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Ameliorative and Anti-Inflammatory Properties of *Thuja occidentalis* in Phenytoin-Induced Hepatic and Renal Dysfunctions in Male Albino Rats

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Abstract

Phenytoin (PHT) is one of the most used anticonvulsants in the developing world, but lack of monitoring and concurrent medications can easily cause harmful negative effects on the liver and kidneys. Herbal medicines have consistently drawn the interest of researchers in contrast to drug therapies, which have a number of negative effects. The current study looked into the protecting impacts of *Thuja occidentalis* (TO) against phenytoin-exposed rats' hepatic and renal damage. In the current work, young, non-woody *Thuja occidentalis* L. was extracted using a newly made methanolic solution. This study used distillation-based methods to obtain branches with leaves. The study involved of three groups of eight rats per group. Group I rats acted as a control group, whereas group II take 75 mg/kg of PHT alone. Groups III received PHT parallel to TO 200 mg/kg for 14 consecutive days. Liver and kidney tissues prepared for histological examination and serum samples separated for biomarkers. (I) liver injury indicator enzymes, alanine aminotransferase ALT, aspartate aminotransferase AST and alkaline phosphatase ALP; (II) kidney injury indicators as urea and creatinine. (III) inflammatory markers, interleukins (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) were subsequently determined. The findings showed that PHT exposure group elicited significant increases ($p < 0.05$) in ALT, AST, ALP, urea and creatinine levels. Moreover, some hepatic and renal histological changes were observed. However, rats co-treated with TO, had their functional indices of the liver and kidneys returned to nearly normal levels. In conclusion, *Thuja occidentalis* might guard the liver and kidneys from damage brought on by PHT medication.

Keywords: Phenytoin; *Thuja occidentalis*; Hepatotoxicity; Nephrotoxicity; Cytokines.

1. Introduction

Phenytoin (diphenylhydantoin) still one of the most used first-line Anti-Epileptic Drugs (AEDs) either as monotherapy or as adjunctive treatments for the treatment of partial or secondary generalized seizures (Perucca *et al.,* 2018). The regulation of sodium and calcium channels that are voltage-dependent is thought to be how it works. In addition to being processed by the CYP450 system and inducing numerous enzymes in this system, phenytoin is approximately 90% protein bound. As a result, when changing medication regimens, careful consideration should be given to adequate dosing and monitoring of drug levels because phenytoin can enhance the metabolism of many routinely prescribed medicines (Bansal *et al.,* 2015). Long-term use of AEDs, especially those from the older group (e.g., valproate or VPA, phenytoin or PHT, carbamazepine or CBZ, etc.), is linked to undesirable side effects, such as metabolic and endocrine consequences (Hamed, 2015),

vascular, cognitive, behavioral (Hamed *et al.,* 2013), bone disease (Hamed, 2016) and non-alcoholic fatty liver (Verrotti *et al.,* 2009). One negative side effect of various AEDs has been observed to be kidney malfunction or damage. Phenytoin toxicity is frequently observed in patients with hepatic and renal impairment as well as with substantial consumption (Craig, 2005). The authors discovered multinucleated histiocytes in the renal interstitium, exact lymphocyte sensitization with PHT (a clinically typical delayed hypersensitivity reaction), and negative renal immunofluorescence investigations for immune reactants showing cell-mediated renal damage generated by PHT. After using PHT, a youngster developed interstitial nephritis, according to Hyman *et al.* (1978).

Northern white cedar, *Thuja occidentalis* L., a member of the Cupressaceae family, is commonly known as arborvitae and sometimes "tree of life". TO has been used to treatment liver conditions, diarrhea, psoriasis, enuresis, amenorrhea, cystitis, bullous bronchitis, uterine carcinomas, and rheumatism in traditional medicine (Akkol *et al.,* 2015). Intestinal worms, cancer, and fungal diseases have all been treated with essential leaf oil (Biswas *et al.,* 2011). The vital oil of TO has been utilized in folk medicine since ancient times. Thujone-containing TO has been used to treat uterine cancer, rheumatoid arthritis, psoriasis, bronchial catarrh, and hepatoprotection. Homeopathy uses the mother tincture of TO to treat a variety of illnesses. TO is one of the principal treatments for psychotic constitutions in homeopathy, as well as for snakebite, smallpox, and vaccination-induced toxicity, as well as for the growth of pathological vegetation (Nash, 2002).

