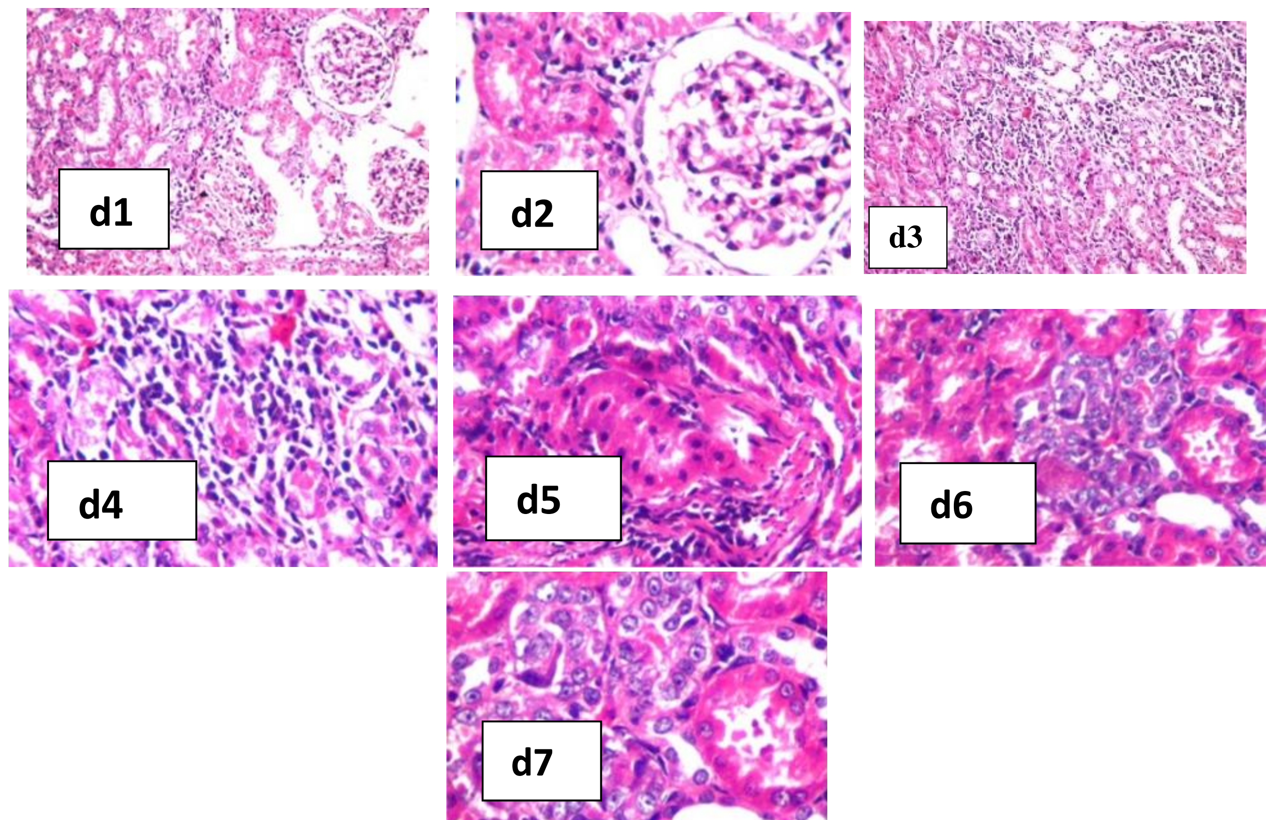


Figure (d): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of AL- induced toxicity with protein malnutrition showing degenerative change in the tubular lining epithelium while the glomeruli showed swelling and vacuolization of the endothelial cells lining the tufts (d1, d2). The corticomedullary junction showed focal inflammatory cells infiltration in between the tubules (d3, d4). Coagulative necrosis was observed in the lining epithelium of some tubules (d5), while hyperplasia and dysplasia with prominent nucleoli were detected in others (d6, d7).

