Research Article

A comparative Study on the Renoprotective Effects of Vitamin E versus Sodium hydrosulfide on Paracetamol-Induced Renal Damage in rats

Adel H. Saad* and Sara N. Abdel-Hafez**
* Department of Physiology, Faculty of Medicine, Minia Universiy,, Egypt
** Department of Histology, Faculty of Medicine, Minia Universiy,, Egypt

Abstract
Background and aim: Paracetamol is a widely used analgesic antipyretic medication. At high doses paracetamol can induce kidney damage. The current study aimed to compare the renoprotective effects of vitamin E versus sodium hydrosulfide (NaHS), a prominent hydrogen sulfide donor on acute overdose paracetamol-induced renal damage. **Methods**: Thirty-two male albino rats were randomly divided into 4 groups (8 rats each) including**: (1) Control group (C):** received no treatment just the vehicle. **(2) Paracetamol group (P)**: in which rats received paracetamol at a dose of 2 gm/kg body weight as a single dose orally by oral gavage, **(3) Paracetamol + Vitamin E (E):** in which each rat received pretreatment plus vitamin E with a dose of 100mg/kg body weight as a single dose by intraperitoneal injection 24 hours before receiving paracetamol, and **(4) Paracetamol + NaHS (H):**in which each rat received pretreatment plus NaHS with a dose level of 56µmole/kg once daily for 2 days by intraperitoneal injection. The second dose is given 1 hour before receiving paracetamol. At the end of the experiment, blood samples were collected for estimation of serum levels of renal injury markers; urea, creatinine, as well as serum tumour necrosis factor-α (TNF-α), B-cell leukemia/ lymphoma-2 (Bcl-2), and reduced glutathione (GSH) levels. Kidney samples were collected for histopathological examination as well as chemical estimation of malodialdehyde (MDA). **Results**: Induction of acute renal injury caused marked deterioration in kidney functions as evidenced by histopathological examination, significant high serum levels of renal injury markers, TNF-α and tissue MDA along with significant reduction in Bcl-2 and GSH levels as compared with the control group. Administration of vitamin E and NaHS significantly abolished the adverse effects of renal injury as evidenced by histopathological examination, significant reductions in renal injury markers, TNF-α, along with increased Bcl-2 levels. Also there was improvement in oxidative status in terms of reduced MDA levels along with preserved GSH. Vitamin E produced more significant improvement on kidney injury as evidenced by percentage changes in different parameters and restoration of normal kidney architecture. **Conclusion:** Paracetamol overdose can induce kidney damage. Vitamin E and NaHS proved to be protective against this damage mostly via anti-inflammatory, antioxidant, and antiapoptotic effects. Vitamin E was more powerful in its protection; combination of both drugs can be used, as a new combination method which may be more effective against acute kidney injury.
Key Words: Paracetamol, renal damage, Vitamin E, Sodium hydrosulfide

Introduction
Paracetamol is a frequently used pain killer. It is generally regarded as a safer drug with regard to kidney function compared to non-steroidal anti-inflammatory drugs, which are known to contribute to the development of acute kidney injury (AKI)[(1)]. However, little is known about the association between Paracetamol and AKI. Previous case reports[(2,3)] and cohort studies[(4,5)] had suggested an association between suprathe-rapeutic doses of paracetamol and AKI.

Scientists had found that oxygen free radicals play an important role in AKI and cause extensive damage to DNA, proteins, and carbohydrates. During AKI, oxygen free radicals have the potential to activate signaling pathways, such as the nuclear factor kappa B (NFkB) pathway and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway, in addition to having the ability to further activate some transcription factors (AP-1, Nrf2, and P50) as well as activate an antioxidant response and

inflammation. This response elicits oxidative and nitrosative stress, which then leads to cellular damage[6]. Furthermore, ROS could induce lipid peroxidation (LPO) and result in a large production of secondary products, such as malondialdehye which is known as second messenger of free radicals. High concentration of MDA in kidney tissue indicates renal toxicity and eventually lead to increased membrane rigidity as well as abnormal endothelial function, which may be involved in the pathophysiology of AKI[7].

Vitamin E is a component of vegetable oils that is found in nature in four different forms: α, β, γ, and δ-tocopherol. Vitamin E is the main antioxidant vitamin transported in the blood flow by the lipid phase of plasma lipoprotein particles[8].

The α-tocopherol represents over 90% of total tocopherol. It is the one that has the strongest biological antioxidant activity and is widely distributed in tissues and plasma. While vitamin E has various biological functions including enzymatic activity, gene regulation, and inhibition of platelet aggregation, the most important role of vitamin E is considered to be strong antioxidant. As reactive oxygen species have been known to play an important role in the development of AKI. Vitamin E is able to bind to various reactive oxidant species such as superoxide free radicals, and it is possible to prevent damage caused by reactive oxygen species[9].

H_2S is a member of the growing family of gasotransmitters. Once regarded as a noxious molecule predominantly present in the atmosphere, H_2S is now known to be synthesized endogenously in mammals. It regulates a variety of physiological processes such as vasorelaxation, neuromodulation, and inflammatory responses. It is recognized as an important signaling molecule that has the ability to neutralize a variety of ROS as well as increase cellular glutathione levels through activation of gamma-glutamylcysteine synthetase, and reduction of the disulfide bonds. Patients with cystathionine beta-synthase deficiency tend to produce a lesser amount of H_2S; suggesting that these patients are likely more prone to oxidative stress-mediated damages due to the excessive production of homocysteine that induces oxidative stress through the formation of ROS, including superoxide anion and hydrogen peroxide [10].

