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RESEARCH ARTICLE

THE EFFECT OF TAURINE ON METHOTREXATE INDUCED HEPATORENAL TOXICITY IN RATS.

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Keywords:-
Methotrexate; taurine, oxidative stress, apoptosis.

Abstract

The aim of the work: Hepatorenal toxicity is a major side effect for methotrexate. Taurine is a natural compound with multiple pharmacological activities such as antioxidant, antiapoptotic and anti-inflammatory effects. This study investigated the effect of taurine on methotrexate-induced toxic effects in male albino rats.

Methods: 40 male albino rats were divided into 4 groups (10 rats each): (control group): Rats injected with1ml saline intraperitoneally (i.p) for 6 days, (methotrexate treated group): Rats injected daily with 1ml saline i.p for 5 days, at the 6th day they were injected i.p with a single dose of methotrexate (20 mg/kg), (taurine treated group): Rats injected with taurine 100 mg/kg daily i.p for 6 days, (taurine+methotrexate treated group): Rats injected i.p with taurine 100 mg/kg daily for 5 days at the 6th day after the last dose of taurine, rats were injected i.p with a single dose of methotrexate (20 mg/kg).

Results: Methotrexate administration resulted in hepatorenal toxicity, oxidative stress, lipid peroxidation and apoptosis. These results were confirmed by histopathology and by detection of DNA fragmentation by gel electrophoresis. Taurine administration before methotrexate improved hepatorenal functions caused a reduction in oxidative stress, lipid peroxidation, and elevation in the activity of antioxidant enzymes. This was confirmed by the histopathological findings of hepatic and renal tissue and by the reduction in DNA fragmentation.

Conclusion: Taurine supplementation had beneficial effects on liver and kidney functions, with marked reductions in oxidative stress and apoptosis induced by methotrexate.

Introduction:-

Methotrexate (MTX) is utilized at high doses in malignancies, essentially in leukemias. Over the past 50 years, low doses of MTX were applied in different diseases including rheumatoid arthritis and psoriasis. However, toxic effect of MTX limits its use ***(Braun and Rau, 2009).***

MTX cytotoxicity is not limited only to cancer cells but it affects cells with high rate of proliferation as hematopoietic stem cells in the bone marrow and the rapidly-dividing cells in the gut mucosa. Moreover, MTX toxicity affects other organs as liver and kidney ***(Aithal, 2011).***

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The toxic impact of MTX is caused by the generation of oxygen free radicals which affects the cell membrane lipid bilayer and cause lipid peroxidation. In addition, these free radicals cause mitochondrial dysfunction ***(Çakır et al., 2011).***

There is tendency to use substances that could reduce MTX toxic effect and enhance its effectiveness. Therefore amelioration of oxidative stress or increasing cellular antioxidants by natural antioxidants most probably treats and/or protects against MTX-induced toxicity ***(Hemeida and Omar, 2008).***

Taurine is a sulfur containing amino acid (2-aminoethanesulfonic acid). It is nonessential amino acid, present freely in most mammalian cells, it represents 50% of free amino acids in inflammatory cells ***(Huxtable, 1992).***

Dietary cysteine, methionine and their metabolism in the liver are the main sources of taurine in the body ***(Marcinkiewicz and Kontny, 2014).***

Taurine has antioxidant properties; it can maintain stability of cell membrane, act as a free radical scavenger, has a role in calcium regulation, and also share in immunological response ***(Al-Asmari et al., 2016).***

Taking the above facts as a main priority, this study was designed to explore the effect of taurine against methotrexate-induced hepatorenal toxicity in rats.

Materials and Methods:-

Experimental design:-

The present work was carried out on 40 male albino rats ranging in weight between 150-200 gm. The rats were housed in isolated animal cages (5 in each cage), in a standard animal laboratory room temperature and exposed to alternate cycles of 12h light- darkness, had free access to tap water and pelleted laboratory chow all over the period of the work. All rats are fed normal diet composed of protein 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch**.** It was obtained from El Gomhoureya Company For Trading Drugs & Medical Supplies**.** All procedures were done according to instructions of the ethical committee of Faculty of Medicine code no (30550/10/15), Tanta University.

The animals were acclimatized for two weeks, and after acclimatization rats were randomly divided into four groups (10 rats each):-

Group I:-

Control group:-

The animals of this group were injected daily with 1ml saline i.p for 6 days.

Group II:-

Methotrexate treated group:-

The animals of this group were injected daily with 1ml saline i.p for 5 days. At the 6^{th} day, they were injected with a single dose of methotrexate (20 mg/kg) i.p ***(Çakır et al., 2011).*** Methotrexate was obtained from Techno Pharma Co. under the trade-name Methotrexate vial (50mg/2ml)

Group III:-

Taurine treated group:-

The animals of this group were injected with taurine 100 mg/kg daily i.p for 6 days ***(El-Sayed et al., 2011).*** Taurine was supplied by Sigma Company as a dry-frozen, pale white, sterilized powder. Each vial of sterilized taurine powder contains 500mg. The powder was weighed and dissolved in 5ml saline to get a solution.

Group IV:-

Tautine & Methotrexate- Treated Group:-

The animals of this group were injected i.p with taurine 100 mg/kg daily for 5 days ***(El-Sayed et al., 2011).*** At the 6^{th} day after the last dose of taurine, rats were injected with a single dose of methotrexate (20 mg/kg) i.p**.**

At the end of the experimental period the animals fasted overnight, then, all rats were anaesthetized by i.p injection of pentobarbital (50 mg/kg) ***(Samson et al., 1957)*** and blood samples were obtained by cervical dislocation. Blood samples were collected in clean test tubes, and centrifuged at 3000 rpm for 15 minutes and the separated sera were

then transferred into clean cuvette tube stored at -80^{o}C and used for measurements of the following: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were assayed by using commercial kit that was supplied by Egyptian Company for Biotechnology kit number 264 001 according to the method described by ***(Reitman and Frankel, 1957).*** Creatinine was measured according to the method described by ***(Bartles et al.,1972***) it was obtained from Biodiagnostic CO. Egypt kit number CR 1251.Urea was assayed by Modified Urease –Berthlot Method described by ***(Tiffany et al.,1972),*** it was obtained from Biodiagnostic CO. Egypt kit number UR2110. Blood urea nitrogen (BUN) was estimated by the equation described by ***(Deacon, 2009).*** Tumor necrosis factor alpha (TNFα) was assayed by Rat TNFα ELISA kit (Shanghai Sunred Biological Technology Co. Ltd, China. Catalog no 201-11-0765) according to manufacturer's protocol ***(Maskos et al., 1998).***