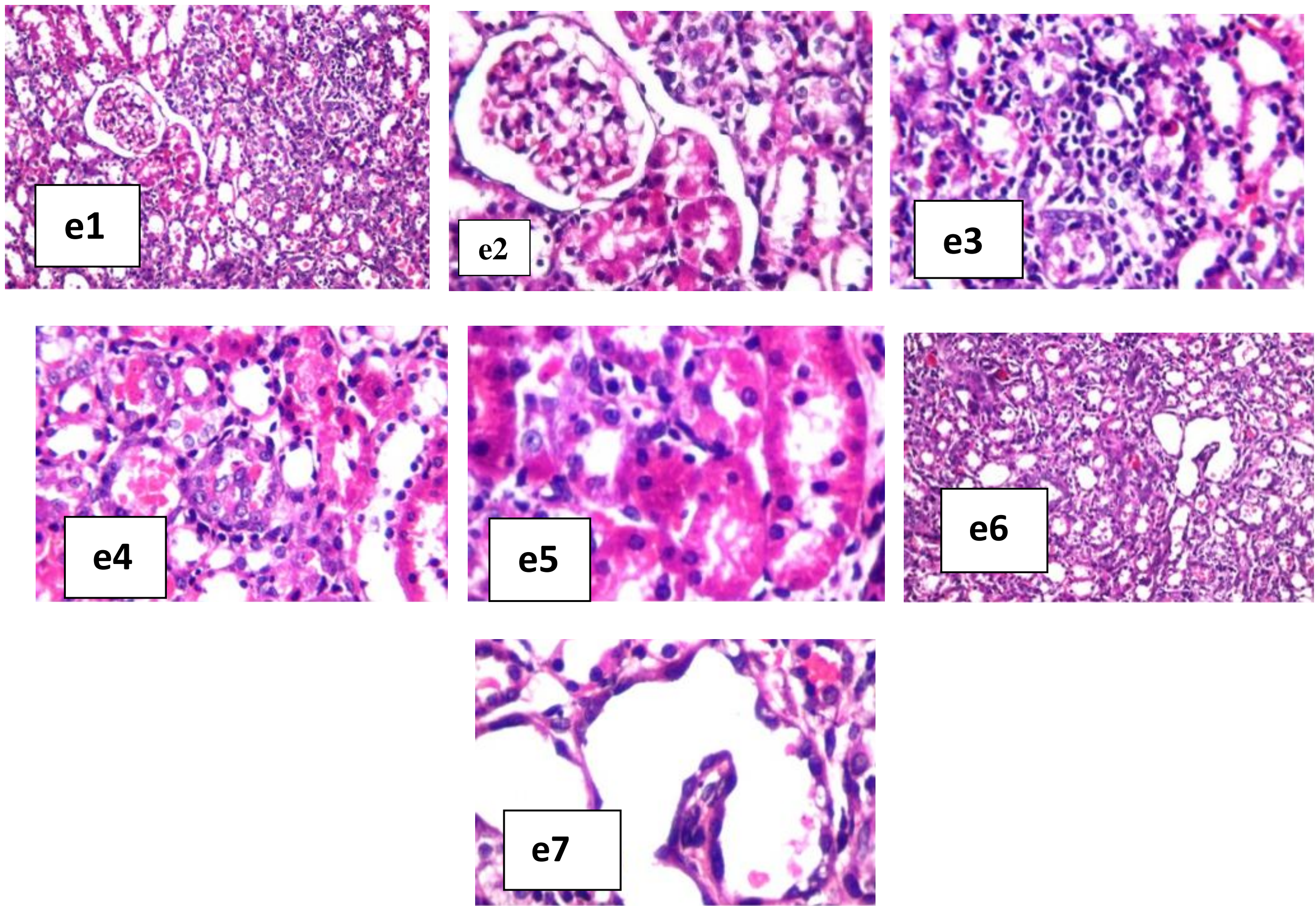


Figure (e): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of Al-induced toxicity with electric shock showing vaculization and swelling in the endothelial cells lining the glomerular tufts associated with focal inflammatory cells infiltration in between the degenerated and necrosed renal tubules (e1, e2, e3). Coagulative necrosis was detected in the tubular lining epithelium (e4, e5). The corticomedullary portion showed proliferation in the tubular lining epithelium with polyps formation and cystic dilatation (e6, e7).

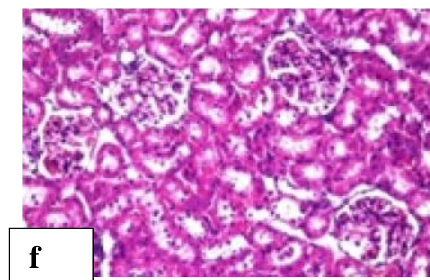


Figure (f): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of control group with EGCG showing normal histological structures of the glomeruli and tubules at the cortex

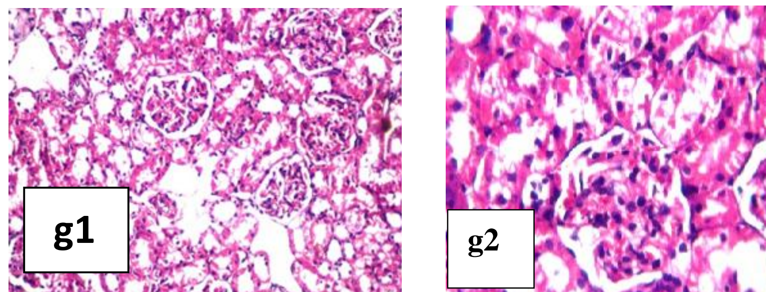


Figure (g): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of Al-induced toxicity with EGCG showing degeneration and coagulative necrosis in the tubular lining epithelium (g1, g2).

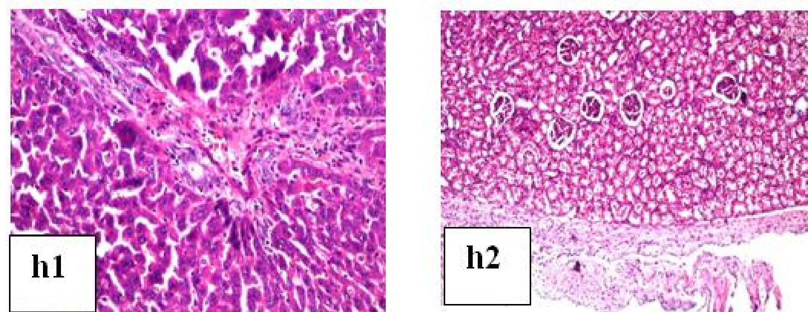


Figure (h): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of AL- induced toxicity with social isolation and EGCG showing thickening in the renal capsule by oedema and inflammatory cells infiltration (h1, h2)