Therefore, the present study was designed to investigate and compare the potential protective effects of vitamin E versus NaHS on acute renal injury produced by a single overdose of paracetamol and the possible underlying mechanisms mediating such effects in adult male albino rats. We focused on the effects of both drugs at multiple levels including structural modifications, oxidative stress markers, inflammatory markers, and apoptosis.

Materials and Methods

Animals:

Thirty-two adult male albino rats of local strain, weighing 150–250 g, about 12-15 weeks old, were included in the present study. Rats were purchased from the National Research Centre, Cairo, Egypt. Animals were housed in groups (8 rats each) in stainless steel cages to provide adequate space for free movement and wandering (40 cm × 40 cm × 25 cm) at room temperature with natural dark/light cycles, and were fed a standard diet of commercial rat chow (Nile Company, Egypt) and tap water *ad libitum* for 2 weeks for acclimatization. All experimental procedures done to the rats were in accordance with our institutional guidelines. The protocol was ethically approved by The Laboratory Animals Maintenance and Usage Committee of the Faculty of Medicine, Minia University.

Experimental design

The rats were randomly divided into Four experimental groups (n = 8) as follows:

1. **Control group (C):** The rats of this group received saline by oral gavage and were left freely wandering throughout the period of the experiment.
2. **Paracetamol group (P):** The rats in this group received paracetamol at a dose of 2 gm/kg body weight given as a single dose orally by oral gavage [11].
3. **Paracetamol + Vitamin E (E)**: The rats in this group received pretreatment of vitamin E with a dose of 100mg/kg body weight that is received as a single dose that is received by intraperitoneal injection 24 hours before receiving paracetamol[12].
4. **Paracetamol + NaHS (H):** The rats in this group received pretreatment of NaHS with a dose level of 56μmole/kg once a day for 2 days

by intraperitoneal injection. The second dose is given 1 hour before receiving paracetamol [13].
At the end of all experiments, rats were sacrificed by decapitation after overnight fasting and blood samples were obtained. Blood samples were collected in tubes and left to clot at room temperature then centrifuged at 3000 rpm for 15 min in a cooling centrifuge. The supernatant serum was then withdrawn into labeled eppendorf tubes and stored at -20° C till the time of the assay.
The kidneys were excised, one of them was stored at −80 °C for further biochemical assay and the other kept in formalin for histo-pathological examination.

Biochemical analyses
- Urea, creatinine, by enzymatic colorimetric commercial kits (Biodiagnostic, Egypt) following the manufacturers' instructions.
- TNF-α by ELISA method (CUSABIO, China).
- B-cell leukemia/ lymphoma-2 (Bcl-2) by enzyme-linked immunosorbent assay kit (ELISA) (Calbiotech, USA).
- Serum reduced glutathione (GSH) by Direct colorimetric method [14] (ELABSCIENCE – CAT No. E-BC-K030).

Preparation of tissue homogenates
Specimens from kidneys were homogenized in potassium phosphate buffer 10 mM pH7.4 .The ratio of tissue weight to homogenization buffer was 1:10. The homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for determination of renal contents of Malodialdehyde (MDA) according to the method of[15].

Histological study
Multiple small specimens of renal tissue were obtained from each animal then rapidly fixed in 10% neutral-buffered formalin, dehydrated, cleared with xylene. After that specimens were embedded in paraffin wax. The sections (5 - 7μm thick) were stained with Hematoxylin and Eosin stain (H&E) to evaluate the histo-pathological structure [16].

Photography
The photography was performed in Histology department, Faculty of medicine, Minia University. Digital camera adapted to an Olympus (U.TV0.5XC-3) light microscopy used in this study. Images were carried out using Adobe Photoshop.

Morphometric study
The scorings was performed in 8 non overlapping fields from each slide. The renal sections were graded according to the histo-pathological damage occurring in glomeruli, tubules, vessels using a semi-quantitative scale. An overall histo-pathological score for each kidney was calculated[17].

Statistical analysis
Data were expressed as mean ± standard error of the mean (M ± SEM). Statistical analysis was performed using Graph pad Prism V8 program (GraphPad Software, Inc. San Diego, USA) and significant difference between groups was done by one-way ANOVA followed by Tukey-Kramar post hoc test for multiple comparisons. A p value ≤ 0.05 was considered statistically significant.

Results

Effect of overdose Paracetamol administration with and without treatment on renal injury markers (serum urea and creatinine):
As shown in table 1, administration of a single overdose of paracetamol resulted in significant increase in renal injury markers (serum urea and creatinine) as compared with control group. On the other hand, administration of both vitamin E and NaHS reversed the condition and caused significant decrease in renal injury markers as compared with control group.

Table 1: Effect of overdose Paracetamol administration with and without treatment on renal injury markers (serum urea and creatinine):

Parameter	C	P	E	H
Urea (mg/dl)	15.45±2.45	70.25±5.44[a]	16.37± 1.89 [b]	26.43±3.68[b]
% change from C group		+253.7		
% change from P group			- 76.70	-49.57
Creatinine (mg/dl)	0.75±0.06	3.25±0.9 [a]	0.93±0.4 [b]	0.98±0.6 [b]
% change from C group		+333.3		
% change from P group			-71.38	-69.84

Data are expressed as mean ± SEM of eight rats per group. [a], significant difference from control group; [b], significant difference from P group. C: Control, P: Paracetamol, E: Vitamin E, and H: Sodium hydrogen sulfide. $p \leq 0.05$.

Effect of overdose Paracetamol administration with and without treatment on serum TNF-α, Bcl-2, GSH, and tissue MDA:

As shown in table 2, administration of a single overdose of paracetamol resulted in significant increase in the serum inflammatory marker, TNF-α and oxidative stress tissue marker, MDA along with significant decrease in serum antiapoptotic factor Bcl-2 and GSH (another oxidative stress marker) levels as compared with control group. On the other hand, administration of both vitamin E and NaHS reversed the condition and caused significant decrease in TNF-α and MDA along with significant increase in serum Bcl-2 and GSH levels as compared with control group.