Additionally, TO exhibits hepatoprotective, antioxidant, antibacterial, anti-fungal, anti-diabetic, anti-inflammatory and anticancer potential, among other properties (Bhan *et al.,* 2011; Caruntu *et al.,* 2020), antioxidant (Naz *et al.,* 2022). According to reports, TO has a protective influence against radiation-induced toxicity and can boost spleen cell proliferation as well as serum TNF-α, IL-6 and IL-1 activity (Belal *et al.,* 2005).

Cytokines are crucial immune response mediators and have been shown to excite immune cells (Kelly *et al.,* 2002). Immune cells like T lymphocytes and macrophages are primarily responsible for secreting cytokines and chemokines, which are implicated in immune-mediated hepatotoxicity and are followed by inflammation or the infiltration of lymphocytes to hepatocytes (Oo and Adams, 2010). The transcriptional factors listed below stimulate the synthesis of cytokines: Interferon-γ and IL-12 are secreted by T-box expressed in T cells (T-bet), IL-4, IL-5, and IL-13 are secreted by GATA-binding domain-3 (GATA-3), and ROR-γt is required for the development of Th17 cells, which largely secrete IL-17 (Steinman, 2007).

The liver gathers metabolites from the blood, neutralizes them, and then removes the harmful byproducts of metabolism (Mescher, 2010). Hepatotoxicity has been linked to the use of phenytoin (Bjo¨rnsson, 2008) and also reported to be toxic to the kidneys (Hyman *et al.,* 1978). The goal of the current investigation was to investigate the hypothesis that TO might be able to control the biochemical and histological changes that PHT therapy in rats' liver and kidneys causes.

2. Materials and Methods

2.1 Experimental animals

Twenty-four adult male albino rats from King Abdul-Aziz University's central animal facility in Jeddah, Saudi Arabia, were used in this investigation. Animals weighing (180 – 200 gm) housed at room temperature (30 ± 5 ºC) in stainless steel cages, a 12/12 hour light/dark cycle, and enough ventilation and obtained complete diet pellets and water ad-libitum. Before the trial began, we observed the animals for around two weeks to make sure there were no concurrent infections.

2.2 Chemicals

Phenytoin (Phenytoin Sodium) was imported under the license of (Pfizer Manufacturing Deutschland GmbH, Betriebsstatte Freiburg, Mooswaldallee 1, D-79090 Freiburg, Germany) from El-Nahdi

pharmacy (Almadinah Almonawarah, Saudi Arabia). The tablets with the brand name Epanutin contained 100 mg per tablet.

2.3 Plant materials and extraction

Leaves of TO were acquired from a local market (Almarwani for Herb) in Al-Madinah Al-Monawarah. The leaves were systematically distinguished and confirmed, where a voucher specimen number (*Thuja* 3/2021) was deposited in Department of Pharmacognosy and Pharmaceutical Chemistry, college of Pharmacy, Taibah University.

The methanolic extract of the air-dried fine powder of leaves prepared as follow: dried powder was defatted using chloroform for 72 hours and then maceration was conducted using methanol for 72 hours with intermittent shaking for methanolic extract preparation. Filtration was done and then distillation was performed to remove the solvent. The product hence obtained was reduced to brown-colored granules by distillation on rotary evaporator for further solvent elimination. This part of the sample was the methanolic extract. The extract was refrigerated for storage.

2.4 Dose and treatment procedure

The PHT dose was 75 mg/kg body weight (Owoeye *et al.,* 2015). This dose was dissolved in normal saline 0.9% and given orally every day for 14 days by gastric intubation. The dose of TO used was 200 mg/kg body weight (Sunila *et al.,* 2011) for 14 consecutive days, this dose was dissolved in normal saline 0.9% and administered orally through gastric intubation.

2.5 Animal grouping

Animals were disaggregated into three groups, with eight rats in every group, as follows:

1. The 1st group served as a healthy control group and received normal saline 0.9%.

2. The 2nd group received PHT orally of a dose (75 mg/kg b. w.) daily for 14 days

3. The 3rd group received PHT orally of a dose (75 mg/kg b. w.) daily for 14 days, then after 1 h treated with TO orally of a dose (200 mg/kg b. w.) daily for 14 days.

