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RESEARCH ARTICLE

THE PROTECTIVE EFFECTS OF THYMOQUINONE AGAINST VALPROATE-INDUCED NEPHROTOXICITY IN RATS.

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Abstract

Background: Valproate is commonly used to treat epilepsy seizures. This study was designed to examine the protective role of thymoquinone against nephrotoxicity induced by valproate through estimating the oxidative status, kidney function parameters and histopathological alteration in the kidney of rats.

Methods: Forty rats were divided into four groups (n=10): control group; thymoquinone group was administered with thymoquinone (10 mg/kg); the valproate group was orally given 500 mg/kg; and the valproate + thymoquinone group was co-administrated with thymoquinone following valproate for twenty-eight days.

Results: Valproate administration impaired the balance between oxidants and antioxidants as evedienced by markedly increase in malondialdehyde (MDA), nitric oxide (NO), nuclear factor kappa-B (NF-kB) coupled with the decrease of glutathione (GSH) and catalase (CAT). Biochemical findings showed a marked increase in the urea and creatinine levels following valproate intoxication. Also, histological examination revealed congested glomerular capillaries with increased cellularity of meningeal cells admixed with congested blood vessels in the interstitial tissue. In contrast, thymoquinone administration ameliorated the histopathological damages and biochemical alterations produced by valproate.

Conclusion: These findings suggest that co-administration of thymoquinone following valproate ameliorated the changes in the oxidative damage, inflammation, and histopathological alterations in renal tissues which may be by facilitating valproate biomethylation and excretion.

Introduction:-

Epilepsy is the most common primary neurological disorder; about 50 million epileptic patients in the world with 177 thousands cases in KSA have been recorded (Khan 2015). Epileptic patients suffering from uncontrolled repetitive seizures, these seizures results from the disturbance in the electrochemical activities in the brain tissue (Brahmane et al. 2010). Treatment of epilepsy was improved by several of the third generation antiepileptic drugs during the past three decades (Kanner 2016). Nevertheless, resistance to antiepileptic drugs as well as intolerability in 20-30% of the patients led to serious demands for developing new drugs or strategies for epilepsy treatment [4].

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Valproic acid (valproate), 2-propylpentanoicacid or dipropylacetic acid (Aktas et al. 2010), is commonly used to treat epilepsy seizures (Silva et al. 2008). Valproate is mainly effective in preventing absence seizures, partial seizures, and generalized seizures (Berkovic et al. 1989). However, long-term use of valproate is associated with several diseases (de la Microvasculatura et al. 2019; Nwidu and Ibor 2019; Nwidu and Oboma 2019). Serious side effects can include liver dysfunction, pancreatitis, and nephrotoxicity (Frick et al. 1993; Nwidu and Ibor 2019). The drug is involved in rise in the dangerous abnormalities in children if taken during pregnancy (Christensen et al. 2013).

Sanaa et al. (Galaly et al. 2014) reported that valproate administration further exacerbated nephrotoxicity in rat. Furthermore, El-Shenawy and Hamza (2016) indicated that valproate administration was implicated with the disturbance in the oxidative stress. However, nephrotoxicity resulting exposure to epilepsy drugs is caused by increased generation of reactive oxygen species (ROS), which increases oxidative stresses, therefore kidney damage (El-Shenawy and Hamza 2016; Galaly et al. 2014; Silva et al. 2008). Many promising studies have reported that the antioxidants such as catalase and glutathione play an effective role in preventing nephrotoxicity which scavenge lipid peroxidation (LPO) suppressors induced by an increased ROS (de la Microvasculatura et al. 2019; Nwidu and Ibor 2019; Nwidu and Oboma 2019).