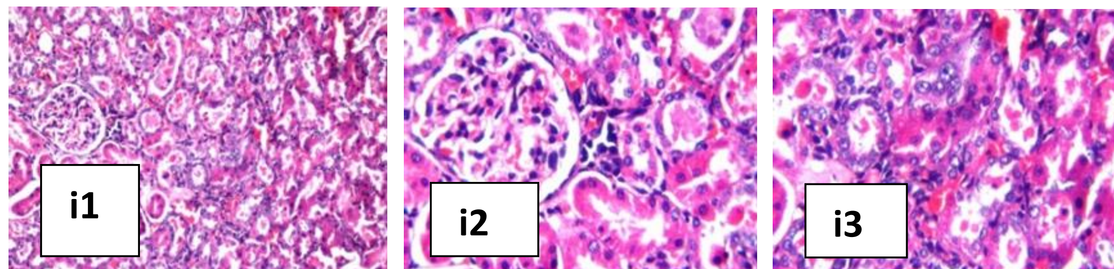


Figure (i): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of Al-induced toxicity with protein malnutrition and EGCG showing that the renal tubules showed degenerative change and coagulative necrosis (i1, i2). Focal area of the tubules showed hyperplasia and dysplasia in the lining epithelium (i3).

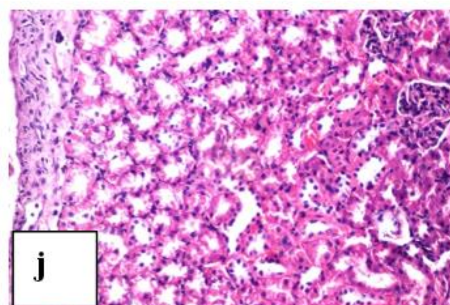


Figure (j): Representative photomicrographs of kidney sections stained by Hematoxylin–Eosin stain (magnification 40 X): Sections taken from kidney of Al-induced toxicity with electric shock and EGCG showing thickening in the capsule by inflammatory cells infiltration and fibroblastic cells proliferation

DISCUSSION

Aluminum is widely used in daily life all over the world especially in developing countries. It has been added mainly to drinking water for purification purposes; it was used also in food especially yellow cheese, salt, herbs, spices and tea, as well as cosmetics, medicines and wares (Reinke *et al*., 2003). The effects of Al intoxication on human health have been progressively worrying in the last few years (Al Kahtani, 2010; Turkez *et al*., 2010). In 2007, Al was included in the priority list of dangerous substances identified by The Agency for Toxic Substances and Disease Registry (ATSDR, 2007). The current study explored the impact of stress or PM on Al-induced nephrotoxicity and hepatotoxicity in rats through assessment of some biochemical parameters in blood in addition to LPO and oxidative stress in these organs, as well as it examined the potential role of EGCG in providing protection and counteracting these detrimental effects. Moreover, to examine whether the alterations of serum transaminases and kidney function tests together with oxidative stress biomarkers were accompanied by comparable changes in the tissue, liver and kidney were subjected to histological analysis. In the present study, injection of rates by $AlCl_3$ (70 mg/kg, IP) daily for consecutive three weeks induced hepatotoxicity and nephrotoxicity that could be manifested by deteriorations in both liver and kidney function tests (ALT, AST, ALP, BUN and creatinine respectively). These results agreed with Kowalczyk *et al*. (2004) and Nicolov *et al*. (2010) who reported that exposure to high concentrations of Al can result in its accumulation in liver and kidneys then deterioration in their functions.

The present study showed a significant increase in BUN and serum creatinine of Al-intoxicated rats and more increased in rats exposed to PM, SI and ES respectively and this indicates kidney function impairment that is further evidenced by the histopathological changes of the kidneys tissue. The increase of BUN and serum creatinine levels can be a consequence of crucial accumulation of Al in kidneys followed by development of renal failure as kidney is the main organ for Al elimination (Kowalczyk *et al*., 2004). These results are in the same line with the results of Al Kahtani *et al*. (2014) who proved that $AlCl_3$ exposure (25 mg/kg BW, IP) markedly increased the BUN and serum creatinine concentrations in

mice. Also, the study of Abdel-Hamid (2013) stated that Al-induced histopathological changes in the kidneys of pregnant rats after 3 months of $AlCl_3$ exposure (150 mg/kg/day). Additionally, Five months of $AlCl_3$ oral exposure (500 mg/kg BW) led to plasma biochemical changes, kidney atrophy, and morphological alterations of the Bowman's capsule, the glomerulus and several different renal tubules. These pathological changes in the renal structure would impair the kidney function (Belaïd-Nouira *et al*., 2013). Al Dera (2016) clearly showed significant alterations in kidney function and histopathological status after $AlCl_3$ exposure associated with increased renal oxidative stress and inflammation suggesting the strong pro-oxidant activity of $AlCl_3$ in spite of its non-redox status (Exley, 2004). Nearly similar findings were found by Guo and Wang (2011) who reported that an increased plasma Al concentration associated with increased oxidative stress and increased inflammation status in hemodialysis patients.