2.6 Ethical consideration

This study was carried out in accordance with the recommendations for the care and the use of laboratory animals. Every effort was made to reduce the quantity of used animals as well as their suffering. The research project has received the confirmation of the Ethical Committee of Taibah University, KSA (IRB Reg. No. COPTU-RIC 20210113) approved the protocol for this study.

2.7 Biochemical study

At the end of the experiment rats were anesthetized by 500 µL of Ketamine-Xylazine intraperitoneal injection (100 and 20 mg/kg body weight, respectively), and blood was obtained from the retro-orbital venous plexus 24 hours following the most recent treatment (Schumann and Klauke, 2003). Then blood was collected and left to coagulate for 40 minutes at room temperature. For biochemical analysis, sera were separated by centrifugation at 5000 rpm for 20 minutes at 20 °C and then frozen at -20 °C. The levels of urea, creatinine, ALP, AST and ALT were then measured with commercially available kits (Muslco SJ, Saudi Arabia).

2.8 Evaluation of inflammatory interleukins

Evaluation of inflammatory cytokine (interleukins) such as some inflammatory markers (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) were tested in serum using enzyme-linked immunosorbent assay (ELISA) kits, as directed by the manufacturer.

2.9 Histopathological examination

Further liver and kidney were fixed in 10% buffered formalin, washed with tap water, and dehydrated in ascending series of 100% ethanol, cleared in xylene then embedded in paraffin wax. Paraffin wax sections with a thickness of 4 microns were cut off. The tissue sections were then put on clean glass slides and stained with hematoxylin and eosin before being examined under a microscope for histological purposes (Bancroft and Gamble, 2002).

2.10 Statistical analysis

The obtained data were analyzed by one-way ANOVA, after that post hoc multiple comparisons LSD's test using the SPSS statistical package v22.0 for Windows (IBM, Armonk, NY, USA). P values less than 0.05 were regarded as statistically significant differences. The mean ± standard error of the mean are used to represent the examined data (SEM).

3. Results and Discussion

3.1 The influence of (TO) on serum liver enzymes (AST, ALT, ALP) and kidney (urea and creatinine) concentration.

In rats, PHT caused liver damage, as demonstrated by significant increases in AST, ALT and ALP to 60.75%, 118.7% and 104 % respectively, as compared to the normal control group. When compared to the PHT - control group, TO significantly reduced high serum AST, ALT and ALP to 16%, 32% and 30.75 % respectively (Table 1) and (Figures. 1, 2 & 3).

In rats, concerning kidney function, PHT caused kidney impairment, as demonstrated by significant increases in serum urea and creatinine to 80.35 % and 216 % respectively, as compared to the normal control group. Urea and creatinine levels in the blood are well-known indicators of acute renal damage. When compared to the PHT - control group, TO reduced raised serum urea by 31.83 % and reduced elevated serum creatinine by 33.8 % respectively (Table 1) and (Figures. 4 & 5).

3.2 The influence of (TO) on serum inflammatory cytokines level.

The level of inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in serum of PHT-treated rats revealed a significant increment ($p < 0.05$) as compared with controls. However, PHT administration resulted in significant elevation of the level of (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in serum to 738 %, 123.14 %, 690.8%, 519.26%, 119.1 %, 198.7 % and 111.85 % respectively in comparison to the typical control group. Combined treatment with TO significantly reduced ($p < 0.05$) the elevated level of (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in serum to 66.86 %, 61 %, 81.65%, 77.65%, 34.33 %, 60.5 % and 44.74 % respectively in comparison to PHT- control group (Tables 2 & 3) and (Figure 6. a, b, c, d, e, f & g).

3.3 Histopathological examination of hepatic and renal tissues

The normal control rats' hepatic sections show a normal histological image, with normal architecture, normal portal venule (PV), normal hepatic artery (HA) and a bile duct (BD) lined by simple cuboidal epithelium. Lymphatic vessels (L) with thin wall are also seen in the portal triad (Figure 7. a).