After animals were sacrificed part of the liver and kidney were instantly removed, washed three times in ice-cold saline and blotted on filter paper, and homogenized in 50mM potassium phosphate (pH 7.4). The homogenate was centrifuged in 7000×g for 10 min at 4°C and supernatant were stored at -80^{o}C and used for measurement of oxidative stress by determination of tissue reduced glutathione (GSH) levels which was measured using Biodiagnostic Kit No.GR 25 11(BiodiagnosticCo., Egypt) that is based on the spectrophotometric method of ***(Beutler, 1963).*** Malondialdehyde (MDA) was assayed by using Biodiagnostic Kit No MD 25 29 based on the spectrophotometric method of ***(Ohkawa et al., 1979).*** Catalase (CAT) was measured using Biodiagnostic Kit No. CA25 17 which is based on the spectrophotometric method described by ***(Aebi, 1984).*** Caspase-3 was assayed using RayBio Kit No. 68CL-Casp3-S100 which is based on a spectrophotometric method described by ***(Porter and Janicke, 1999).*** Nitrite was assayed using Biodiagnostic Kit No. NO 25 33 which is based on the spectrophotometric method described by ***(Montgomery and Dymock, 1961).***

Another part of liver and kidney was separated for detection of DNA damage by gel electrophoresis. DNA extraction kits (EZ-10 Spin Column Animal Genomic DNA Miniprep Kit) was obtained from Bio Basic Inc Co. Canda, NY, USA kit code 41105504m DNA which is based on the protocol described by ***(Kasibhatla et al., 2006).*** Another part from liver and kidney from all rats of all groups were fixed in 10% neutral buffered formalin (pH 7.2) and embedded in paraffin for histopathological examination. Paraffin-embedded tissues were sectioned into 4µm thickness slices by microtone and stained with hematoxylin-eosin (H&E) stain.

Then, the sacrificed animals were packed in a special package according to safety precautions and infection control measures.

Results:-

Results were expressed as Mean ± SD and all statistical comparisons were made by means of one-way ANOVA test, followed by Tukey's post hoc analysis, and p values less than 0.05 were considered statistically significant. The analysis was performed by statistical package for the social science software (SPSS version 22.0.).

Effect of taurine on MTX hepatotoxicty:-

AST, ALT are markers of liver dysfunction. As shown in Fig. 1, levels of AST& ALT were significantly ($p<0.05$) increased in the MTX group compared to the control group. Administration of taurine to normal rats did not change the levels of AST& ALT compared to rats in control group. In addition, pretreatment with taurine prevented MTX-induced hepatotoxicity and induced a significant ($p<0.05$) decrease in AST and ALT levels in taurine+MTX treated group when compared with MTX group (Fig. 1A and B).

Effect of taurine on MTX renal toxicity:-

Creatinine, urea, and BUN are markers of renal dysfunction. As shown in Fig. 2, levels of creatinine, urea and BUN were significantly ($p<0.05$) increased in the MTX group compared to the control group. Administration of taurine to normal rats did not change the levels of creatinine, urea, and BUN compared to rats in control group.

In addition, pretreatment with taurine significantly prevented MTX-induced renal toxicity as it caused significant decrease ($p<0.05$) in creatinine, urea, and BUN levels in taurine+MTX group compared to MTX group (Fig. 2A, B and C).

Effect of taurine on MTX induced inflammation:-

MTX induced elevation of inflammatory markers as shown in Fig. 3, levels of TNFα was significantly ($p<0.05$) increased in the MTX group compared to the control group. Administration of taurine to normal rats did not change

the levels of TNFα compared to rats in control group. In addition, pretreatment with taurine significantly decreased ($p<0.05$) TNFα in taurine+MTX group when compared with MTX group (Fig. 3).

Effect of taurine on oxidative stress induced by MTX:-

Effects of taurine on antioxidant enzymes:-

The effect of taurine on the activity of antioxidant enzymes GSH and CAT content is illustrated in Fig. 4. The GSH content and activity of CAT were significantly ($p<0.05$) decreased in the MTX group compared to the control group in both hepatic and renal tissue (Fig. 4A, B, C and D). Administration of taurine to normal rats caused significant elevation ($p<0.05$) of GSH in both hepatic and renal tissue but did not change hepatic and renal tissue CAT compared to rats in control group as shown in (Fig. 4A and B).

Pretreatment with taurine significantly increased ($p<0.05$) GSH content and CAT activity in taurine+MTX group when compared to the MTX group in both hepatic and renal tissue (Fig. 4A, B, C and D).

Effects of taurine on oxidative stress parameters:-

The effect of taurine on oxidative stress parameters such as MDA, caspase, and nitrite levels is illustrated in Fig.5.
As shown in Fig. 5A&B the concentration of MDA, an end product of lipid peroxidation, was significantly ($p<0.05$) increased in hepatic and renal tissue of the MTX group compared to the control group. Administration of taurine to normal rats didn't change tissue MDA. In contrast, pretreatment with taurine significantly ($p<0.05$) decreased hepatic and renal MDA concentration in Taurine+MTX group compared to the MTX group.

As shown in Fig. 5C&D administration of MTX significantly increased hepatic and renal tissue caspase-3 level compared to the control group ($p<0.05$). Administration of taurine to normal rats did not change tissue caspase-3 level. Pretreatment with taurine significantly decreased ($p<0.05$) hepatic and renal caspase-3 level in taurine+MTX group compared to the MTX group.

As shown in Fig. 5E&F administration of MTX significantly increased ($p<0.05$) hepatic and renal tissue nitrite concentration compared to the control group ($p<0.05$). Administration of taurine to normal rats did not change tissue nitrite level compared to control group. Pretreatment with taurine significantly decreased ($p<0.05$) hepatic and renal nitrite level in taurine+MTX compared to the MTX group.