Thymoquinone (TQ, 2-isopropyl-5-methyl-benzoquinone) is the main ingredient substance of Nigella sativa seed oil that displays potent anti-inflammatory, and antioxidant effects (Al-Brakati et al. 2019). Several promising studies provided biological evidence that thymoquinone has an effective role in the treatment of chemical-induced nephrotoxicity (Al-Brakati et al. 2019). However, the mechanism(s) of kidney dysfunction/injury induced by valproate is not clear. So, the present work achieved to evaluate the protective role of thymoquinone against nephrotoxicity induced by valproate through estimating the oxidative status, kidney function parameters, and histopathological alteration in kidney of rats.

Materials and methods:-

Experimental animals

Forty male Wister albino rats (200-250 grams) were used in this study. The rodents were obtained from the Animal House of King Fahd for medical research, King Abdulaziz University, Jeddah, Saudi Arabia. The rodents were kept in 12 hours light/dark cycle and housed in cages and offered water and laboratory food for one week before starting the experiment for acclimatization.

Drugs and reagents

Chemicals

Thymoquinone (CAS number 490-91-5), was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Valproic acid sodium salt was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Urea and creatinine kits were provided by Biodiagnostic Co. (Giza, Egypt).

Animals groups and doses of the treatment

Rats were divided into four groups (10 each) as follows: The control group, the thymoquinone group, the valproate group, and the thymoquinone + valproate group. The rats in control group were administered with physiological saline (0.9% NaCl) for four weeks. The rats in thymoquinone group were given thymoquinone orally at a dose of 10 mg/kg body weight (bw) for four weeks according to Albrakati et al. (Al-Brakati et al. 2019). The rats in the valproate group were administered with valproate (500 mg/kg BW) for four weeks according to previous study of El-Shenawy et al. (El-Shenawy and Hamza 2016). The rats in valproate + thymoquinone group were given valproate and thymoquinone for four weeks. In this group rats were received valproate with the same dose as in the valproate group, three hours later, rats were re-treated with thymoquinone with the same doses as in the thymoquinone group. The experiment was conducted at the laboratories of the Faulty of Medicine, Taif University, Taif, Saudi Arabia.

Blood samples and tissue specimens

All the rats were euthanized by pentobarbital (100 mg/kg i.p.) followed by decapitation 24 hours after the last treatment and their blood was collected and stored at -80°C till the beginning of the experiments.

Histology study

The kidney was placed in 10% neutral buffered formalin. Following fixation, specimens were dehydrated, embedded, and then cut at 12 µm thickness using Leica microtome (Leica RM 2025; Nassloch, Germany).

Specimens were stained with hematoxylin and eosin stain (Bancroft and Gamble 2008). Finally, the slides were examined using a Nikon Eclipse E200-LED (Tokyo, Japan) microscope with 400× magnification.

Kidney functions markers
Serological levels of urea and creatinine were assayed using commercially available kits sourced from Randox Laboratories (Crumlin, UK) according to the manufacturer's protocol.

Kidney oxidative damage
Kidney homogenates were subjected to a thiobarbituric acid reactive substance formation assay to determine the level of malondialdehyde (MDA) formed (Janero 1990). Nitrate–nitrite–nitric oxide (NO) and glutathione (GSH) levels were determined after protein removal according to the methods described by Green et al (Green et al. 1982).

Antioxidant status
Kidney homogenate supernatants were used to determine the activity of catalase (CAT) according to the method described by Aebi (1984).

Quantification of renal NF-κB
The nuclear factor-κB (NF-κB) level was performed using ELISA kits in kidney supernatants according to the manufacturer's protocols. Briefly, kidney supernatants were prepared using a protease inhibitor cocktail (catalogue number: P8340; Sigma-Aldrich) from kidney samples that were collected and frozen at -20 °C until analysis. Pro-inflammatory cytokine levels were measured in duplicate and expressed as picogram per gram tissue.

Statistical analysis
The values were expressed as the mean ± S.D. for the 6 rats in each group. Differences between groups were assessed by one way analysis of variance (ANOVA) using SPSS (version 13.0). Significant differences among means were evaluated using Duncan's Multiple Range Test.

Ethical considerations
All experiments protocols including the use animals were approved by the committee of research ethics for laboratory animal care, anatomy department, school of medicine, Taif University.