Hepatic section from PHT- treated rats showing dilatation and congestion of (PV*) and terminal branches of (HA*). Also, (BD*) and (L) appear dilated. Moreover, perivenular inflammatory cell infiltration (double arrow)

Table 1. Effects of TO on serum AST, ALT, creatinine and urea levels

Groups	Hepatic function test			Renal function test	
	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dl)	Urea (mg/dl)
Normal control	133.25 ± 0.85	40.6 ± 0.66	84.26 ± 0.59	2.5 ± 0.09	43.61 ± 0.57
PHT-control (75 mg/kg)	214.20[a] ± 2.45	88.79[a] ± 1.18	171.86[a] ± 1.95	7.9[a] ± 0.19	78.67[a] ± 0.9
PHT +TO (200 mg/kg)	179.87[a,b] ± 1.01	60.37[a,b] ± 0.55	119.02[a,b] ± 1.04	5.23[a,b] ± 0.07	53.63[a,b] ± 0.54

Data is offered as mean ± SEM
[a]Significantly diverse from Normal control group at $p < 0.05$ (LSD's test).
[b]Significantly diverse from PHT-control group at $p < 0.05$ (LSD's test).

is noticed. Some hepatocytes in the region of the portal tract exhibit vacuolar degeneration in the form of foamy vacuolated cytoplasm (C*) and ill-defined nuclei (N*) (Figure 7. b).

For the toxic PHT group treated with TO reveals slight dilatation in (PV) and the terminal branches of (HA). Less dilatation in (BD) and (L). Many hepatocytes (H) in the region of the portal tract are conserved, whereas few cells (H*) are affected. Generally, histopathological image and liver looks nearly normal (Figure 7. c).

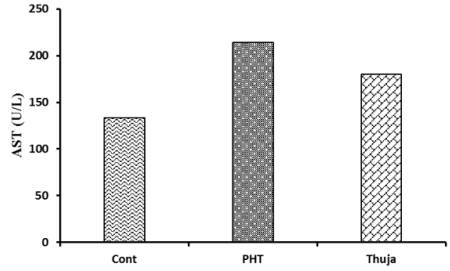

Figure 1. Effect of TO on PHT-treated rats' serum AST activity

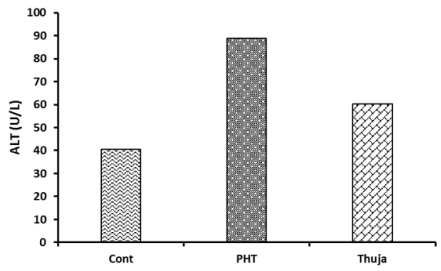

Figure 2. Effect of TO on PHT-treated rats' serum ALT activity

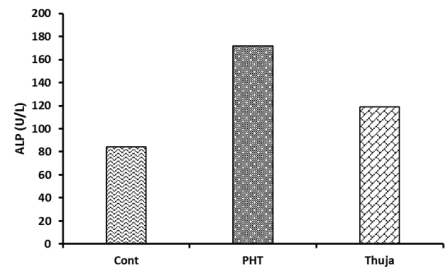

Figure 3. Effect of TO on PHT-treated rats' serum ALP level

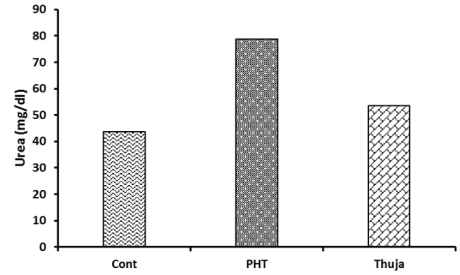

Figure 4. Effect of TO on PHT-treated rats' serum urea concentration

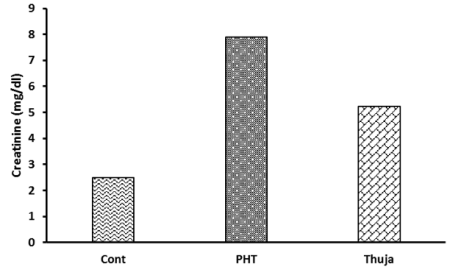

Figure 5. Effect of TO on PHT- treated rats' serum creatinine concentration

Table 2. Effects of TO on serum inflammatory cytokines level

Groups	hIL-1α (pg/ml)	hIL-1β (pg/ml)	hIL-4 (pg/ml)	hIL-6(pg/ml)
Normal control	177.42 ± 2.21	1162.12 ± 22.06	141.22 ± 0.69	1141.21 ± 0.59
PHT-control (75 mg/kg)	1486.74 [a] ± 44.46	2593.11[a] ± 13.34	1116.81[a] ± 5.09	7067.76[a] ± 28.84
PHT +TO (200 mg/kg)	492.73[a,b] ± 15.27	1010.98[a,b] ± 17.1	204.88[a,b] ± 5.49	1579.45[a,b] ± 14.54