Effect of taurine on MTX induced apoptosis:-

These results were confirmed by DNA gel electrophoresis with the typical ‘‘ladder’’ pattern of DNA fragmentation in hepatic and renal tissue in group treated with MTX. Pretreatment with taurine abrogated MTX induced DNA fragmentation while taurine alone had no effect (Fig. 6).

liver and kidney histopathology:-

The control group presented livers with a normal architecture of the liver cells (Fig. 7A). In the MTX group, liver tissue from all of rats showed portal congestion, infiltration of inflammatory cells and apoptotic bodies (Fig. 7 B& C). In addition, administration of taurine did not cause any detectable alteration in the liver structure (Fig 7D). In the taurine+MTX treated group (Fig. 7E), the histopathological lesions were effectively attenuated.

As regard to the kidney, control rats also presented kidney with normal renal architecture as regard to renal tubules and glomeruli (Fig. 8A). Renal tissue from all of the MTX treated rats showed degeneration of renal tubules and inflammatory cellular infiltration (Fig. 8B). In addition, administration of taurine did not cause any detectable alteration in the renal structure (Fig 8C). In the taurine+MTX treated group (Fig. 8D), the histopathological lesions were effectively attenuated.

Discussion:-

The results of the present study revealed that MTX induced hepatorenal toxicity as demonstrated by elevation of serum AST, ALT, creatinine, urea and BUN. It caused elevation of serum TNFα. Also, MTX treatment caused oxidative tissue damage, as assessed by increased lipid peroxidation (MDA), caspase, and nitrite and decreased GSH, CAT levels in the liver and kidney. These results were confirmed by DNA fragmentation as well as by histopathological findings.

Hepatorenal toxic effect was also reported by ***Çakır et al., (2011) and David et al., (2016),*** as it was found that there was elevation of AST, ALT, ALP and total protein and elevation of creatinine, urea, reduction in the activity of CAT and GSH and elevation of MDA in hepatorenal tissue, in rats receiving MTX when compared with control ones.

Also, ***El-Sheikh et al., (2015)*** observed deterioration in hepatorenal function by MTX, and proved the apoptotic effect of MTX by upregulation of caspase-3 in liver and kidney, as in our results.

The histopathological findings of the present study are in agreement with ***El-Sheikh et al., (2015) and David et al., (2016)*** as they reported that MTX causes congested portal vein, inflammatory cellular infiltration, and cell apoptosis in liver. It also causes degeneration of renal tubules and inflammatory cellular infiltration in the kidney.

The elevation of enzyme activities (AST&ALT) could be attributed to the damaged structural integrity of the liver (possibly by oxidative stress and lipid peroxidation), that leads to leakage of these cytoplasmic enzymes into the blood ***(Sakeran et al., 2014 and Mehrzadi et al., 2018).***

The hepatotoxic effect of MTX was explained by ***Bath et al., (2014),*** who stated that MTX causes activation of stellate cells which are vitamin A-storing lipocytes found in the perisinusoidal areas, when activated by chronic liver injury they form into myelofibroblasts, which secrete collagen and other matrix protein such as fibronectin which causes liver fibrosis and cirrhosis.

The nephrotoxic effect of MTX is caused by crystal nephropathy as MTX is mainly excreted by the kidney; toxicity is evoked by precipitation of MTX or its metabolite 7-hydroxy methotrexate in the renal tubules as they are less soluble in acidic medium. This nephrotoxicity contributes to delayed methotrexate elimination, which further increases the toxicity ***(El-Sheikh et al., 2015 and Ulusoy et al., 2016).***

Ahmed et al., (2015) also clarified the nephrotoxic impact of MTX by coordinate direct harmful impact of MTX on renal tubules and ROS generation in the kidney, with consequent cell damage.

With respect to inflammatory effect of MTX it was discovered that nuclear factor Kappa (NF-κB) assumes part in the pathophysiology of MTX-induced toxicity as reported by ***Abo-Haded et al., (2017)*** who observed that MTX induced marked activation of NF-κB pathway which is transcriptional factor that translocates into the nucleus and binds to DNA and up-regulates the transcription of many inflammatory genes.

In agreement with our results, ***Çakır et al., (2011) and Abo-Haded et al., (2017)*** suggested that the toxic effect of MTX may be due to production of oxygen free radicals which mediate oxidative reactions. These highly reactive species react with biological macromolecules producing lipid peroxides, inactivating proteins and mutating DNA.

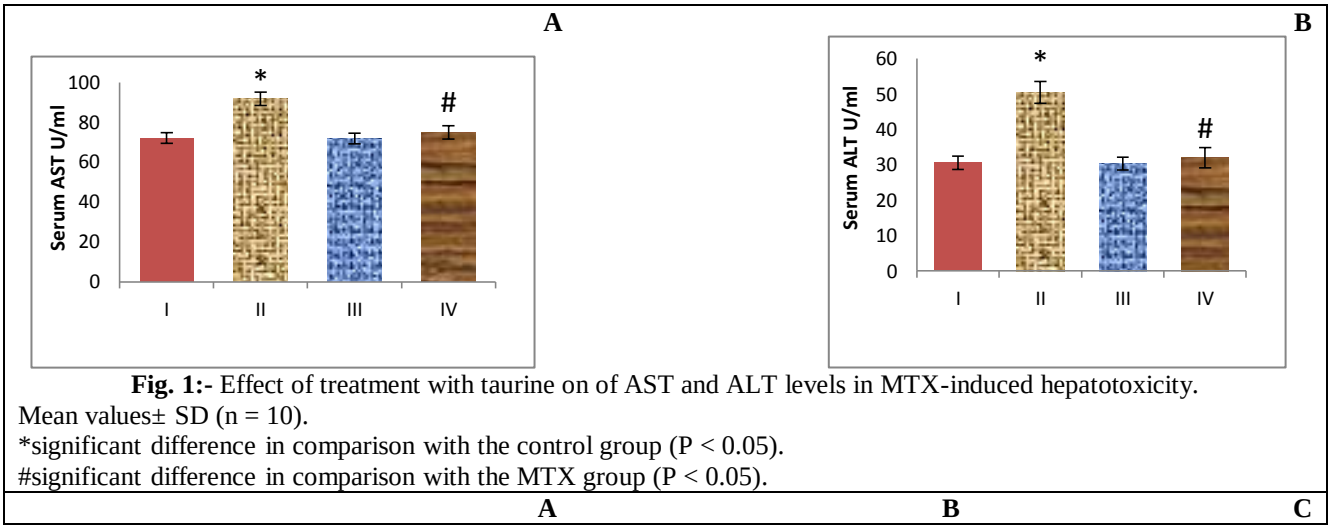


Fig. 1:- Effect of treatment with taurine on of AST and ALT levels in MTX-induced hepatotoxicity.
Mean values± SD (n = 10).
*significant difference in comparison with the control group (P < 0.05).
#significant difference in comparison with the MTX group (P < 0.05).

