ROLE OF TRIMETAZIDINE IN PREVENTING ACETAMINOPHEN-INDUCED HEPATIC INJURY IN MICE

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ABSTRACT

Acetaminophen is one of the most common pharmaceuticals associated with both intentional and accidental poisoning. Acetaminophen-induced hepatotoxicity is the most frequent cause of fulminant liver failure, and can have a mortality rate 90%. The present work aimed at the investigation of the possible effect of trimetazidine in preventing acetaminophen-induced hepatotoxicity and comparing it with the traditionally used N- acetyl cysteine for the same purpose.

The present study was carried out on 40 mice. Mice were divided into 2 main groups. Group I: consisted of 10 animals considered as control group and received saline. Group II: consisted of 30 animals which were subdivided into 3 equal subgroups (10 mice/

subgroup) as follows : subgroup (IIA): served as acetaminophen only treated group, in a dose of 500 mg/kg intragastrically. Subgroup (IIB): acetaminophen treated mice treated with N-acetyl cysteine in a dose of 200mg/kg intragastrically one hour before administration of acetaminophen. Subgroup (IIC): acetaminophen treated mice treated with trimetazidine in a dose of 20 mg/kg intragastrically one hour before administration of acetaminophen. Liver cell integrity was monitored by measurement of serum glutamate pyruvate transaminase (SGPT). Glutathione (GSH) in blood and malondialdehyde (MDA) in serum were assessed. Liver cell integrity was also confirmed by histopathological examination for assessing the distribution and extent of cell death. Mice treated with acetaminophen only

showed a significant increase in SGPT & MDA; and a significant reduction in GSH. Histopathologically, acetaminophen produce many grades of necro inflammatory lesions.

Pre-administration of N-acetyl-Cystein or trimetazidine before acetaminophen treatment produced a significant reduction in SGPT and MDA, furthermore, it produced a significant increase in GSH level and also protected the liver against acetaminophen-induced injury.

In conclusion, the present work demonstrated that trimetazidine ameliorates the acetaminophen induced lesion to a similar extent by N-acetyl-Cysteine.

INTRODUCTION

Acetaminophen (paracetamol) is the most widely used analgesic and anti-pyretic agent in the world; it is contained in more than 100 products. As such, acetaminophen is one of the most common pharmaceuticals associated with both intentional and accidental poisoning. It is also a component of many over the counter medications.

It has an excellent safety profile in

therapeutic doses, but hepatotoxicity can develop with over dose or in persons with enhanced susceptibility (1,2). Acetaminophen-induced hepatotoxicity as the result of either deliberate over dose or accidental overdose with chronic alcohol abuse is the most frequent cause of fulminant liver failure in the united kingdom⁽⁶⁾ and the united states ⁽⁴⁾ and can have a mortality rate 90% ^(7,8). However, there are few reports of accidental over dose due to recurrent ingestion of high therapeutic doses in children. ^(9,3,4,10,11).

Acetaminophen in therapeutic doses is rapidly metabolized in the liver principally through glucuronidation and sulfation and only a small portion is oxidized by cytochrome P-450 2E, to generate a highly reactive and cytotoxic intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) ^(12,13,14,15), which is quickly conjugated by hepatic glutathione to yield a harmless water soluble product mercapturic acid. However, after acetaminophen overdose the capacity for glucuronidation and sulfation is exceeded and a large amount of NAPQI is formed and after glutathione is depleted, NAPQI binds covalently to hepatic parenchymal cell proteins

and DNA with resultant liver injury (13,15).

because of its ability to act as a precursor of intracellular GSH. (24).

However metabolic activation of acetaminophen and NAPQI binding to target proteins and DNA appear to be necessary but not sufficient for hepatotoxicity (16,17). Evidences indicate that the generation of reactive oxygen species (18), and nitric oxide (19), lipid peroxidation (20), mitochondrial dysfunction (21), disruption of calcium homeostasis (22) and induction of apoptosis (23), are all mechanisms that may be involved in acetaminophen-induced hepatotoxicity.