Regarding serum hepatic function biomarkers, the activities of ALT, AST and ALP were significantly increased in Al-intoxicated rats. These liver changes are obviously more prominent in Al-intoxicated rats under different stressful conditions including PM, SI and ES than those rats exposed to $AlCl_3$ alone. These results provide an evidence of $AlCl_3$ hepatotoxic effect. As a matter of fact, the elevation in transaminases are encountered in conditions causing hepatocellular damage, loss of functional integrity of the cell membrane, and necrosis such as in chemically induced liver injury (Ninh *et al*., 2003). Cell injury in Al toxicity may be explained by the interactions between oxidative stress and hepatic damage which may accelerate hepatodegenerative disorders and elevate liver enzymes (Tripathi *et al*., 2008). The increased activity of ALT, an enzyme localized in the cytoplasm, and ALP, a membrane bound enzyme related to the transport of various metabolites, in Al-treated rats indicated hepatic dysfunction probably due to Al-induced damage to cell membranes while the observed increase in the activity of AST, an enzyme localized in mitochondria, in Al-treated rats may have resulted from Al-induced loss of functional integrity of mitochondrial liver cell membranes. These results are in harmony with Zlatkovic *et al*. (2014) and accompanied by congestion of central vein, sinusoidal dilatation and lipid accumulation in liver revealed by our histopathological studies which are similar to those findings reported by Geyikoglu *et al*. (2013). Here we should refer to Rahman *et al*. (2000) who concluded that the increase in the activities of different enzymes in blood might be due to the necrosis of liver, revealed here by DNA fragmentation, showing the stress condition of the treated animals. The current data showed a significant increase in serum TG and TC with a significant decrease in HDL in $AlCl_3$ administered group compared to the control group which worsened in combination with different studied stressful conditions. This apparent dyslipidemia is certainly due to impaired liver function that is probably due to couple of events. Firstly, Al shows high affinity for phosphate groups and binds to the phospholipid head through electrostatic forces, which may induce conformational changes in the lipid bilayer of liver cell plasma membrane (Martin, 1986). Secondly, LPO that causes damage to cell membranes then loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability (Nehru and Anand, 2005). All these events lead to leakage of the enzymes from the liver cells and consequently impaired liver cell functions.

In the view of the present data, administration of $AlCl_3$ resulted in marked elevation in oxidative stress as indicated by increasing levels of NO and LPO (measured as MDA level) and decreasing SOD and TAC. These alterations in oxidative stress were aggravated in combination with SI, PM or ES. These results may be referred to the effect of Al on declining the expression of mRNA of endogenous antioxidants (Gonzalez *et al*., 2007; Manal *et al*., 2010). $AlCl_3$ also generates ROS including hydroxyl radicals, superoxide anion and H_2O_2 as well as, it undergoes redox cycling (Storz *et al*., 2005). An increase of ROS generation accelerated peroxidation of membrane lipids and caused dramatic alterations in antioxidant status (El-Demerdash, 2004). Moreover, the increased generation of free radicals can also lead to protein modifications and reduction of sulphhydryl groups in susceptible amino acids (Halliwell and Gutteridge, 1990). It has been reported that psychological stress enhances lipid peroxidation as clinical studies have demonstrated elevated MDA levels in patients with affective disorders (Ozcan *et al*., 2004). Nearly similar findings were obtained by Jyoti *et al*. (2007) who declared that administration of $AlCl_3$ in a dose of (50mg/kg/day) in drinking water for a month induced oxidative damage with subsequent LPO. Parallel to our results, Stevanovic *et al*. (2009) recorded significant increase in NO and MDA concentrations and reduced glutathione content at 3hrs, and 30 days after treatment of wistar rats with $AlCl_3$ intrahippocampal injections. The significant increase of MDA levels in Al-treated isolated rats suggests increased oxidative stress caused by Al and/or chronic SI stress. MDA is produced by LPO. Furthermore, LPO may be coupled to deleterious effects to the cell membrane, causing increased permeability observed by ALT and AST leakage into the serum (Yajima *et al*., 2009). In addition, Zlatkovic *et al*. (2014) observed significantly increased oxidative stress in the liver of chronically-isolated rats as compared to vehicle-treated controls. Also, Djordjevic *et al*. (2010) concluded that chronic SI led to disproportion between O_2^-metabolizing SODs and peroxide metabolizing enzymes, thus promoting H_2O_2 accumulation and pro-oxidative state in liver. Mohale and Chandewar (2012) reported that long term SI significantly altered oxidative stress markers in liver and subsequent liver damage occurred as levels of ALT and AST increased in rats subjected to long term SI compared to short term SI. Thus, stressful conditions may alter membrane permeability that leads to release of these transaminases. Additionally, PM rats treated with $AlCl_3$ showed more deterioration in liver and kidney functions, this may be due to increased oxidative stress since dietary protein is an important source of essential amino acids that can be used as intracellular antioxidants. Therefore, its restriction may lead to an increase in oxidative damage by diminishing antioxidant defences of the tissue (Feoli *et al*., 2008).

Nitric oxide production may play several roles in renal pathophysiology, including induction of tubular damage. Prevention or reduction of NO generation reduces NO renal injury, and the increased generation of NO is capable of inducing intracellular oxidizing reaction and cell death (Chatterjee *et al*., 2002). In the same line, Liu *et al*. (2016) mentioned that ROS causes cell damage and apoptosis of the rat kidney cells, which suppresses the glomerular filtration function, the reabsorption function of the renal tubule and induces oxidative stress of the kidney leading to an inhibition of kidney function. Add to previous studies, ROS could impair