A B C

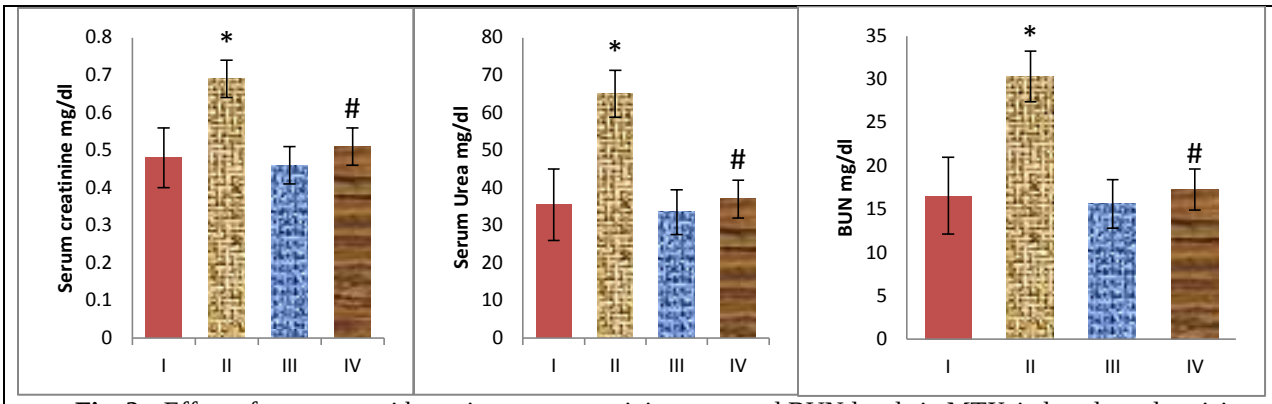


Fig. 2;- Effect of treatment with taurine on on creatinine, urea and BUN levels in MTX-induced renal toxicity.
Mean values ± SD (n = 10).
*significant difference in comparison with the control group (P<0.05).
#significant difference in comparison with the MTX group (P<0.05).

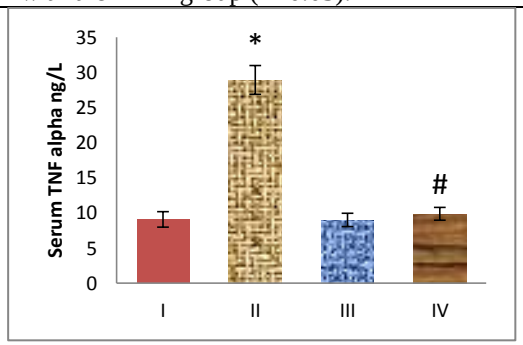
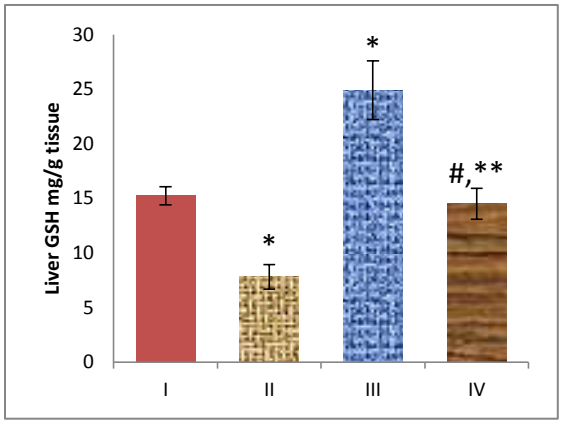


Fig. 3:- Effect of treatment with taurine on serum TNFα levels in MTX-induced hepatorenal toxicity.
Mean values ± SD (n = 10).
*significant difference in comparison with the control group (P<0.05).
#significant difference in comparison with the MTX group (P<0.05).

A



B

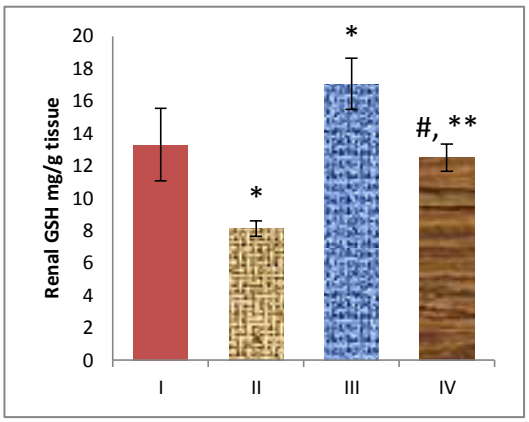
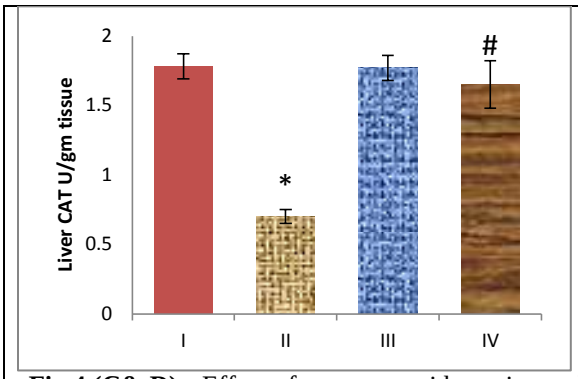


Fig.4 (A& B):- Effect of treatment with taurine on hepatic and renal GSH levels in normal rats and in MTX-induced hepatorenal toxicity.
Mean values ± SD (n = 10).
*significant difference in comparison with the control group (P<0.05).
#significant difference in comparison with the MTX group (P<0.05).
** significant difference in comparison with the taurine group (P<0.05).