Results:-

MDA level in the kidney tissue
MDA was used as a marker of oxidative damage in this study. Analysis of the results showed that valproate markedly increased **(P<0.05)** the MDA level in the valproate group **(Fig. 1)** as compared to the control group and/or thymoquinone group. On the other hands, the rats treated with thymoquinone following valproate showed a significant decrease **(P<0.05)** in MDA level when compared to valproate group **(Fig. 1).**

CAT activity in the kidney tissue
Results show a marked increase **(P<0.05)** in the CAT activity in the valproate group **(Fig. 1)** as compared to the control group. Whereas, the rats treated with thymoquinone following valproate showed a significant decrease **(P<0.05)** in CAT activity as compared to valproate group **(Fig. 1**).

NO level in the kidney tissue
Results showed a marked increase **(P<0.05)** in the NO level in the valproate group **(Fig. 1)** as compared to the control group. In contrast, the rats treated with thymoquinone following valproate showed a significant decrease **(P<0.05)** in NO level as compared to valproate group **(Fig. 1**).

GSH content in the kidney tissue
Results showed a marked decrease **(P<0.05)** in the GSH content in the valproate group **(Fig. 1)** as compared to the control group. Meanwhile, the treated rats with thymoquinone following valproate showed a significant increase **(P<0.05)** in GSH level as compared to valporate group **(Fig. 1**).

NF-κB in the kidney tissue

Valproate-intoxicated group exhibited a significant increase **(P< 0. 05)** in the NF-κB as compared with the control group as shown in **Fig. 1.** In contrast, the rats treated with thymoquinone following valproate showed a pronounced decrease in NF-κB level **(P< 0. 05)** when compared to valproate treated group.

Analysis of serum urea and creatinine levels

Urea and creatinine are nitrogenous end products of metabolism. To assess the protective role of thymoquinone following valproate exposure, kidney function test was achieved. Analysis results of urea and creatinine revealed a significant increase **(P< 0. 05)** in urea and creatinine levels respectively, in valproate treated rats as compared with rats in the control group. Meanwhile, a significant **(P< 0. 05)** decrease in levels of urea and creatinine in rats treated with thymoquinone following valproate as compared to valporate treated group **(Table 1)**.

Histological results

Histological examination of the control group and thymoquinone group revealed the normal histoarchitecture of glomerular capillaries with normal cellularity and basement membrane as shown in **Fig. 2 A** and **B**, respectively. Histopathological examination of the valproate group showed congested glomerular capillaries with increased cellularity of meningeal cells admixed with swollen eosinophilic columnar lining of renal tubules and congested blood vessels in the interstitial tissue as seen in **Fig. 2 C**. In contrast, co-administration of thymoquinone following valproate showed improvement histoarchitecture of the glomerular and renal tubules and attenuate the congested of the glomerular capillaries as shown in **Fig. 2 D**.

Discussion:-

Recently, several studies have shown renal injury following valproate administration in experimental models (Gad 2018a). The treatment with valproate for long term increased creatinine and urea levels may be due to ROS-stimulated oxidative damage in renal tissue (Al-Amoudi 2017). Therefore, lead to impairment kidney function resulting damaged cellular structure of their tissue (Ghorbani et al. 2018).

The histopathological examination of the current study showed hydropic changes in Bowman's capsule and the epithelial cells of renal convoluted tubules in the valproate group. These changes may result from the accumulation of metabolites of valproate in the renal tissue. Rising level of lipid peroxidation led to increase the production of epoxides, hydroperoxides, and MDA, via interact with cellular proteins as DNA, therefore causing cellular damaged and then nephrotoxicity (El-Shenawy and Hamza 2016). These findings may be resulted oxidative damage induced by ROS (Nwidu and Ibor 2019).These results are an agrement with previous findings of El-Shenawy and Hamza (2016) who reported that valproate causes nephrotoxicity in rats resulted from the extreme accumulation of valproate and its metabolites in the renal tissue.