- **Table 2: Effect of overdose Paracetamol administration with and without treatment on serum TNF-α, Bcl-2, GSH, and tissue MDA**

Parameter	C	P	E	H
Serum TNF-α (pg/ml)	15.45±2.23	49.50±3.22[a]	19.33±2.42[b]	20.25±2.12[b]
% change from C group		+220.38		
% change from P group			-60.95	-59.09
Serum Bcl-2(ng/L)	71.45±2.89	52.77±3.67[a]	67.90±3.60[b]	62.40±2.97[b]
% change from C group		-26.14		
% change from P group			+28.67	+18.24
Serum GSH(mg/dl)	14.23±1.56	7.45±1.89[a]	12.24±2.70[b]	10.12±1.90[b]
% change from C group		-47.64		
% change from P group			+64.29	+35.83
Renal MDA(nmol/mg tissue)	36.45±3.20	67.70±2.80[a]	45.23±4.20[b]	52.30±4.23[b]
% change from C group		+85.73		
% change from P group			-33.19	-22.74

Data are expressed as mean ± SEM of eight rats per group. [a], significant difference from control group; [b], significant difference from P group. C: Control, P: Paracetamol, E: Vitamin E, and H: Sodium hydrogen sulfide. $p \leq 0.05$.

Results of histological examination:

H & E results:

The renal tissue of control group displayed normal lobular organization. The renal corpuscles were seen with their Bowman's spaces. The proximal convoluted tubules (PCT) were noticed containing narrow lumen while the distal convoluted tubules (DCT) showed wide lumen (Figure 1).

H & E sections from paracetamol group showed various morphological changes, there was sever disturbed normal lobular architecture. The dilated blood vessels containing hemosiderin pigment and the distorted renal corpuscle were clearly noticed. Higher magnification of some sections showed numerous vacuolated and pyknotic cells (Figure 2).

While, the Vit E group showed restoration of the normal lobular architecture except at focal areas which showed mild congested blood vessels. With higher magnification, little vacuolated and pyknotic cells were seen (Figure 3).

Additionally, in H2S group disturbed normal lobular architecture was frequently seen. With higher magnification, distorted renal corpuscle, vacuolated and pyknotic cells were seen (Figure 4).

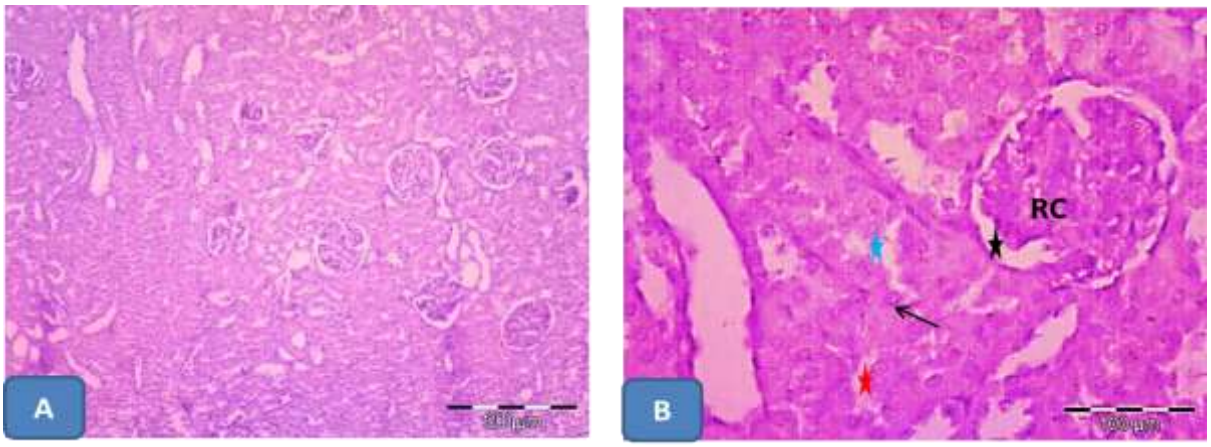

Figure 1: Photomicrographs of the renal tissue of control group showing: **A)** normal lobular architecture. **B)** Higher magnification showing normal renal corpuscle (RC) with its Bowman's space (black star). Notice the PCT (red star) and DCT (blue star) containingthevesicularnuclei. (H&E;AX100;BX400)