C **D**



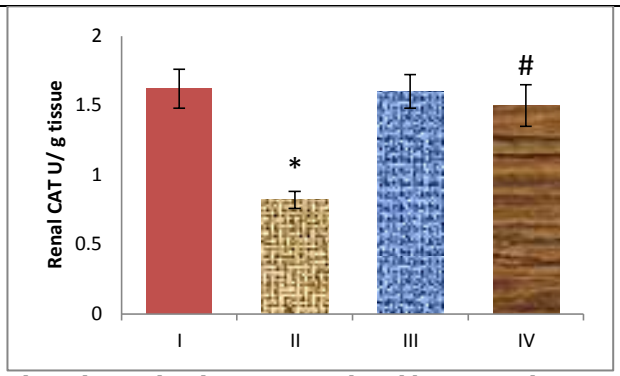
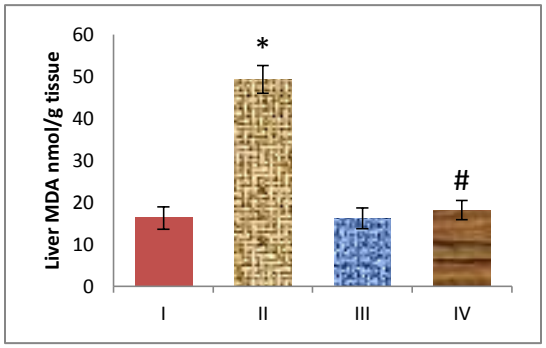


Fig.4 (C& D):- Effect of treatment with taurine on hepatic and renal CAT levels in MTX-induced hepatorenal toxicity. Mean values ± SD (n = 10).
*significant difference in comparison with the control group (P<0.05).
#significant difference in comparison with the MTX group (P<0.05).

A



B

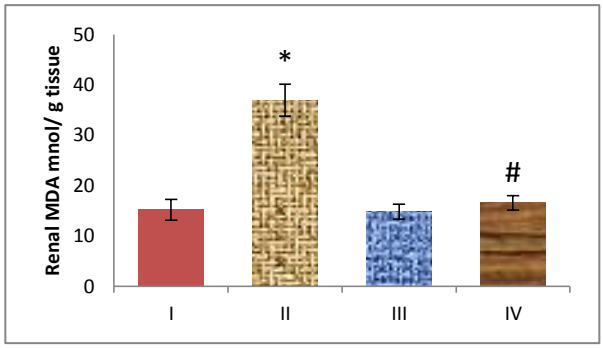
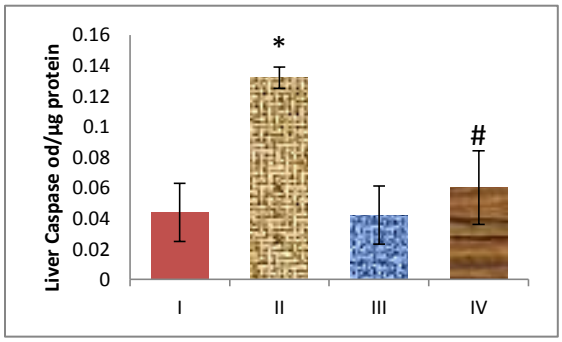


Fig.5 (A& B):- Effect of treatment with taurine on hepatic and renal MDA levels in MTX-induced hepatorenal toxicity. Mean values ± SD (n = 10).
*significant difference in comparison with the control group (P<0.05).
#significant difference in comparison with the MTX group (P<0.05).

C



D

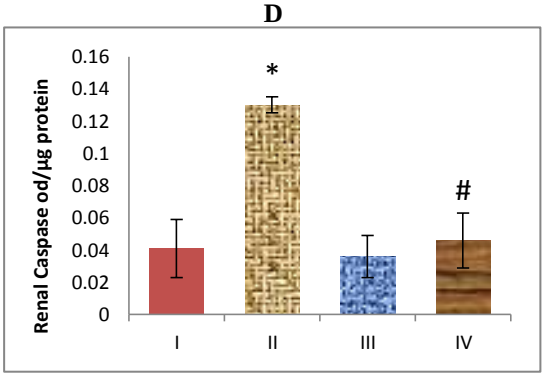
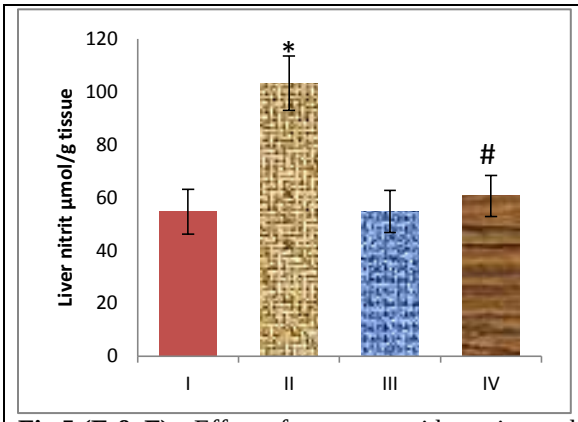


Fig.5 (C & D):- Effect of treatment with taurine on hepatic and renal caspase-3 levels in MTX-induced hepatorenal toxicity.
Mean values ± SD (n = 10).
*significant difference in comparison with the control group (P<0.05).
#significant difference in comparison with the MTX group (P<0.05).