cell function and induce apoptosis through activation of cell apoptosis signalling pathways (Valko *et al*., 2007; Chambers and LoGrasso, 2011). As shown in our study, increased ROS production may indicate extent of potential damage of DNA integrity and serve as an index of increased apoptosis in $AlCl_3$ treated rats and rats exposed to different stressful conditions with $AlCl_3$ treatment. These results are in harmony with Said *et al*. (2005). DNA fragmentation is a hallmark of apoptosis. Controlled cellular apoptosis provides an effective means to ensure the removal of unwanted DNA (Zhang and Xu, 2002). Thus, correction of the oxidant/antioxidant fluctuation is an essential goal to minimize nephrotoxicity and hepatotoxicity. The present study showed significant increase in levels of inflammatory mediators (TNF- , IL-6 and NF- B) in Al-intoxicated rats which became more increased in association with SI, PM or ES. These significant increases may be related to the oxidant/antioxidant imbalance observed in this study while ROS generation and loss of antioxidant defence play an important role in tissue injury. Many of these reactive molecules activate the signalling mechanisms that result in TNF production. TNF- is a proinflammatory cytokine capable of up-regulating its own expression, as well as the expression of other genes important in the inflammatory response (Donnahoo *et al*., 2000). TNF- increases inducible nitric oxide synthase (iNOS) activity and this causes NO production (Sanders *et al*., 2001). Inducible or endothelial nitric oxide synthase is found in hepatocytes, Kupffer cells and endothelial cells. Drug-activated Kupffer cells that produce signalling molecules such as NO and superoxide anion activate NF- B, resulting in an increased synthesis of ROS and NO. Furthermore, increased generation of NO activates COX-2 expression which is regulated by transcriptional factor NF- B (Na *et al*., 2006). Activation of NF- B following SI may be due to the accumulation of peroxides causing perpetual NF- B activity (Kobayashi *et al*., 2008).

No single mechanism emerges to explain all the systemic effects of $AlCl_3$. One of the mechanisms involves free radical-induced oxidative cell injury in Al-toxicity (Yousef and Salama, 2009). As a matter of fact, interactions between oxidative stress and liver and kidney tissues accelerate the progression of chronic hepatodegenerative and nephrodegenerative disorders (Tripathi *et al*., 2008). On the contrary, increasing antioxidant capacity plays an important role as hepatoprotective (Pushpavalli *et al*., 2008). Epigallocatechin-3-gallate, the main and most significant polyphenol in green tea, has shown numerous health promoting effects acting through different pathways; as antioxidant, anti-inflammatory and anti-atherogenic agent, showing gene expression activity, functioning through growth factor-mediated pathways, the mitogen-activated protein kinase-dependent pathway, the ubiquitin/proteasome degradation pathway as well as eliciting an amyloid protein remodelling activity (Mereles and Hunstein, 2011). The results of the current study showed that EGCG treatment in Al-intoxicated rats reduces MDA, NO and caspase-3 levels and restores enzymatic activity of SOD and TAC in kidney and liver as well as liver and kidney function tests. Also, rats supplemented with EGCG and subjected to SI, PM or ES had reduced pro-oxidant state, oxidative damage, and improved renal and hepatic functionality, indicating an attenuation of oxidative injury and dysfunctions mediated by these stress conditions. The protective effect of EGCG may be combined with an ability to normalize early intracellular events linked to the progression of oxidative damage as it has phenol rings that act as electron traps to scavenge peroxy radicals, superoxide anions, and hydroxyl radicals and prevent oxidation of iron. Therefore, it has been suggested that in addition to the reduction of iNOS expression, EGCG may block peroxynitrite and nitrite production through inhibition of oxidative reactions (Kim *et al*., 2000). Furthermore, modulation of NF- B activation might play a pivotal role in the protective effect of EGCG. TNF- /NF- B activation interplay occurs in oxidative stress (Guha *et al*., 2000). Here we notice a serious reduction in TNF- , IL-6 and NF- B levels in EGCG-treated rats, thus TNF- dependent gene are not activated and tissue damage is reduced. EGCG ameliorates the overproduction of pro-inflammatory cytokines and mediators as it inhibits transcription factor-mediated gene activation such as that via NF- B (Ahmad *et al*., 2000). Inhibition of NF- B mediated gene activation is the central phenomenon that explains the convergence in the antioxidant activity of the green tea catechins. NF- B, in response to ROS, activates transcription of many pro-inflammatory and anti-apoptotic/survival genes (Schoonbroodt and Piette, 2000). The ROS-scavenging activity of EGCG inhibits NF- B activation, leading to inhibition of expression of these proinflammatory and survival genes. In addition, EGCG has been shown to directly inhibit proteasome activity (Nam *et al*., 2001), leading to accumulation of the NF- B inhibitory protein (I B). Inhibition of NF B-mediated gene activation is also the likely mechanism of inhibition of iNOS observed with EGCG which mediates its anti-inflammatory actions (Singh *et al*., 2002). Moreover, a study done by Navarro-Perán and his colleagues (2008) declared that EGCG suppressed TNF- -induced NF- B activation through adenosine as EGCG by inhibiting folic acid uptake can disturb the metabolism of this vitamin in Caco-2 cells, producing the release of adenosine then adenosine blocks TNF- -stimulated NF- B activation resulting in suppression of NF- B. Thus, by modulating NF- B activation, EGCG combats inflammation. The toxic effects of Al appear to be mediated, at least in part, by free radicals (Abubakar *et al*., 2003). As known, DNA damage mainly develops related with oxidative stress. It is noteworthy to mention that oxidative stress, which has been strongly a potent apoptotic inducer. Causing cell death process, DNA cleavage always occur by topoisomerase II agents that increase topoisomerase II-mediated DNA cleavage. Dietary polyphenols as EGCG act against the enzyme by at least two different mechanisms. Firstly, redox-independent topoisomerase II poisons interacting with the enzyme in a noncovalent manner. Conversely, others inhibit DNA cleavage in a redox-dependent manner that requires covalent adduction to topoisomerase II (Bandele *et al*., 2008).