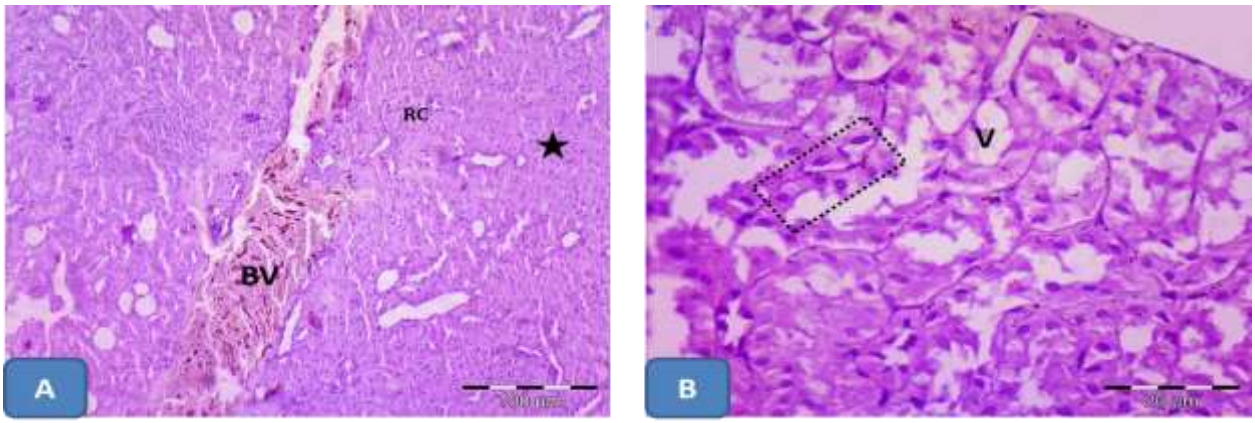

Figure 2: Photomicrographs of the renal tissue of paracetamol group showing: **A)** Disturbed normal lobular architecture (star). Notice the dilated blood vessels (BV) containing hemosiderin pigment (circle) and the distorted renal corpuscle (RC). **B)** Higher magnification showing numerous vacuolated (V) and pyknotic (rectangle) cells. H& E;AX100; BX400

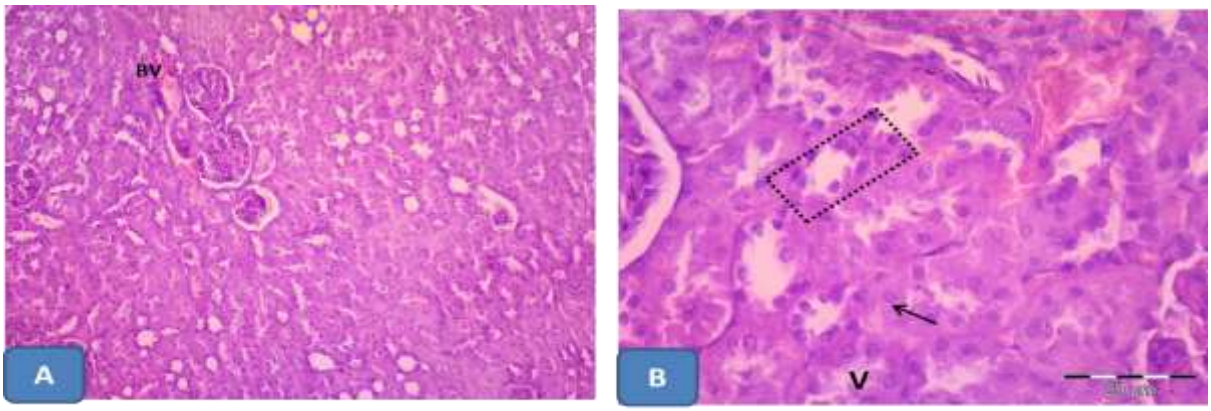

Figure 3: Photomicrographs of the renal tissue of Vit E group showing: **A)** Apparent normal lobular architecture except at focal areas showing mild congested blood vessels (BV). **B)** Higher magnification showing little vacuolated (V) and pyknotic (rectangle) cells. H& E;AX100;BX400

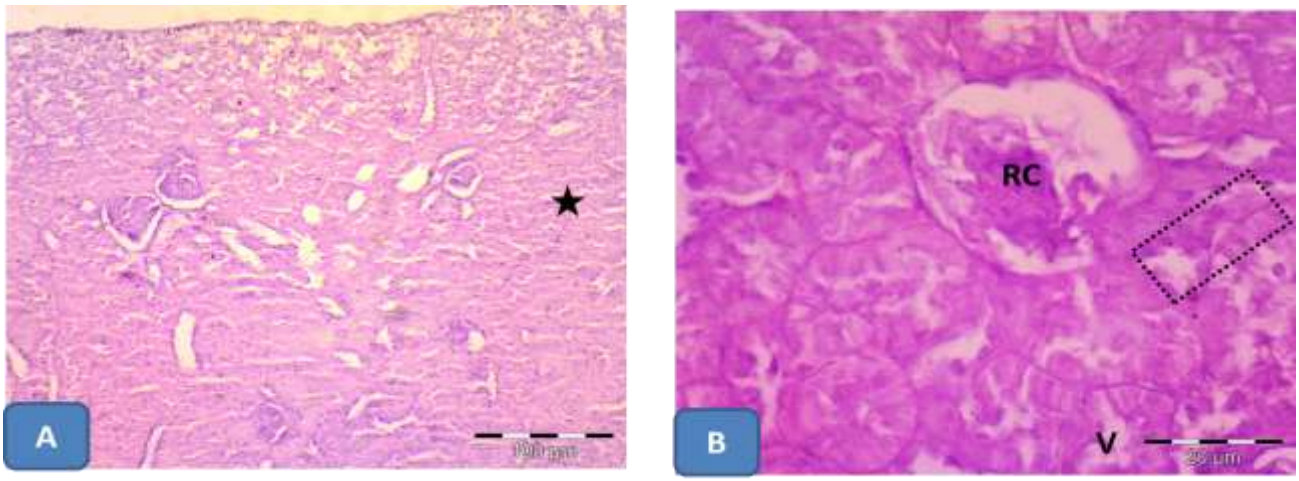

Figure 4: Photomicrographs of the renal tissue of H2S group showing: **A)** Disturbed normal lobular architecture (star). **B)** Higher magnification showing distorted renal corpuscle (RC) ,vacuolated (V) and pyknotic (rectangle) cells. H & E;AX100; BX400

Morphometric study:

In this current work, there was a highly significant increase in renal tissue damage (according to the histo-pathological scoring) in paracetamol group if compared with the control group ($P<0.001$). It was a highly significant between paracetamol group and group treated with Vit. E ($P<0.001$). While, it was a weak significant between paracetamol group and group treated with H2S ($P<0.05$) (Table 3).