E F



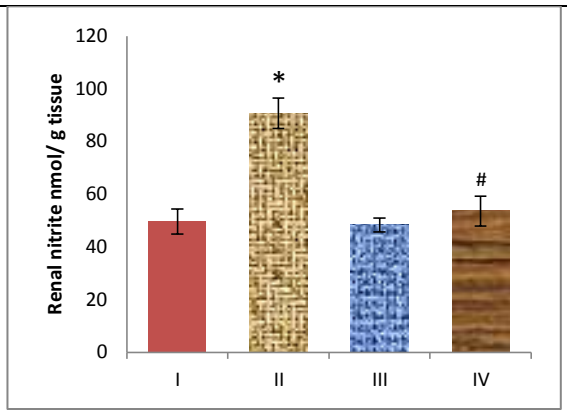


Fig.5 (E & F):- Effect of treatment with taurine on hepatic and renal nitrite levels in MTX-induced hepatorenal toxicity. Mean values ± SD (n = 10).
*significant difference in comparison with the control group (P<0.05).
#significant difference in comparison with the MTX group (P<0.05)

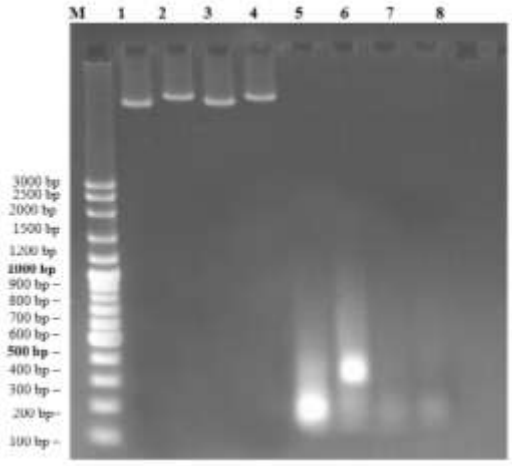
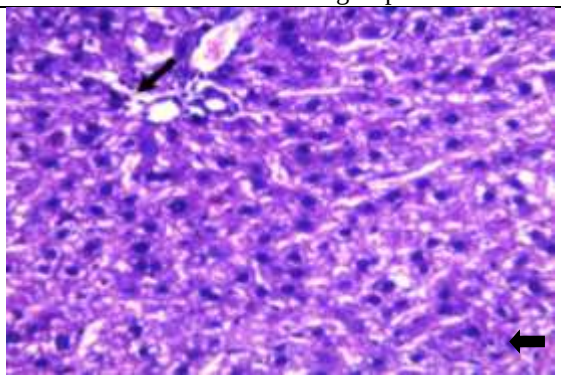


Fig (6):- Lane (M): represents (100-3000 bp DNA ladder)**. Lane (1):** represents DNA extracted from the hepatic tissue of control group and shows no apoptotic fragmentation**. Lane (2):** represents DNA extracted from the renal tissue of control group and shows no apoptotic fragmentation. **Lane (3):** represents DNA extracted from the hepatic tissue of taurine-treated group and shows no apoptotic fragmentation. **Lane (4):** represents DNA extracted from the renal tissue of taurine-treated group and shows no apoptotic fragmentation. **Lane (5):** represents extracted DNA from the hepatic tissue of methotrexate-treated group and shows apoptotic DNA fragmentation at approximately (200 bp) **Lane (6):** represents extracted DNA from the renal tissue of methotrexate-treated group and shows apoptotic DNA fragmentation at approximately (400 bp) **Lane (7):** represents DNA extracted from the hepatic tissue of taurine+methotrexate treated group and shows a reduction of DNA apoptotic fragmentation. **Lane (8):** represents DNA extracted from the renal tissue of taurine+methotrexate treated group and shows a reduction of DNA apoptotic fragmentation.



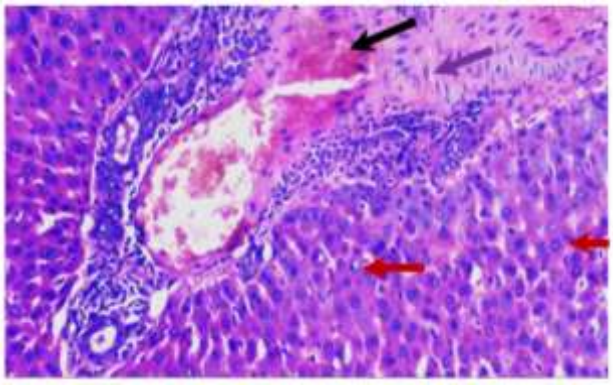


Fig 7A:- (group I) Photomicrograph of a section

from liver tissue showed normal liver tissue with a normal central vein, blood sinusoids and hepatocytes ( ) (H & E× 100).

Fig.7B:- (group II): Photomicrograph of a section from liver tissue showed congested portal vein ( ), inflammatory cellular infiltration ( ) and apoptotic bodies ( ) (H & E × 100).

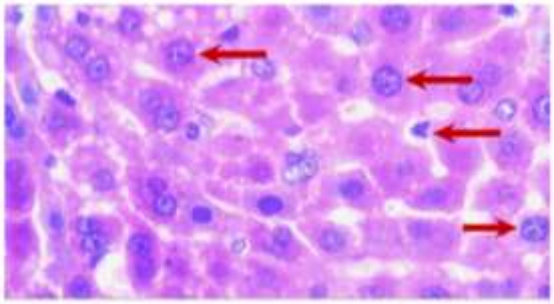


Fig. 7C: (group II):- Photomicrograph of a section from liver tissue showed apoptotic bodies ( ) (H & E × 200).

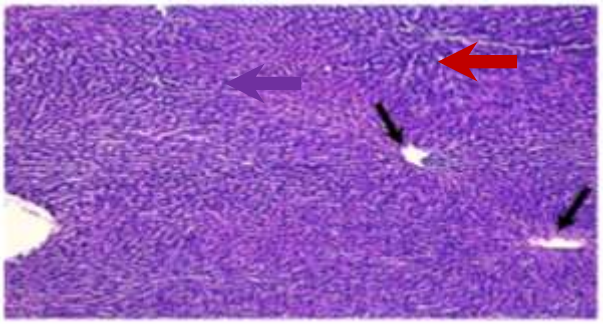


Fig. 7D:- (group III): Photomicrograph of a section from liver tissue showed normal liver tissue as control ( ) (H & E × 100).

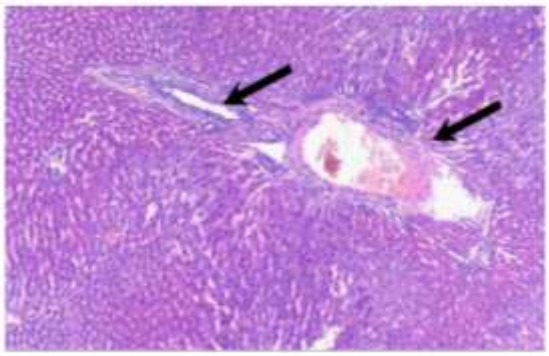


Fig. 7E:- (group IV): Photomicrograph of a section from liver tissue showed Normal hepatocytes with less congested portal vein and little inflammatory cellular infiltration ( ) (H & E × 100).