Data is offered as mean ± SEM
[a]Significantly diverse from Normal control group at $p < 0.05$ (LSD's test).
[b]Significantly diverse from PHT-control group at $p < 0.05$ (LSD's test).

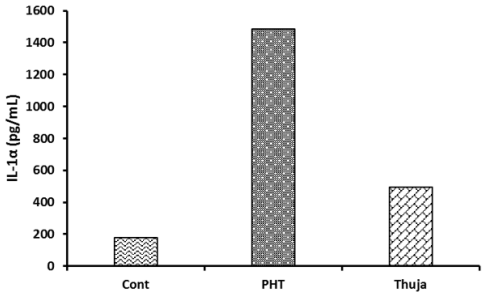

Figure 6. a) Effect of TO on PHT-treated rats' serum IL-1α level.

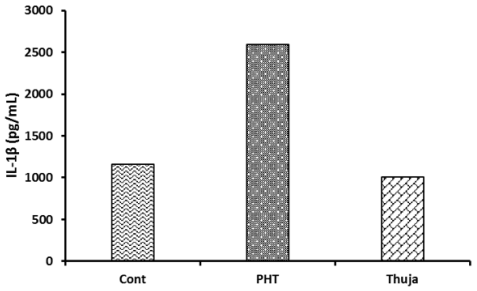

Figure 6. b) Effect of TO on PHT- treated rats' serum IL-1β level.

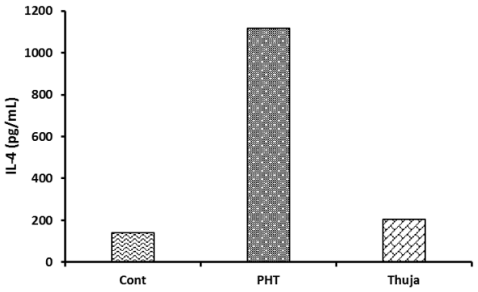

Figure 6. c) Effect of TO on PHT-treated rats' serum IL-4 level.

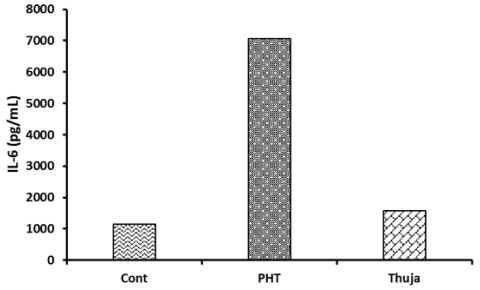

Figure 6. d) Effect of TO on PHT- treated rats' serum IL-6 level.

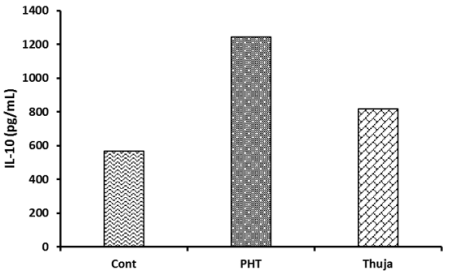

Figure 6. e) Effect of TO on PHT- treated rats' serum IL-10 level.

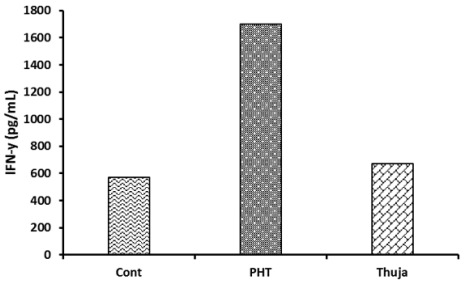

Figure 6. f) Effect of TO on PHT- treated rats' serum IFN-γ level.