Several studies showed the nephroprotective roles of thymoquinone in amelioration histopathological alteration and antioxidants levels in renal tissue against nephrotoxicity induced by several toxic agents such as the arsenic. The nephroprotective effect of thymoquinone was approved by histological examination. Histopathological examination showed that co-administrated of thymoquinone following valproate ameliorated tubular degeneration, inflammatory cell infiltration, haemorrhage and swelling of the convoluted tubule. These results showed that the thymoquinone may have a bioactive role in protecting renal tissue from valproate-induced renal toxicity by elevating the biomethylation process of valproate.

Disturbance in antioxidant enzymes and oxidative stress are linked to inhibiting the lipid peroxidation (Taka et al. 2015). Valproate treatment raise nuclear alterations in kidney tissue via inhibition of histone deacetylase an anticancer agent which leads to detaches the chromatin structure (Kramer et al. 2003). Additionally, activate pro-inflammatory NF-kB leads to promotes production of NO synthase (iNOS) (Abdel Moneim 2016). Also, increase NO depletes levels leads to reduce intracellular levels, therefore raising oxidative stress in kidney tissue (Al-Brakati et al. 2019).

Biochemical results of the oxidative stress and antioxidant markers showed that valproate intoxication compromised the antioxidant system defence confirmed by produced a markedly increase in MDA, NO, NF-kB coupled with a decrease of GSH and CAT. Massive increase of NO lead to reacts with superoxide anions to form peroxynitrite which leads to renal cells oxidation (El-Mahmoudy et al. 2002). These results were agreement with the previous results reported by Maneenin et al. (2019)who showed that valproate intoxication compromised the antioxidant

system defence approved in rats by increasing the levels of LPO coupled with reducing in the enzymatic activity and total antioxidant capacity.
Thymoquinone is a phytochemical compound derivative from the *Nigella sativa* (Gholamnezhad et al. 2016). It has shown anti-inflammatory and antioxidant effects (Inci et al. 2013). Furthermore, several studies have been shown that thymoquinone has protective effect against chemical-induced nephrotoxicity (Jones 2010; Srinivasan et al. 2010). Improvement of the histopathological alteration in intoxicated rats may be ascribed to significantly decreased oxidative stress via enhancement antiradical, antioxidant, anti-inflammatory provoked from metabolism of drugs and other toxic substances (Fuchs and Milbradt 1994).

Our results showed that co-administrated of thymoquinone following valproate ameliorate the histopathological alteration in renal tissue. This finding may be resulted from reduced the overproduction of NO which save antioxidant defence mechanisms in intoxicated rats with valproate. In addition, results of the biomarkers showed also reduced the antioxidant system by decreasing GSH content (Gadea et al. 2004). The protective effects of thymoquinone saved GSH content at near-normal levels, which have the ability to detoxify of kidney tissue toxicity induced by valproate, therefore improve antioxidant defence system. Valproate caused the nephrotoxicity maybe via increased ROS formation and thus participate to apoptosis (Gad 2018b). In this regards, thymoquinone led to increasing state GSH levels, which confer enhanced nephroprotection against valproate intoxication (Chaudhary et al. 2015). Thymoquinone primarily reduces ROS and then inhibits oxidation that could lead to nephrotoxicity (Elmaci and Altinoz 2016). Our results are in agreement with the work of Al-Brakati et al. (2019) who reported that thymoquinone following arsenite ameliorated nephrotoxicity in female rats by facilitating arsenite biomethylation and excretion via promotes the antioxidant system, counteracting oxidative stress. These findings are in agreement with the results of this study, which showed that thymoquinone treatment markedly inhibited the overproduction of NO, therefore, saved the antioxidant defence mechanisms in the renal tissue of rats treated with valproate intoxication.