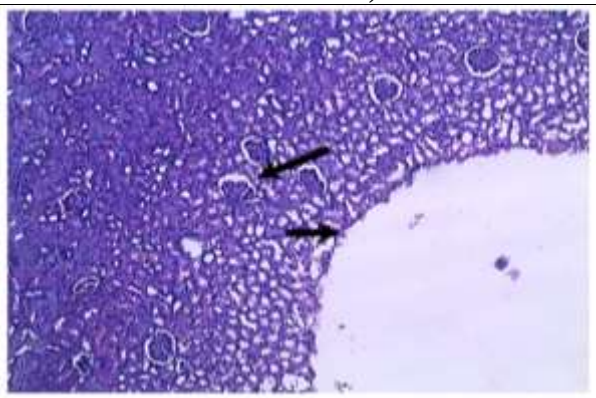


Fig.8A: (group I) Photomicrograph of a section from renal tissue showed normal glomeruli, tubules and interstitium ( ) (H & E × 100).

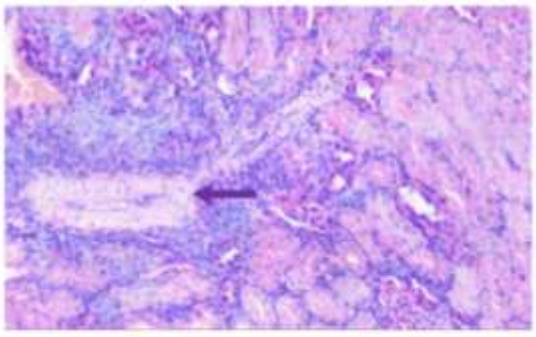


Fig. 8B:- Photomicrograph of a section from renal tissue showed tubular degeneration with inflammatory cellular infiltration ( ) (H & E × 200).

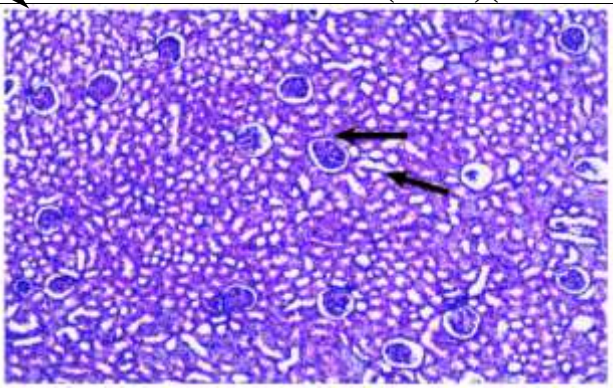


Fig. 8C (group III): Photomicrograph of section from renal tissue showed normal renal glomeruli, tubules and interstitium as control ( ) (H & E × 100).

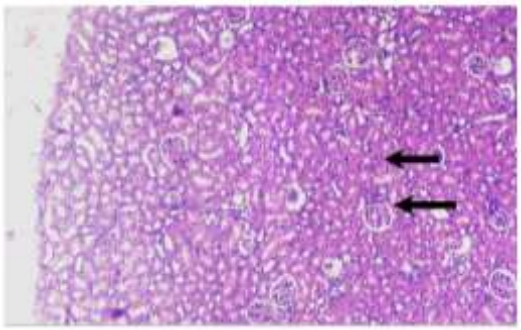


Fig.8D:- (group IV): Photomicrograph of section from renal tissue showed normal glomeruli, tubules and little inflammatory cellular infiltration (⬅) (H & E × 100).

Abo-Haded et al., (2017) demonstrated that MTX induced a decrease in mRNA of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and Nrf2 binding capacity which is a transcriptional activator that can serve as a sensor for oxidative stress. Nrf2 regulates activation of defensive genes and induces antioxidant enzymes as CAT, SOD, and GPx leading to suppression of injury triggered by ROS.

The significant decrease of tissue GSH in MTX treated group was clarified by ***Jahovic et al., (2003)*** who stated that MTX causes exaggerated inhibition of glucose-6-phosphate dehydrogenase (G6PD) and this, in turn, results in a decrease of NADPH availability with subsequent inhibition of glutathione reductase activity and finally an inhibition of GSH cycle.

The significant increase in tissue MDA level in MTX treated indicates lipid peroxidation mediated by oxygen free radicals which target cell lipids, especially those within membrane bilayers, containing a large quantity of unsaturated fatty acids, nucleic acids and proteins causing its damage ***(Çakır et al., 2011 and El-Sheikh et al., 2015).***

As a respect to nitrosative stress induced by MTX, there was a significant increase of tissue nitrite level in MTX group; this could be explained by up-regulation of iNOS expression caused by MTX as reported by ***(Leitão et al., 2011 and El-Sheikh et al., 2015).***

The apoptotic effect of MTX was explained by ***Czarnecka-Operacz and Sadowska-Przytocka, (2014)*** who stated that MTX is folic acid antagonist which is retained within the cell as a polyglutamate, it binds with an affinity greater than that of folic acid to dihydrofolate reductase and inhibits it. MTX also inhibits AICAR (5-aminoimidazole-4- carboxamide ribonucleotide) transformylase and thymidylate synthase. Inhibition of these enzymes limits the conversion of folic acid to tetrahydrofolate which is essential for DNA synthesis. Inhibition of synthesis of purine and pyrimidine thymidine by MTX results in improper DNA synthesis and subsequent apoptosis. In addition, MTX affects MTHFR (methylenetetrahydrofolat ereductase) and hence the generation of methionine from homocysteine. Excess homocysteine can generate oxidative stress and increases cell sensitivity to the cytotoxic effect of ROS. Homocysteine may activate proinflammatory cytokines ***(Pandit et al, 2012).***

So, several mechanisms could be attributed to the hepatorenal toxic effect of MTX; cell inflammation, neutrophil migration and increased cytokine concentration that may lead to hepatorenal cell apoptosis and necrosis and release of toxic agents that induce cellular damage. Also, increase in tissue NO and MDA together with decrease GSH levels indicates an imbalance of oxidant and antioxidant systems.