Table 3: Histo-pathological injury scores for kidneys from all studied groups:

Parameter	C	P	E	H
Histological score	0.25±0.16	15.25±0.88[a]	4.63± 0.50 [b]	2.00±0.70 [b]

Data are expressed as mean ± SEM of eight observations per group. [a], significant difference from control group; [b], significant difference from P group. C: Control, P: Paracetamol, E: Vitamin E, and H: Sodium hydrogen sulfide. $P \leq 0.05$.

Discussion

Paracetamol is one of the most commonly used analgesic and antipyretic drugs, available without a prescription, both in mono- and multi-component preparations. It is the drug of choice in patients that cannot be treated with non-steroidal anti-inflammatory drugs (NSAID), such as people with bronchial asthma, peptic

ulcer disease, hemophilia, salicylate-sensitized people, and children under 12 years of age, pregnant or breastfeeding women. It is recommended as a first-line treatment of pain associated with osteoarthritis [18].

As there is a fact that there is no drug without side effects, paracetamol, especially when taken regularly and in large doses (> 4 g/day), there is a risk of serious side effects. Paracetamol overdose causes acute renal failure, and chronic exposure to paracetamol has been linked to chronic renal failure[19]. An official medical package leaflet published in Japan stated that paracetamol is contraindicated in patients with severe renal impairment in order to prevent further renal damage [20].

As a type of nephropathy, acute kidney injury (AKI) has a high morbidity and mortality rates in clinical investigations. However, the treatment options for this intractable disease are still currently limited. Reactive oxygen species (ROS) have been shown to play a key role in AKI, causing extensive damage to DNA, proteins, and carbohydrates [6]. Looking at in vitro data, paracetamol in therapeutic doses is said to induce fibroblast proliferation, possibly resulting in kidney injury, but its clinical implications have not yet been proven[21].

In the present study, we aimed to investigate and compare the possible renoprotective effects of vitamin E versus NaHS (a prominent hydrogen sulfide donor) on single acute overdose paracetamol-induced renal damage in adult male albino rats.
Oral administration of single overdose of paracetamol resulted in marked kidney damage as evidenced by histopathological examination, significant high serum levels of renal injury markers (urea and creatinine), TNF-α and tissue MDA along with significant reduction in Bcl-2 and GSH levels as compared with the control group.

Our findings are in agreement with other previous studies[7] who said that, at therapeutic doses, paracetamol is metabolized via glucuronidation and sulfation reactions in the liver which result in the water-soluble metabolites that are excreted via the kidney. When large doses of paracetamol are ingested, there is more severe GSH depletion as well as massive production of metabolites such as lipid peroxides, which increase the toxicity, leaving large amounts of reactive metabolite unbound. This process disrupts homeostasis and initiates apoptosis and kidney dysfunction. Progressive increase in the production of reactive species of oxygen (ROS) and subsquent decrease in antioxidants after CKD appears to be the key features for the pathophysiology of CKD. Hence, a significant increase in oxidative stress may stimulate cell hypertrophy and proliferation and inflammatory-cell infiltration [22].

Oxidative stress could accelerate renal injury progression by inducing cytotoxicity. In agreement with this is the observation that plasma malonyldialdehyde values are accompanied by increases in renal malonyldialdehyde levels in rats with renal mass reduction, suggesting that plasma ROS levels could reflect local ROS production in kidneys[23].
In the present work, significant increase in both serum creatinine and urea on paracetamol administration is correlated with[24], who found that paracetamol promotes increase of urea and creatinine leading to uremia. Increased plasma urea is due to the higher rate of plasma urea production, which exceeds the rate of urea clearance due to renal cytotoxicity resulting in malfunction of the kidney. Tissue creatinine breakdown increases plasma creatinine level when nephrotoxicity occurs

Our data showed that paracetamol administration resulted in significant decrease in the antiapoptotic marker; Bcl-2. Induction of apoptosis and viability of the cells is regulated by the expression of Bcl-2 through maintenance of cytochrome c release from the mitochondria[25]. Our results are correlated with[26] who found that paracetamol induced apoptosis of cultured murine tubular epithelial cells through a caspase-mediated mechanism that involves caspase-9 and caspase-3 in a cytochrome c and Smac/DIABLO–independent manner. Caspase-12 has been reported to cleave caspase-9 *in vitro* in the absence of cytochrome c[27]. Also,[25], found that, the oxidative stress by hydrogen peroxide led to suppression of Bcl-2 level in MG63 cells (osteosarcoma cells)**.**[28] have evaluated inflammatory markers as well as markers of oxidative stress in a group of patients with CKD stages. Renal dysfunction was accompanied by higher levels of lipid hydroperoxide and oxidized LDL as compared with subjects with normal function. In addition,

they have observed higher CRP, TNF- α, and IL-1b levels in uremic patients than in age-matched subjects with normal renal function. A correlation was also found between oxidized LDL and CRP, supporting the inflammation oxidative stress link. Inflammation is reported to be central common events in the pathogenesis of AKI [29]. TNF-α is an important pro-inflammatory cytokine that is a marker of AKI severity[30]. All these data are correlated with our results in the form of significant increase of TNF-α after paracetamol administration. This may be due to renal damage alone or due to the toxic effects of ROS. Also, this increase in TNF-α resulted in more kidney damage.

Administration of vitamin E significantly abolished the adverse effects of renal injury as evidenced by histopathological examination, significant reductions in renal injury markers, and TNF-α, along with increased Bcl-2 levels. Also there was improvement in oxidative status in terms of reduced MDA levels along with preserved GSH.