To further confirm the nephrotoxicity following valproate treatment, the kidney function biomarkers were examined. Our results revealed increased creatinine and urea levels in the treated rats in the valproate group. These findings may be resulted from the oxidative damage mediated by ROS, which leads to damaged cellular structure, resulted from leakage of kidney biomarkers through impaired cellular membranes (El-Demerdash et al. 2009; Zhao et al. 2014). In this regards, it has been reported that high levels of creatinine and urea are indicative of nephrotoxicity (Kumar et al. 2003).

Conclusion:-

The current study concluded that thymoquinone maintained the architectural and functions of the renal tissue against nephrotoxicity induced by valproate through promoted the antioxidant system which counteracts oxidative stress.

Ethical approval

We declare that experiments protocols including the use animals were approved by the Committee of Research Ethics for Laboratory Animal Care, Anatomy Department, School of Medicine, Taif University (approval no, 40-36-0191).

Declaration of conflict interests

We declare that we have no conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1:-Shows change in urea and creatinine level in the four groups: control group, thymoquinone group, valproate group, and the valproate + thymoquinone group.

Parameters	control	Thymoquinone	Valproate	Valproate + Thymoquinone
Urea (mg/dL)	28.32 + 0.13	26.33 + 1.16	38.13 + 1.24 *	29.11 + 1.54 *
Creatinine (mg%)	0.61 + 0.22	0.60 + 0.21	0.92 + 0.03 *	0.85 + 0.01 *