However, the results of the present work showed significant improvement of hepatic and renal function in rats injected with taurine. This improvement was evidenced by the significant reduction in serum level of AST, ALT, creatinine, urea, BUN and TNFα, tissue level of MDA, caspase, nitrite and significant increase of tissue level of GSH and CAT in taurine+MTX treated group when compared with MTX group. There was also a significant reduction in DNA fragmentation in the hepatic and renal tissue of rats in taurine+MTX treated group.

These results were confirmed by histopathological findings of hepatorenal tissue in taurine+MTX group.

These results are in agreement with ***El Kader et al., (2015)*** who investigated the protective role of taurine, against oxidative stress induced by gamma irradiation and found that taurine, significantly improved radiation-induced injury in hepatic, cardiac, and renal tissues.

Moreover, ***El-Sayed et al., (2011)*** studied the protective effectt of taurine against aluminum-induced hepatotoxicity. Also, ***Zhang et al., (2014)*** studied whether taurine could reduce the hepatotoxicity of iron overload. They found that taurine can reduce hepatic oxidative stress, preserve liver function and inhibit hepatocyte apoptosis.

In addition, ***Demircioglu et al., (2011)*** reported that taurine has renoprotective effect as pretreatment with taurine can prevent oxidative changes in renal tissue caused by ischemia reperfusion.

In agreement with histopathological finding, ***Al-Asmari et al., (2016)*** studied the ameliorative effect of taurine on hepatorenal damage induced by 5 fluorouracil and observed that infiltration of inflammatory cells, necrosis, and renal tubular degeneration were attenuated by taurine.

Cysteine is the common precursor of taurine and GSH biosynthesis, so treatment with taurine might increase the GSH levels as a result of directing more amount of cysteine into GSH biosynthesis, so this mechanism explains how taurine increases antioxidant cellular capacity ***(El Kader et al., 2015)***.

Ahmad et al., (2015) stated that taurine enhances the synthesis of GSH and as it stimulates the activity of G6PD, an enzyme that generates NADPH which is required by glutathione reductase to convert oxidized glutathione into GSH.

Another mechanism of antioxidant effect of taurine was explained by ***El-Sayed et al., (2011)*** who suggested that taurine stimulate the nitrosylation of GSH into nitrosoglutathionen which is much potent antioxidant than GSH itself.

Taurine has the ability to conjugate with MDA, the end product of lipid peroxidation, so it stabilizes the lipid bilayer and decreases the vulnerability of the membranes lipids to toxic insult induced by ROS ***(Ahmad et al., 2015)***.

The reduction in tissue nitrite level was explained by ***Kim & Cha, (2014)*** who demonstrated that Tau-Cl inhibits production of NO through depressing iNOS evidenced by decreased expression of iNOS mRNAs upon taurine administration.

Issabeagloo et al., (2011) & Devi et al., (2016) stated that taurine removes HOCL by reacting with it to form taurinecholoramine (TauCl) and prevents the direct attack of this oxidant on cell membranes.

The anti-inflammatory effect of taurine was due to the formation of TauCl which decreases translocation of NFκB into the nucleus. Transcription of iNOS and TNFα genes is critically dependent on the NFκB transcription factor signaling pathway ***(Kim et al., 2015)***.

Mcleay et al., (2017) explained the antioxidant effect of taurine due to the presence of sulphur side chain group which can accept the unpaired electron of ROS.

Ahmad et al., (2015) stated that taurine itself does not directly quench classical ROS and free radicals but its metabolic precursor, hypotaurine, has been shown as an efficient radical scavenger.

The antiapoptotic effect of taurine may be due to the reduction in ROS which in turn prevents opening of mitochondrial permeability transition pore (mPTPs) and subsequent mitochondrial swelling ***(Zhang et al., 2014)***.

Taurine ensures an efficient flux of reducing equivalents through the respiratory chain, thereby preventing the diversion of electrons to the acceptor oxygen; so it inhibits the generation of superoxide anion ***(Jong et al., 2012)***.

Taurine has antiapoptotic effect as it reduces caspase-3 activation and subsequent DNA fragmentation. This could be due to the ability of taurine to inhibit oxidative stress, which in turn reduces pro-apoptotic pathway activation

(Bax, Bcl-XS) and prevents the loss of the anti-apoptotic pathway (Bcl-2, Bcl-XL) and prevents subsequent caspase activation ***(Depboylu et al., 2008).***

Taurine antioxidant activity suppresses extrinsic apoptotic pathway by decreasing in the gene expression of Fas receptors (FasR, apoptosis antigen 1or tumor necrosis factor receptor superfamily member) and subsequent caspase activation ***(Nagai et al., 2016).***

Taurine also suppresses intrinsic apoptotic pathway by stabilizing mitochondrial membrane and elevating level of GSH since high levels of cytoplasmic GSH maintain cytochrome c in a reduced or inactive state. This prevents DNA damage and reduces apoptosis ***(Devi &Anuradha, 2010)***.

Ahmed et al., (2015) demonstrated that excess Ca^{2+} during oxidative stress conditions cause opening of mPTPs. Taurine prevents Ca^{2+} overload via Na/Ca exchanger so, it is cytoprotective that protects cells from injury and subsequent necrosis.

So, taurine could improve the hepatic and renal functions by its antioxidant effect which increase the CAT activity and GSH level which are powerful antioxidant, Consistent with the antioxidant properties, taurine could reduce lipid peroxidation and MDA level, it also has anti-inflammatory effect by reducing TNFα and it has anti-apoptotic effect through reduction of tissue caspase and DNA fragmentation.

Conclusion:-

We conclude that taurine has ability to reduce MTX-induced hepatorenal oxidative injury through its anti-inflammatory and antioxidant and antiapoptotic effects, which were evaluated both biochemically and histologically.

Recommendation:-

Thus, our data suggest that taurine may be used therapeutically in patients receiving other toxic chemotherapeutic agents to prevent hepatic and renal toxicity. Significant improvement of anti-cancer drugs side effects can increase the tolerance of these drugs and increase the therapeutic effect of these drugs for oncology or rheumatology patients.

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