Conclusion

Aluminum- induces hepatic and renal dysfunction as well as genetic damage. Stress enhances nephrotoxicity and hepatotoxicity induced by Al more than PM. Administration of EGCG attenuates these toxicity particularly when accompanied by SI, PM or ES. The effect of EGCG is mainly due to its ability to modulate both oxidative stress and the inflammatory process. Consequently, long-term consumption of green tea is advised especially in populations more subjected to stress and/or PM to prevent or at least to minimize aluminum nephrotoxicity and hepatotoxicity.

REFERENCES

Abdel-Hamid GA 2013. Effect of vitamin E and selenium against aluminum-induced nephrotoxicity in pregnant rats. *Folia HistochemCyto*, 51:312–319.

Abubakar, MG, Taylor A, Ferns GA. 2003. Aluminium administration is associated with enhanced hepatic oxidant stress that may be offset by dietary vitamin E in the rat. *Int. J. Exp. Pathol*., 84, 49–54.

Ahmad N, Gupta S, Mukhtar H. 2000. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells. *Archives of Biochemistry and Biophysics*, 376 (2), 338–346.

Ahmed S, Rahman A, Hasnain A, Lalonde M, Goldberg VM and Haqqi TM. 2002. Green tea polyphenol EGCG inhibits the IL-1 beta- induced activity and expression of COX-2 and NOS-2 in human chondrocytes. *Free RadicBiol Med*, 33 (8): 1097-1105.

Al Dera HS. 2016. Protective effect of resveratrol against aluminum chloride induced nephrotoxicity in rats. *Saudi Med J*., 37(4): 369 – 378.

Al Kahtani MA, Abdel-Moneim AM, El-Sayed WM. 2014. The influence of taurine pretreatment on aluminum chloride induced nephrotoxicity in Swiss albino mice. *Histol Histopathol.,* 29:45–55.

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(3): 94. doi: 10.4172/2161-0460.C1.016.

ATSDR (Agency for Toxic Substances and Disease Registry) 2007. Notice of the revised priority list of hazardous substances that will be the subject of toxicological profiles. Fed. Regist. 73:12178-12179.

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

Berihu BA, Afwerk M, Debeb YG, Gebreslassie A. 2015. Review on Histological and Functional Effect of Aluminium Chloride on Cerebral Cortex of the Brain. *International Journal of Pharma Sciences and Research (IJPSR),* 6(8): 1105 – 1116.

Chambers JW. and LoGrasso PV 2011. Mitochondrial c-Jun N-terminal kinase (JNK) signaling initiates physiological changes resulting in amplification of reactive oxygen species generation. J Biol Chem 286:16052–16062.

Chatterjee PK, Patel NSA, Kvale EO. 2002. Inhibition of inducibile nitric oxide synthase reduces renal ischemia/reperfusion injury. *Kidney Int*., 61:862–871.

Coffin CS, Fraser HF, Panaccione R, Ghosh S. 2011. Liver diseases associated with anti-tumor necrosis factor-alpha (TNF-) use for inflammatory bowel disease. *Inflamm. Bowel Dis*., 17:479–484.

Devbhuti P, Saha A, Sengupta C. 2009. Gentamicin induced lipid peroxidation and its control with ascorbic acid. *Acta Pol. Pharm*., 66, 363–369.

Diehl AM 1991. Nutrition, hormones, metabolism and liver regeneration. Seminars Liver Dis. ll: 315-320.

DNA Cleavage Enhancement

Donnahoo KK, Meldrum DR, Shenkar R, *et al*. 2000. Early renal ischemia, with or without reperfusion, activates NF_B and increases TNF- bioactivity in the kidney. *J Urol*., 163:1328–1332.

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 Elem Med Biol*., 18:113–21.

Exley C. 2004. The pro-oxidant activity of aluminum. *Free Radic. Biol. Med*., 36: 380–387.

Feagins LA, Flores A, Arriens C, Park C, Crook T, Reimold A, Brown G. 2015. Nonalcoholic fatty liver disease: A potential consequence of tumor necrosis factor-inhibitor therapy. *Eur. J. Gastroenterol. Hepatol*., 27:1154–1160.

Feoli AM, Leite MC, Tramontina AC, Tramontina F, Posser T, Rodrigues L,Swarowsky A, Quincozes-Santos A, Leal RB, Gottfried C, Perry ML,Goncalves CA. 2008. Developmental changes in content of glial marker proteins in rats exposed to protein malnutrition. *Brain Res*., 1187:33-41.

Geyikoglu F, Turkez H, Bakir TO, Cicek M. 2013. The genotoxic, hepatotoxic, nephrotoxic, haematotoxic and histopathological effects in rats after aluminium chronic intoxication. *ToxicolInd Health*, 29:780–791.

Gonzalez, M.A.; Alvarez, M. L.; Pisani, G.B.; Bernal, C.A.; Roma M.G; and Carrillo, M.C. 2007. Involvement of oxidative stress in the impairment in biliary secretory function induced by intraperitoneal administration of aluminum to rats. *Biol Trace Elem Res*., 116(3):329-48.

Guha M, Bai W, Nadler JL, Natarajan R. 2000. Molecular mechanisms of tumor necrosis factor- gene expression in monocytic cells via hyperglicemia-induced oxidant stress-dependent and –independent pathways. *J BiolChem*., 275:17728–17739.