In the present study, the antioxidant effects of vitamin E are correlated with previous results which found that oral supplementation of α-tocopherol was found to maintain the cellular redox status by maintaining the activities of SOD, CAT, GPX, glutathione reductase and down-regulation in the levels of lipid peroxides, hydroxyl radical and hydrogen peroxides generation[31]. Also, [32] found that, in the non-hydrophobic portion of α-tocopherol, there is the hydroxyl radical (HO), whose atom of hydrogen is easily removable. So, when peroxyl free radicals are generated during lipid peroxidation, they are likely to combine with fatty acids of the tail of vitamin E, thus stopping to withdraw electrons from membrane fatty acids. So, vitamin E acts as a scavenger of free radicals due to its structural characters**.**

Strong attenuation of the oxidative stress by vitamin E may be the cause of improvement of kidney injury and consequently the reduction of renal injury markers, the inflammatory marker TNF-α and the increase in the antiapoptotic factor Bcl-2.

However, it is important to note that the biological functions of vitamin E are not just limited to antioxidant capabilities as vitamin E has other biological functions, such as enzymatic activity and gene regulation, which may be relevant for the acute renal damage therapy[6].

In recent years, scientists have gradually focused on the mechanisms at the subcellular level for vitamin E action. For example, a possible mitochondrial mechanism of dimethoate-induced nephrotoxicity may be that membrane-bound ATPases and plasma biomarkers are disturbed, and then, the administration of vitamin E ameliorates the toxic effects of this pesticide in the renal tissue because of its antioxidation mechanism[33].

Intraperitoneal injection of NaHS produced a significant improvement in the renal injury as evidenced by histopathological examination, significant reductions in renal injury markers, and TNF-α, along with increased Bcl-2 levels. Also there was improvement in oxidative status in terms of reduced MDA levels along with preserved GSH.

Improvement of oxidative stress in NaHS group can be explained by stimulation of the major antioxidant enzymes (CAT and SOD), direct neutralization of peroxide, depletion of ferrous iron, a catalytic agent of the Fenton reaction and transient depletion of free cysteine; the reducing agent that fuels the Fenton reaction[34]. H_2S also preserves the mitochondrial structure and function by decreasing mitochondrial oxygen consumption and increasing complex I and complex II efficiency. Mitochondrial swelling is decreased and matrix density and mitochondrial biogenesis are increased when H_2S is received[35]. Through these mechanisms H_2S improved balance between reduced glutathione (GSH), oxidized glutathione (GSSG) and attenuated formation of lipid hydroperoxides.

Antioxidant effects of H_2S resulted in attenuation of kidney damage and decrease both renal injury markers and TNF-α levels. In addition,[36] found that H_2S inhibits the progression of apoptosis by increasing the protein expression of heat shock protein (HSP-90) and Bcl-2, which correlates with the result of our study. This indicates that H_2S protects the kidney through an upregulation of intracellular antioxidant and antiapoptotic signaling pathways**..**

Comparing between vitamin E and NaHS renoprotective effects, we found that vitamin E is more effective as regard degree of restoration of renal architecture in histopathological examination and the percentage differences in different measurable parameters. This may be due to the strong antioxidant effect of vitamin E or the other protective effects of vitamin E such as enzymatic activity and gene regulation or its subcellular mechanisms.

In conclusion, the results of the present study clearly demonstrated the effectiveness of exogenous vitamin E and NaHS in improvement of AKI induced by paracetamol. Proposed mechanisms may include the suppression of oxidative stress, inflammation, and apoptosis. Based on the benefits associated with vitamin E, such as strong antioxidant function, low toxicity, rare side-effects, low cost and can be exclusively obtained from the diet, therefore, vitamin E has promise as a potential therapeutic agent in the management of AKI. The combination of vitamin E with NaHS can be used, as new combination method which may be more effective against AKI because this combination may has a synergistic effect on the protection against oxidative processes

References

1- Griffin M. R., Yared A., Ray W. A. (2000): Nonsteroidal antiinflammatory drugs and acute renal failure in elderly persons. Am J Epidemiol.;151(5):488-496.

2- Loh C., and Ponampalam R. (2006): Nephrotoxicity associated with acute paracetamol overdose: a case report and review of the literature. Hong Kong J.; 13(2):105–110.

3- Mazer M., Perrone J. (2008): Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management; J Med Toxicol.;4(1):2–6.

4- Chen Y. G., Lin C. L, Dai M. S., et al. (2015): Risk of acute kidney injury and long-term outcome in patients with acetaminophen intoxication: a nationwide population-based retrospective cohort study. Medicine; 94(46):e2040.

5- Stollings J. L., Wheeler A. P., Rice T. W. (2016): Incidence and characterization of acute kidney injury after acetaminophen overdose. J Crit Care. ; 35:191–194.

6- Liu P., Fengc Y., Wanga Y., Zhoua Y. and Zhao L. (2015): Protective effect of vitamin E against acute kidney injury Bio-Medical Materials and Engineering; 26 (2015) S2133–S2144.

7- Mandal, A., Patra A. , Mandal S., Roy S. and Mahapatra S.D. et al., (2015): Therapeutic potential of different commercially available synbiotic on acetaminophen-induced uremic rats. Clin. Exp. Nephrol., 19: 168-177.

8- Waniek, S., di Giuseppe, R., Esatbeyoglu, T., Plachta-Danielzik, S., Ratjen, I., Jacobs, G., Nothlings, U., Koch, M., Schlesinger, S., Rimbach, G., and Lieb, W. (2017): Vitamin E (alpha- and gamma-Tocopherol) Levels in the Community: Distribution, Clinical and Biochemical Correlates, and Association with Dietary Patterns. *Nutrients* 10.

9- Cho K. S., Ko I. K., and Yoo J. J. (2018): Bioactive Compounds for the Treatment of Renal Disease. Yonsei Med J. Nov; 59(9):1015-1025.