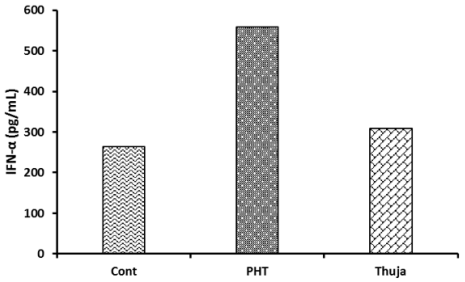

Figure 6. g) Effect of TO on PHT- treated rats' serum IFN-α level.

Table 3. Effects of TO on serum inflammatory cytokines level

Groups	hIL-10 (pg/ml)	IFN-γ (pg/ml)	IFN-α (pg/ml)
Normal control	568.08 ± 1.79	569.52 ± 0.88	263.52 ± 0.33
PHT-control (75 mg/kg)	1244.67[a] ± 11.73	1701.19[a] ± 4.73	558.27[a] ± 2.49
PHT + TO (200 mg/kg)	817.32[a,b] ± 2.03	671.47[a,b] ± 4.12	308.49[a,b] ± 1.77

Data is offered as mean ± SEM
[a]Significantly diverse from Normal control group at $p < 0.05$ (LSD's test).
[b]Significantly diverse from PHT-control group at $p < 0.05$ (LSD's test).

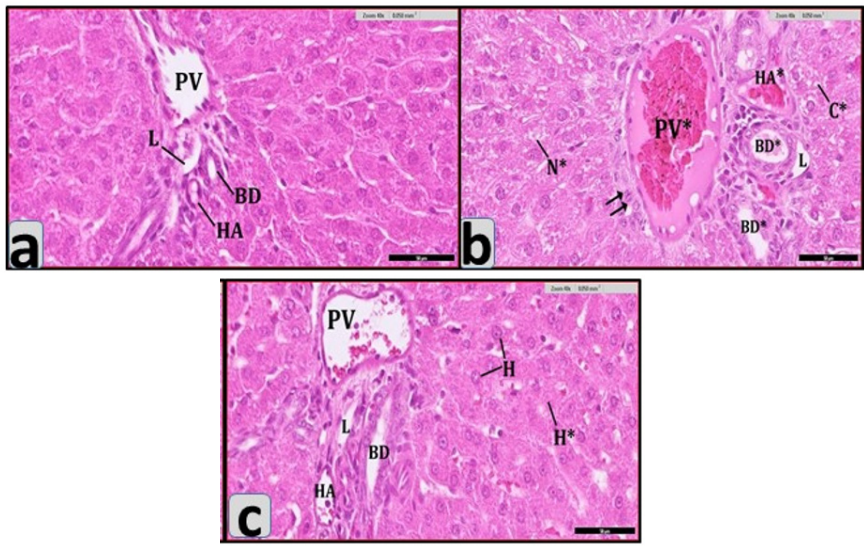

Figure 7. H&E-stained photomicrographs of the livers of control, PHT, and TO-treated rats showing:
a) Normal hepatocytes with normal portal venule (PV), the hepatic artery (HA) and a bile duct (BD).
b) The dilatation and congestion of (PV*) and the hepatic artery (HA*). Also, (BD*) and lymphatic vessels (L) appear dilated. Moreover, perivenular inflammatory cell infiltration (double arrow) is noticed.
c) A slightly dilatation in the portal venule (PV) and the hepatic artery (HA), the bile ducts (BD) and lymphatics (L).

The renal sections of the normal control rat's reveals normal renal corpuscles, tubules and arcuate arteries (AA) in the renal interstitium at the cortico-medullary junction. The juxtamedullary renal corpuscle shows conventional Bowman's capsule (BC) with its space (BS) and glomerulus (G) alongside the cells of the juxtaglomerular apparatus (JGA). In addition, the proximal tubules (PT) and distal tubules (DT) appear normal. (Figure 8. a).

Renal section from PHT- treated rat reveals focal tubular epithelial necrosis of (PT*) and (DT*) together with intratubular eosinophilic hyaline casts (arrow) that obstruct the tubular lumen. In addition, interstitial edema (E), leukocytic infiltration (double arrow) and focal areas of hemorrhage (Hg) are noticed. In addition, (AA*) appear congested. Moreover, congestion (G*) is observed. (Figure 8. b).