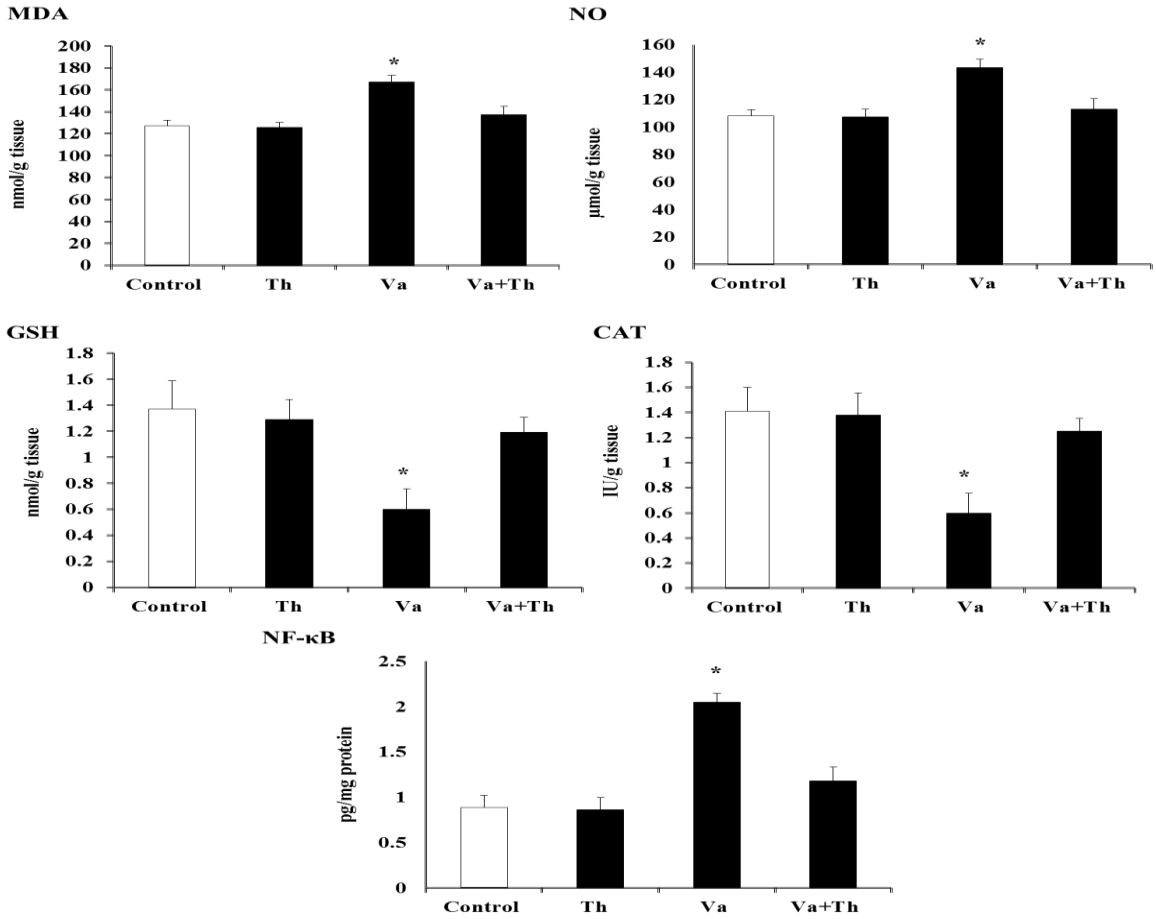

Fig 1:-Shows oxidant and antioxidant markers of rat renal tissue of control group, valproate group (Va), thymoquinone group (Th) and Va + Th group. Results are presented as mean ± S.D., n= 10. *P < 0.05.

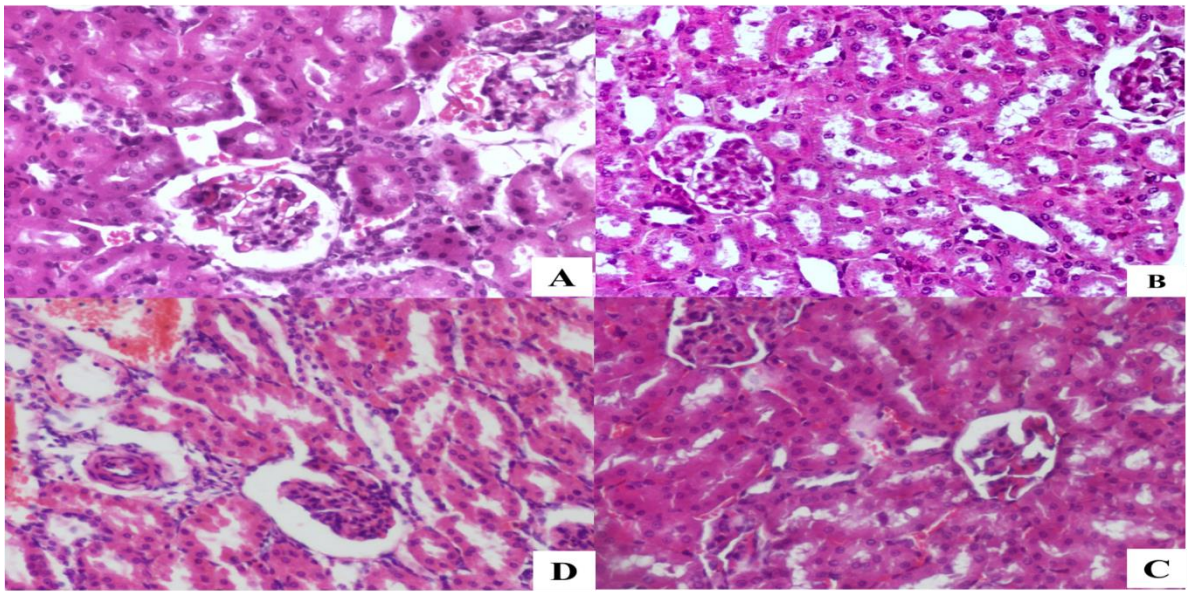

Fig 2:-A. Light photomicrography of kidney tissue of control group (A) and thymoquinone group (B); show normal structure of glomerular capillaries with normal cellularity and basement membrane. Valproate treated group (C)

shows congested glomerular capillaries with increased cellularity of meningeal cells admixed with congested blood vessels in the interstitial tissue. Thymoquinone + valproate treated group (D) shows improvement histological structure of the glomerular and renal tubules and attenuate the congested of the glomerular capillaries. H & E, x400.

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