Guoa C-H and Wang C-L 2011. Plasma aluminum is a risk factor for oxidative stress and inflammation status in hemodialysis patients. *Clinical Biochemistry*, 44(16): 1309 – 1314.

Halliwell B, Gutteridge JMC. 1990. Role of free radicals and catalytic metal ions in human disease: an overview. *Meth Enzymol*., 186:1–85.

Higuchi Y 2003. Chromosomal DNA fragmentation in apoptosis and necrosis induced by oxidative stress. *Biochemical Pharmacology*. 66: 1527–1535.

Jianhua Zhang and Ming Xu 2002. Apoptotic DNA fragmentation and tissue homeostasis. *TRENDS in Cell Biology,* Vol.12 No.2 February 2002.

Jyoti A, Sethi P and Sharma D 2007. Bacopamonniera prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. *Journal of Ethno Pharmacology*, 111: 56–62.

Kim S, Lee MJ and Hong J. 2000. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutrition and Cancer*, 37 (1): 41–48.

Kobayashi Y, Nishikawa M, Hyoudou K, Yamashita F, Hashida M. 2008. Hydrogen peroxide-mediated nuclear factor kappa B activation in both liver and tumor cells during initial stages of hepatic metastasis. *Cancer Sci*., 99, 1546–1552.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. 2001. Method for the measurement of antioxidant activity in human fluids. *J ClinPathol.,* 54 (5):356-361.

Kowalczyk E, Kopff A, K dziora J, Błaszczyk J, Kopff M, Niedworok J, Fijałkowski P. 2004. Effect of Long-Term Aluminium Chloride Intoxication on Selected Biochemical Parameters and Oxidative--Antioxidative Balance in

Experimental Animals. *Polish Journal of Environmental Studies*, 13: 41-43.

Li S, Tan H-Y, WangN, Zhang Z-J, Lao L, Wong C-W and Feng Y. 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int J Mol Sci*., 16(11): 26087–26124.

Li, L *et al*. 2001. Endonuclease G (EndoG) is an apoptotic DNase when released from mitochondria. Nature 412, 95–99

Liu J, Wang Q, Sun X, Yang X, Zhuang C, Xu F, Cao Z, Li Y 2016. The Toxicity of Aluminum Chloride on Kidney of Rats. Biol Trace Elem Res.

Mahieu ST, Gionotti M, Millen N and Elias MM. 2003. Effect of chronic accumulation of aluminium on renal function, cortical renal oxidative stress and cortical cortical renal organic transport in rats. *Archives of Toxicology*, 77: 605–612.

Manal S, Azza H, Eman T. 2010. The Protective Effect of Vitamin E against the neurotoxic Effect of Aluminum Cholorid in Male Albino Rat. *J Am Science*, 6(10): 978-991.

Martin, RB. 1986. The chemistry of aluminum as related to biology and medicine. *Clin. Chem*., 32:1797–806.

Martindale JL and Holbrook, NJ. 2002. Cellular response to oxidative stress: Signalling for suicide and survival. *J Cell Physiol.,* 192: 1–15.

Mereles D and Hunstein W. 2011. Epigallocatechin-3-gallate (EGCG) for Clinical Trials: More Pitfalls than Promises? *Int. J. Mol Sci*, 12(9), 5592-5603.

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

Mohale DS and Chandewar AV 2012. Effect of Social Isolation on Oxidative Stress and Transaminase Level. *Asian Journal of Biomedical and Pharmaceutical Sciences,* 2(15), 41- 44.

Na, H.J., Lee, G., Oh, H.Y., Jeon, K.S., Kwon, H.J., Ha, K.S., Lee, H., Kwon, Y.G., Kim, Y.M., 2006. 4-O-Methylgallic acid suppresses inflammation-associated gene expression by inhibition of redox-based NF-kappa B activation. *Int. Immunopharmacol*, 6, 1597–1608.

Naidu Ps, Singh A and Kulkarni SK 2002. Carvedilol attenuates neuroleptic-induced orofacial dyskinesia: possible anti-oxidant mechanisms. *Br J Pharmacol*, 136: 193-200.

Nam S, Smith DM and Dou QP 2001. Ester bond-containing tea polyphenols potently inhibit proteasome activity in vitro and in vivo. *Journal of Biological Chemistry*, 276 (16), 13322–13330.

Navarro-Perán E, Cabezas-Herrera J, Sánchez-del-Campo L, García-Cánovas F and Rodríguez-López JN. 2008. The anti-inflammatory and anti-cancer properties of epigallocatechin-3-gallate are mediated by folate cycle disruption, adenosine release and NF- B suppression. *Inflamm Res*, 57: 472–478

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

Neelam, Uday PK and Kaldhar M. 1999. Risk of aluminium toxicity in Indian context. ICMR Bulletin 29: 8.

Nehru B and Anand P. 2005. Oxidative damage following chronic Aluminum exposure in adult and pup rat brains. *J. Trace Elem. Med. Biol*., 19:203-208.

Nieto N, Greenwel P, Friedman SL, Zhang F, Dannenber A, Cederbau AI 2000. Ethanol and arachidonic acid increase a2 (I) collagen expression in rat hepatic stellate cells overexpessing cytochrome P450 2E1: role of H2O2 and cyclooxygenase-2. *J. Biol. Chem*., 275, 20136–20145.