For the toxic PHT group treated with TO displays conserved (AA), many preserved (PT) and (DT). However, the interstitium shows minute focal areas of hemorrhage (Hg). Few congested (G*), few injured (PT*) and (DT*) are noticed. Generally, histopathological image and kidney looks nearly normal appearance (Figure 8. c).

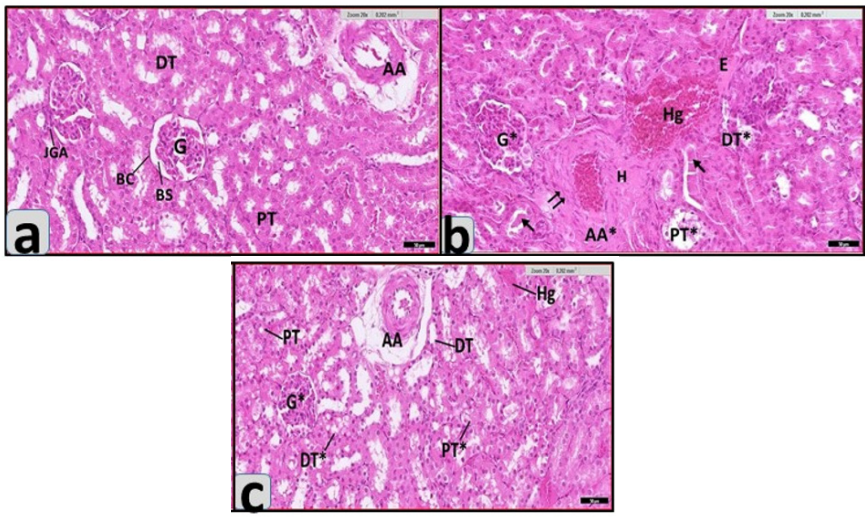

Figure 8. H&E-stained photomicrographs of the kidneys of control, PHT, and TO-treated rats showing:

a) Normal renal corpuscles, tubules, arcuate arteries (AA), Bowman's capsule (BC) with its space (BS), glomerulus (G), the proximal convoluted tubules (PT) and distal convoluted tubules (DT).

b) The focal tubular epithelial necrosis of (PT*) and (DT*) together with eosinophilic hyaline casts (arrow) that obstruct the tubular lumen. Also, interstitial edema (E), leukocytic infiltration (double arrow) and focal areas of hemorrhage (Hg) are noticed.

c) The conserved arcuate artery (AA), many preserved (PT) and (DT). However, the interstitium shows minute focal areas of hemorrhage (Hg). Few congested glomeruli (G*), few injured (PT*) and (DT*) are noticed.

3.4 Discussion

It has long been understood that the liver plays a crucial role in the metabolism of many medications, and in recent years, interest in how drugs affect liver metabolism has grown. This attention has not been least among the medication classes known as anticonvulsants. Severe anticonvulsant medicine studies have shown the numerous negative effects that long-term phenytoin administration in epileptic individuals cases (Minardi, 1975). However, phenytoin is still the medication of choice for reducing generalized seizures (Johannessen, 1980). In human liver microsomes, PHT is converted to an arene oxide and a catechol metabolite, indicating that reactive metabolites are probably involved in hepatotoxicity (Munns *et al.,* 1997). Phenytoin is utilized as a first-line pharmacological therapy for controlling seizures in the emergency room because it may quickly reach emergency treatment levels and is used in both oral and parenteral forms. Gingival hyperplasia, hirsutism, acne, and coarsening of the facial skin are the main adverse effects of phenytoin (Hung and Shih, 2011).

In this study, when compared to the normal control group, PHT treatment significantly increased levels of the liver injury enzymes ALT, AST, ALP also, urea and creatinine. These levels were significantly decreased to near normal levels in the group that received TO, indicating that TO has the ability to modulate the effects of PHT, probably due to its already well-known antioxidant and anti-inflammatory properties (Bhan *et al.,* 2011; Stan *et al.,* 2019).