10- Majumder, A., Singh, M., George, A. K., Behera, J., Tyagi, N., and Tyagi, S. C. (2018). Hydrogen sulfide improves postischemic neoangiogenesis in the hind limb of cystathionine-beta-synthase mutant mice via PPAR-gamma/VEGF axis. *Physiol Rep* 6, e13858.

11- McGill, M. R., Williams, C. D., Xie, Y., Ramachandran, A., and Jaeschke, H. (2012): Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfun-ction, and oxidative stress in the mechanism of toxicity. Toxicology and applied pharmacology; 264, 387-394.

12- Karakilcik, A. Z., Hayat, A., Zerin, M., and Cay, M. (2003): Effects of intra-peritoneally injected selenium and vitamin E in rats anesthetized with halothane. J Trace Elem Med Biol 17, 33-8.

13- Yuan, Y., Zheng, J., Zhao, T., Tang, X., and Hu, N. (2017): Hydrogen sulfide alleviates uranium-induced acute hepato-toxicity in rats: Role of antioxidant and antiapoptotic signaling. Environmental toxicology 32, 581-593.

14- Floreani, M.; Petrone, M.; Debetto, P. and Palatini, P. (1997): A comparison Between Different Methods for the Determination of Reduced and Oxidized Glutathione in

Mammalian Tissues. Free Radical Research, 26: 449-455.

15- Ohkawa H., Ohishi N., Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

16- Bancroft, J.D., Layton, C., 2013: The hematoxylins and eosin. Bancroft's Theory and Practice of histological techniques, 7th ed. [Oxford]: Churchill Livingstone Elsevier, c2013.

17- Crowley, S.D., Vasievich, M.P., Ruiz, P., Gould, S.K., Parsons, K.K., Pazmino, A.K., Facemire, C., Chen, B.J., Kim, H.-S., Tran, T.T., 2009: Glomerular type 1 angiotensin receptors augment kidney injury and inflammation in murine autoimmune nephritis. The Journal of clinical investigation 119(4), 943-953.

18- Jóźwiak-Bebenista M., Nowak J. Z. 2014): Paracetamol: mechanism of action, applications and safety concern. Acta Pol Pharm.; 71(1):11-23.

19- Fored C. M., Ejerblad E., Lindblad P., Fryzek J. P., Dickman P. W., Signorello L. B., Lipworth L., Elinder C. G., Blot W. J., McLaughlin J. K., Zack M. M., and Nyren O. 2001: Acetaminophen, aspirin, and chronic renal failure. N Engl J Med; 345: 1801–1808.

20- Hiragi S., Yamada H., Tsukamoto T., Yoshida K., Kondo N., Matsubara T, Yanagita M., Tamura H., and Kuroda T. (2018): Acetaminophen administration and the risk of acute kidney injury: a self-controlled case series study. Clin Epidemiol. 10: 265–276.

21- Yu Y. L., Yiang G. T., Chou P. L., et al. (2014): Dual role of acetaminophen in promoting hepatoma cell apoptosis and kidney fibroblast proliferation. Mol Med Rep.; 9 (6):2077–2084.

22- Boon A. C., Lam A.K., Gopalan V., Benzie I. F., Briskey D., Coombes J. S., et al. (2015): Endogenously elevated bilirubin modulates kidney function and protects from circulating oxidative stress in a rat model of adenine-induced kidney failure. Sci Rep.; 5:154-82.

23- Quiroz Y., Ferrebuz A., Romero F. et al. (2008): Melatonin ameliorates oxidative stress, inflammation, proteinuria, and progression of renal damage in rats with renal mass reduction. Am J Physiol Renal Physiol; 294: F336–F444.

24- Mayne, P.D. (1994): The Kidneys and Renal Calculi. In: Clinical Chemistry in Diagnosis and Treatment, Mayne, P.D. (Ed.). 6th Edn., Edward Arnold Publications, London, UK., pp: 2-24.

25- Wang Y., Wang W., and Qiu E. (2017): Protection of oxidative stress induced apoptosis in osteosarcoma cells by dihydromyricetin through down-regulation of caspase activation and up-regulation of BcL-2. Saudi J Biol Sci., May; 24(4): 837–842.

26- Lorz C., Justo P., Sanz A., Subira D´, Egido J.S., and Ortiz A. (2004): Paracetamol-Induced Renal Tubular Injury: A Role for ER Stress. J Am Soc Nephrol 15: 380–389.

27- Morishima N., Nakanishi K., Takenouchi H., Shibata T., Yasuhiko Y. (2002): An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c independent activation of caspase-9 by caspase-12. J Biol Chem 277: 34287–34294.

28- Cachofeiro V., Goicochea M., Vinuesa S. G., Oubiñ˜P., Lahera V. and Luno J. (2008): Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease Kidney International; 74 (Suppl 111), S4–S9

29- Gao L., Wu W. F., and Dong L., et al. (2016): Protocatechuic aldehyde attenuates cisplatin-induced acute kidney injury by suppressing nox-mediated oxidative stress and renal inflammation. Front Pharmacol.; 7:479.

30- Mandegary A., Azmandian J., Soleymani S., et al., (2013): Effect of donor tumor necrosis factor-alpha and interleukin-10 genotypes on delayed graft function and acute rejection in kidney transplantation. Iran J Kidney Dis.; 7:135–41.

31- Banudevi, S., Krishnamoorthy, G., Venkataraman, P., Vignesh, C., Aruldhas, M. M., and Arunakaran, J. (2006): Role of alpha-tocopherol on antioxidant status in liver, lung and kidney of PCB exposed male albino rats. Food Chem Toxicol; 44, Jan-Feb; 71(1):11-23.