N-acetylcysteine (NAC), the acetylated variant of the amino acid L-cysteine, is an excellent source of sulfhydryl (SH) groups, and is converted in the body into metabolites capable of stimulating glutathione (GSH) synthesis, promoting detoxification, and acting as free radical scavengers.

Administration of NAC has historically been as a mucolytic agent in a variety of respiratory illnesses; however, it also appears to also have beneficial effects in conditions characterized by decreased GSH or oxidative stress.

NAC also appears to work as an antidote for acetaminophen overdose

Trimetazidine, 1-(2,3,4-trimethoxybenzyl) piperazine, dihydrochloride) a cytoprotective agent, possesses many cytoprotective properties through its action as antioxidant (by its beneficial effect on generation of reactive oxygen species (25,26), its action on lipid peroxidation (26) and also, its effect as intracellular calcium regulator (28) and its protective effect on mitochondrial function (28,29).

In a previous study we demonstrated that trimetazidine has a hepatoprotective activity against ischaemia-reperfusion insult through its cytoprotective effect on mitochondrial dysfunction, on generation of reactive oxygen species and lipid peroxidation, and on intracellular calcium homeostasis (30). These findings led us to investigate the hypothesis that trimetazidine may exert a beneficial effect in preventing acetaminophen induced hepatotoxicity through its cytoprotective properties.

Thus, the aim of this work is to investigate the beneficial effect of trimetazidine in preventing acetaminophen-induced hepatotoxicity and

compare it with the traditionally used N-acetyl cysteine.

MATERIALS AND METHODS

Drugs used :

- 1- Acetaminophen : paracetamol tablets, 500 mg, supplied by pharcom pharmaceuticals company.
- 2- N-acetyl cysteine: Acetylcysteine packs 200 mg, supplied by sedico pharmaceutical company.
- 3- Trimetazidine : vastarel tablets, 20mg, supplied by servier Egypt industries limited company.

Animals used :

Acetaminophen induced liver necrosis has been well documented in mice (31), as they are more susceptible to acetaminophen toxicity (32).

Thus, adult mice were used throughout this study. Forty mice were used; weighing 40 gm each and obtained from animal house. They were housed in plastic cages at 25-27°C.

Experimental protocol :

- 1- Acetaminophen was dissolved in pathogen free normal saline to make a concentration of 25 mg/ml. It was administered orally by a gastric tube in a single hepatotoxic

dose of 500 mg/kg. (33)

- 2- N-acetyl cysteine(NAC): was dissolved in pathogen free normal saline to make a concentration of 10 mg/ml. It was administered orally by a gastric tube in a single dose of 200 mg/kg 1h before administration of acetaminophen. (34,35)
- 3- Trimetazidine (TMZ) was dissolved in pathogen free normal saline to make a concentration of 1mg/ml. It was administered orally by a gastric tube in a single dose of 20mg/kg 1h before administration of acetaminophen.(36)

Treatment :

Forty mice were randomly divided into 2 main groups:

Group I : consisted of 10 animals which were considered as control group and received saline.

Group II : consisted of 30 animals. It was subdivided into 3 equal sub groups (10 mice / sub group) as follows :

Sub group II A : served as acetaminophen only treated group; in a single dose of 500 mg/kg intragastrically. **Subgroup II B :** acetaminophen treated mice,

treated with N-acetyl cysteine in a dose of 200 mg/kg intragastrically 1 hour before administration of acetaminophen.

Subgroup II C : acetaminophen treated mice, treated with trimetazidine in a dose of 20 mg / kg intragastrically 1 hour before administration of acetaminophen. 2 hours after acetaminophen administration, animals were killed, blood & livers were obtained for biochemical analysis and histopathological examination respectively.