The increased levels of liver enzymes in the PHT-administrated rats point to potential injury to the hepatocytes' membranes (Owoeye *et al.,* 2015). Enzymes like AST, ALT, and ALP leakage into the blood circulation may

be caused by toxic injury, ischemia injury, or hepatocyte necrosis in hepatitis, and these enzymes can be utilized as indicators of hepatocellular injury (Giannini *et al.,* 2005), it could be as a result of phenytoin's impact on the function of both mitochondrial and cytosolic enzymes (Lotfy *et al.,* 2015). Our results are in agreement with (Sasaki *et al.,* 2014; Lotfy *et al.,* 2015) and with Bjo¨rnsson (2008) who stated that phenytoin causes liver damage as a part of the hypersensitivity condition. Some epileptic patients receiving phenytoin therapy experienced hepatomegaly and enlarged lymph nodes (Bajoghl, 1961). The ability of TO to shield biological structures from ROS degradation and its ability to stabilize membranes (Sunila *et al.,* 2011; Silva *et al.,* 2017), and this has been established in this investigates. This might be responsible for returning the raised liver enzymes back to near normal levels in our TO injected rats.

Additionally, the injection of phenytoin causes a markedly significant rise in the amount of serum urea and creatinine in PHT-injected rats when compared to normal control rats. These results supported earlier research and showed that PHT therapy resulted in significant kidney impairment (Faria *et al.,* 2019). About 90% of phenytoin binds to the plasma protein, while the remaining 10% is free, which causes the pharmacological effects. Therefore, patients with hypoalbuminemia or end-stage renal disease had higher levels of unbound serum phenytoin (Richens, 1979). This might be linked to the onset of an autoimmune tubulointerstitial nephritis that is accompanied by circulating antitubular basement membrane antibodies and PHT tubular deposit accumulations. Cellular hypersensitivity to PHT was seen concurrently (Hyman *et al.,* 1978). The histological evaluation confirmed the biochemical findings of TO's chemoprotection against PHT toxicity.

As the body's first protective response to harmful stimuli, inflammation serves to repair damaged tissue. This response entails neutrophil extravasation via the capillary network followed by stimulation of macrophages, which yield a diversity of pro-inflammatory cytokines, like TNF-α, and interferons, interleukins, which play a role in the control of inflammatory reactions (Caruntu *et al.,* 2020). Pro-inflammatory cytokine concentrations are a sign of continuing inflammation (Jekarl *et al.,* 2013). It has been demonstrated that blood levels of IL-6 are elevated in both acute and chronic liver disease (Streetz *et al.,* 2003). Additionally, pro-inflammatory cytokine levels in serum and brain fluid samples from epileptic patients have been found to be higher (Sinha *et al.,* 2008). According to the current study, serum from PHT-treated animals had considerably higher levels of inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α). These results are in harmony with prior findings by Sasaki *et al.* (2014) who showed that the PHT-treated animals had higher serum levels of IL-1β and IL-6 than the control group. According to earlier studies, PHT causes significant liver damage in humans that results in extensive necrosis and an inflammatory response (Mullick and Ishak, 1980). Silva *et al.* (2017) study highlighted the anti-inflammatory effect of TO aqueous extract and the polysaccharide fraction in artificial models of acute inflammation. They decreased oxidative stress, COX-2 and iNOS action, and production of the pro-inflammatory cytokines TNF-α and IL-6 (Caruntu *et al.,* 2020). Interestingly, the co-injection of TO and PHT, significantly depressed the levels of these cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) when compared to PHT-control rats. These results were also confirmed by several prior investigations by Sunila and co-workers (2011) and Stan *et al.* (2019) who demonstrated that the TO-treated rats had a decreased expression of serum IL-1β and IL-6. In this context, and in support to biochemical and histological findings, the supplementation of TO prevented PHT-induced inflammation as demonstrated by the decreased levels of (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in blood serum. These findings showed that TO exert a suppressor effects on the inflammatory induction caused by PH medication; consequently plays a central role in its hepato-renal protection.

4. Conclusion

The present study concludes that TO defend against phenytoin induced hepatic and renal injury by raising biochemical parameters such as AST, ALT, ALP, urea, and creatinine, as well as preventing soft tissue destruction and inflammation in relation to PHT treatment. The obtained results are in line with the improvement of histological abnormalities in the liver and kidney organs. Therefore, TO could be used as an adjuvant remedy to protect against phenytoin induced toxicity and consider anti-inflammatory mediators.

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