32- Miguel, F. M., Schemitt, E. G., Colares, J. R., Hartmann, R. M., Morgan-Martins, M. I., and Marroni, N. P. (2017): Action of Vitamin E on Experimental Severe Acute Liver Failure. Arq Gastroenterol 54,123-129.

33- Ben Amara I, Karray A, Hakim A, Ben Ali Y, Troudi A, Soudani N, Boudawara T, Zeghal KM, Zeghal N. (2013): Dimethoate induces kidney dysfunction, disrupts membrane-bound ATPases and confers cytotoxicity through DNA damage. Protective effects of vitamin E and selenium. Biol Trace Elem Res. Dec; 156(1-3):230-42.

34- Fravega, J., Alvarez, R., Diaz, F., Inostroza, O., Tejias, C., Rodas, P. I., Paredes- Sabja, D., Fuentes, J. A., Calderon, I. L., and Gil, F. (2016): Salmonella Typhimurium exhibits fluoro-quinolone resistance mediated by the accumulation of the antioxidant molecule H2S in a CysK-dependent manner. J Antimicrob Chemother Sabja, D., 71, 3409-3415.

35- Wang, P., and Wu, L. (2018): Hydrogen sulfide and nonalcoholic fatty liverdisease. Hepatobiliary Surg Nutr 7, 122-124.

36- Jha, S., Calvert, J. W., Duranski, M. R., Ramachandran, A., and Lefer, D. J. (2008): Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling. American Journal of Physiology-Heart and Circulatory Physiology 295, H801-H806.

دراسة مقارنة لاليات حمايه الكلى بين كلا من فيتامين هـ و;كبريتيد هيدروجين الصوديوم من الأضرار التي يسببها الباراسيتامول في الفئران

عادل حسين سعد و ساره محمد نجيب

قسمى الفسيولوجى والهستولوجى بكليه طب المنيا

الهدف: تهدف الدراسة الحالية إلى مقارنة اليات حمايه الكلى لكلا من فيتامين هـ وهيدروكلوريد الصوديوم (NaHS) أحد الجهات المانحة البارزة لكبريتيد الهيدروجين عند استخدام جرعة واحده زائدة من الباراسيتامول. **الطريقة:** تم تقسيم اثنين وثلاثين من الفئران البيضاء الذكور عشوائيا إلى 4 مجموعات (8 فئران لكل مجموعه): (1) المجموعة الضابطه (C): لا تاخذ أي علاج. (2) مجموعة الباراسيتامول (P): تم اعطاء كل فأر الباراسيتامول بجرعة 2 جم / كجم من وزن الجسم كجرعة وحيدة عن طريق الانبوب الأنف المعدي ، (3) باراسيتامول + فيتامين هـ (E): تم اعطاء كل فأر المعالجة السابقة+ فيتامين هـ بجرعة 100 مج / كج من وزن الجسم كجرعة وحيدة عن طريق الحقن داخل الصفاق قبل 24 ساعة من تلقي الباراسيتامول و(4) باراسيتامول + هيدروكلوريد الصوديوم (H): تم اعطاء كل فأر المعالجة السابقة + هيدروكلوريد الصوديوم بجرعة 56 µmole / كج مرة واحدة في اليوم لمدة يومين عن طريق الحقن داخل الصفاق. تعطى الجرعة الثانية قبل تلقي الباراسيتامول بساعه واحده. **في نهاية التجربة** ، تم جمع عينات الدم لتقدير مستويات المصل من علامات الإصابة الكلوية (اليوريا ، الكرياتينين) ، وكذلك عامل نخر الورم في المصل (TNF-α)، Bcl-2، مستويات الجلوتاثيون المختزل (GSH). تم جمع عينات الكلى للفحص الهستوباثولوجي بالإضافة إلى التقدير الكيميائي للبيروكسيدات الدهنيه (MDA). **النتائج:** تسبب تحريض الإصابة الكلوية الحادة في تدهور ملحوظ في وظائف الكلى كما يتضح من فحص الهستوباثولوجى ، ومستويات مصل الدم المرتفعة من علامات الإصابة الكلوية ، TNF-α ومحتوى الأنسجة من البيروكسيدات الدهنيه MDA جنبًا إلى جنب مع انخفاض كبير في مستويات Bcl-2 و GSH بالمقارنة مع المجموعة الضابطه. اعطاء فيتامين هـ و NaHS ادى الى تحسن ذو دلاله احصائيه على الآثار الضارة التى احدثها الباراسيتامول على الكلى كما يتضح من الفحص الهستوباثولوجي ، وانخفاض علامات الإصابة الكلوية ، TNF-α ، إلى جانب زيادة مستويات Bcl-2. كما كان هناك تحسن في معدل التوتر الناتج من وجود زياده فى مشتقات الاكسجين الحره النشطه من حيث انخفاض مستويات MDA جنبا إلى جنب مع زياده مستوى GSH. أنتج فيتامين (هـ) تحسنا أكثر قيمه في إصابة الكلى كما يتضح من النسبة المئوية للتغيرات في القياسات المختلفة ومستوى ترميم بنية الكلى عند فحص الأنسجة. الخلاصة: اعطاء جرعة زائدة من الباراسيتامول يمكن أن تسبب تلف فى الكلى. أثبت فيتامين هـ و NaHS أنهما واقيان من هذا التلف عبر التأثيرات المضادة للالتهابات ومضادات الأكسدة ومضادات الهدم للخلايا. كان فيتامين (هـ) أقوى في حمايته ؛ يمكن استخدام مزيج من كلا العقارين ، كطريقة مزيج جديدة والتي قد تكون أكثر فعالية ضد إصابة الكلى الحادة لأن هذا المزيج له تأثير تآزري على الحماية من العمليات المؤكسدة