A) Biochemical analysis :

- 1- Serum glutamic pyruvate transaminase(SGPT) was determined in serum using kits from biotic laboratories. Results expressed as international units per litre (IU/L)(37)
- 2- Glutathion(GSH) concentration was determined in blood using the method of Beutler,(38)
- 3- Malondialdehyde (MDA) concentration was determined in blood using draper & Haddy, (39) method

B) Histopathological examination :

The livers were immediately fixed

in 10% neutral formalin, prepared as paraffin block, cut into 4-5 μ thick sections and stained with haematoxylin & Easin. Then, submitted for histopathological examination, and at least 2 sections from each liver were examined.

Paying special attention for necrosis and inflammatory cellular infiltration (necro inflammatory lesion). The distribution and the extent of both necrosis and associated intra lobular inflammatory cellular infiltration were assessed using the classification of Horn et al 40. Necrosis was graded on a semi quantitative scale: 0, normal; 1, rare foci of necrotic cells in centrilobular zones (no more than 1-2 sites per section); 2, few necrotic foci (less than half of centrilobular zones had sites of necrosis), 3, many/diffuse centrilobular zones with necrosis; 4, diffuse centrilobular to midzonal necrosis; and 5, diffuse submassive to massive necrosis (most or almost all of lobule was necrotic).

Intralobular cellular infiltration was graded on a semi quantitative scale 0, no inflammatory cells; 1, rare inflammatory cells (no more than 1-3 cells in 1 or 2 centrilobular zones per section); 2, few inflammatory cells (1-

5 cells in less than 50% of centrilobular zones); 3 moderate inflammatory cells infiltration (5-15 cells in most centrilobular zones); 4, marked inflammatory cell infiltration (greater than 15 cells in most centrilobular to mid-zonal areas) and 5, severe inflammatory cell infiltration (too numerous to count and infiltrating most of the lobule).

Inflammatory cells in portal tracts were assessed semiquantitative as mild, moderate and severe.

STATISTICAL ANALYSIS

- 1- Analysis was done by SPSS program (statistical package for social science) version 10, 1999.
- 2- Multiple comparisons were performed by ANOVA statistical analysis between 2 groups was performed by students independent t test.
- 3- Correlation analysis was done to see if the value of 2 variables are associated or changed correspondingly.

RESULTS

A) Biochemical findings :

- 1- Effect of acetaminophen on biochemical parameter (group IIa): As shown in table (1) & fig (1), it pro-

duced a significant increase in SGPT (Iu/L) (a seven fold increase , $P < 0.05$). Furthermore, it produced a significant increase in MDA (nmol/ml/h) (86.6 ± 1.4 , $P < 0.05$). It also produced a marked reduction in GSH (nmol/mg) a 50 % decrease (33.65 ± 0.15 $P < 0.01$).

- 2- Effect of N- acetyl cystein on acetaminophen induced biochemical changes (group II b) :As shown in table (2,3) & Fig (2,3) NAC pretreatment produced a significant reduction in SGPT (IU/L) (21.7 ± 0.42 $P < 0.05$) as compared to acetaminophen group 91.2 ± 0.55 $P < 0.05$). It also produced a significant decrease in MDA (nmol/ml/h) 27 ± 0.40 , $P < 0.05$) versus acetaminophen only group (group IIa). Moreover NAC produced a highly significant increase in GSH level (nmol/mg) (77.2 ± 0.32 $P < 0.01$) versus acetaminophen only treated group (group IIa).

- 3- Effect of trimetazidine on acetaminophen induced biochemical changes:

Table (2,3) & Fig (2,3) show that TMZ pretreatment produced a significant reduction in SGPT (Iu/L) versus acetaminophen alone. Group IIa and Group IIc ($21.7 \pm$,

0.42 $p < 0.05$) (Fig 1). It also produced a significant decrease in MDA (nmol/ml/h) versus acetaminophen alone (group II a) 28.5 ± 0.48 $p < 0.05$) Fig (2).

4- Comparison between the effect of trimetazidine and N-acetyl cysteine on biochemical changes induced by acetaminophen:

as shown in table (3) & fig (3). There was a non significant difference between trimetazidine and N-acetyl cysteine as regard their effect on SGPT & MDA. However, there was a significant difference as regard their effect on GSH.