Ninh T, Nguyen MD, Scott Braley MD 2003. Comparison of postoperative hepatic functions after laparoscopic versus open gastric bypass. *Am. J. Surg*., 186, 40–44.

Nishikimi M, Appaji N, Yagi K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. *Biochem Biophys Res Commun*., 46 (2):849-854.

Ochmanski W, Barabasz W. 2000. Aluminium-occurrence and toxicity for organisms. Przegl. Lek. 57, 665–668.

Omari J. Bandele, Sara J. Clawson, and Neil Osheroff, 2008. Dietary Polyphenols as Topoisomerase II Poisons: B Ring and C Ring Substituents Determine the Mechanism of Enzyme-Mediated. *Chem Res Toxicol*., June; 21(6): 1253–1260.

Ozcan ME, Gulec M, Ozerol E, Polat R, Akyol O. 2004. Antioxidant enzyme activities and oxidative stress in affective disorders. *Int. Clin. Psychopharmacol*., 19, 89–95.

Palma HE, Wolkmer P, Gallio M, Correa MM, Schmatz R, Thome GR, Pereira LB, Castro VS, PereiraAB, Bueno A, *et al*. 2014. Oxidative stress parameters in blood, liver, and kidney of diabetic rats treated with curcumin and/or insulin. *Mol. Cell. Biochem*., 386:199–210.

Pushpavalli G, Veeraman C, Pugalendi KV. 2008. Influence of Piper betle on hepatic marker enzymes and tissue antioxidant status in D-galactosamineinduced hepatotoxic rats. *J. Basic Clin. Physiol. Pharmacol*., 19, 131–150.

Rahman MF, Siddiqui MK and Jamil K. 2000. Acid and alkaline phosphatase activities in a novel phosphorothionate (RPR-11) treated male and female rats. Evidence of dose and time-dependent response. *Drug and Chemical Toxicology,* 23: 497–509.

Reinke CM, Breitkreutz J, Leuenberger H. 2003. Aluminium in over-the-counter drugs: risks outweigh benefits? *Drug Saf*., 26, 1011–1025.

ROJO AI, SALINAS M, MARTIN D, PERONA R, CUADRADO A. 2004. Regulation of Cu/Zn-superoxide dismutase expression via the phosphatidylinositol 3 kinase/Akt pathway and nuclear factor-kappa B. *J Neurosci.,* 24: 7324–7334.

Sanders DB, Larson DF, Hunter K, *et al*. 2001. Comparison of tumor necrosis factor-alpha effect on the expression of iNOS in macro and cardiac myocytes. *Perfusion*, 16:67–74.

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. *ClinChimActa*., 90(1):37-43.

Schoonbroodt S and Piette J. 2000. Oxidative stress interference with the nuclear factor-kappa B activation pathways. *Biochemical Pharmacology*, 60 (8), 1075–1083.

Shixian Q, VanCrey B, Shi J, Kakuda Y and Jiang Y. 2006. Green tea extract thermogenesis-induced weight loss by epigallocatechin gallate inhibition of catechol-O-methyltransferase. *J Med Food*., 9(4): 451-458.

Singh R, Ahmed S, Islam N, Goldberg VM and Haqqi TM. 2002. Epigallocatechin-3-gallate inhibits interleukin-1beta-induced expression of nitric oxide synthase and production of nitric oxide in human chondrocytes: suppression of nuclear factor kappaB activation by degradation of the inhibitor of nuclear factor kappaB. *Arthritis Rheumatology*, 46 (8), 2079–2086.

Stevanovi ID, Jovanovi , MD, Jelenkovi A, Coli M, Stojanovi I, Ninkovi M. 2009. Effects of L-NAME, a non-specific nitric oxide synthase inhibitor, on AlCl3-induced toxicity in the rat forebrain cortex." *J Vet Sci*., 10 (1):15-22.

Storz P, Do¨ppler H, Toker A. 2005. Protein kinase D mediates mitochondrion to nucleus signaling and detoxification from mitochondrial reactive oxygen species. *Mol Cell Biol.*, 25:8520–30.

SushmaNJ and Rao KJ 2007. Total ATPases activity in different tissues of albino mice exposed to aluminium acetate. *J. Environ. Biol*., 28, 483–484.

Tipoe GL, Leung T-M, Hung M-W, Man-Lung Fung M-L 2007. Green Tea Polyphenols as an Anti-Oxidant and Anti-Inflammatory Agent for Cardiovascular Protection. Cardiovascular & Hematological Disorders-Drug Targets. 7(2): 135-144.

Tripathi S, Somashekar S, Mahdi AA 2008. Aluminum-mediated metabolic changes in rat serum and urine: a proton nuclear magnetic resonance study. *J.Biochem. Mol. Toxicol*., 22, 119–127.

Turkez H, Yousef MI and Geyikoglu F. 2010. Propolis prevents aluminium-induced genetic and hepatic damages in rat liver. *Food and Chemical Toxicology*, 48: 2741–2746.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell B*, 39:44–84

Yajima D, Motani H, Hayakawa M, Sato Y, Sato K, Iwase H 2009. The relationship between cell membrane damage and lipid peroxidation under the condition of hypoxia-reoxygenation: analysis of the mechanism using antioxidants and electron transport inhibitors. *Cell Biochem. Funct*., 27, 338–343.

Yousef MI, Salam A. 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food Chem. Toxicol*., 47, 1168–1175.

Zhang J *et al*. 1998. Resistance to DNA fragmentation and chromatin condensation in mice lacking the DNA fragmentation.

****