B) Histopathological findings :

In comparison to control group, acetaminophen produced many grade of lobular necro inflammatory lesions in liver of all mice. These lesions were ranged from grade 2-3-4. table(4.5) fig. (4,5,6). Also, portal tracts showed severe inflammatory cellular infiltration fig(7).

Pre treatment with either N-acetyl cysteine or trimetazidine effectively decreased the hepatotoxic effect of acetaminophen. The necro inflammatory lesion was detected in less number of mice and they were present in lower grades (0-2) than in acetaminophen only treated group, (table 4,5&fig.8,9,10)

Table (1) : Effect of acetaminophen (500 mg/kg orally) on serum Glutamate pyruvate transferase (GPT) level (Iu/L), serum Malondialdehyde (MDA) level (nmol/m/h) and reduced glutathione (GSH) nmol/mg protein. means $\pm$ SE.

Groups Parameter	Control group	Acetaminophine treated group
SGPT	12.3 $\pm$ 0.47	91.2 $\pm$ 0.55 $P < 0.05$ (0.01)
MDA	18.9 $\pm$ 0.38	86.6 $\pm$ 1.4 $P < 0.05$ (0.01)
GSH	67.4 $\pm$ 0.31	33.65 $\pm$ 0.15 $P < 0.01$

P = significance of difference between acetaminophen treated group versus control group (non treated group).

Table (2): Effect of trimetazidine (20mg/kg orally), N-acetylcysteine (200/kg orally) on serum Glutamate pyruvate transferase (GPT) enzyme level (Iu/L), serum Malondialdehyde (MDA) level (nmol/ml/h) and reduced glutathione (GSH) (nmol/mg mice. (means $\pm$ SE)

	Control group (group I)	Acetaminophen treated mice (group II)		
		Saline treated group (group IIa)	Trimetazidine treated (group IIb)	N.Acetyl cystein treated group (gpIIc)
SGPT	12.3 $\pm$ 0.47	91.2 $\pm$ 0.55 $P_1 < 0.05$ (0.01)	21.7 $\pm$ 0.42 $P_2 < 0.05$	21.7 $\pm$ 0.42 $P_3 < 0.05$
MDA	18.9 $\pm$ 0.38	86.6 $\pm$ 1.4 $P_1 < 0.05$ (0.01)	28.5 $\pm$ 0.48 $P_2 < 0.05$	27 $\pm$ 0.40 $P_3 < 0.05$
GSH	67.4 $\pm$ 0.31	33.65 $\pm$ 0.15 $P_1 < 0.01$	72.2 $\pm$ 0.35 $P_2 < 0.01$	71.2 $\pm$ 0.32 $P_3 < 0.001$

P_1 = Significant of difference between acetaminophine only treated mice versus non treated mice.

P_2 = significance of difference between trimetazidine treated mice versus acetaminophen only treated mice.

P_3 = significance of difference between N-acetyl cystein treated mice versus acetaminophen

Table (3): Comparison between the effect of trimetazidine (20mg/kg orally) and N-acetyl cystein (200 mg/kg orally) on biochemical changes induced by acetaminophen (500 mg/kg orally). Means $\pm$ SE.

parameters	Acetaminophen treated group (group II)		
	Saline treated group (group IIa)	Trimetazidine treated (group IIb)	N.Acetyl cystein treated group (gplIc)
serum Glutamate pyruvate transferase (SGPT)	91.2 $\pm$ 0.55	21.7 $\pm$ 0.42 $P_1 < 0.05$	21.7 $\pm$ 0.42 $P_2 < 0.05$ $P_3 > 0.05$
serum Malondialdehyde (MDA)	86.6 $\pm$ 1.4	28.5 $\pm$ 0.48 $p < 0.05 (0.03)$	27 $\pm$ 0.40 $P_2 < 0.05$ $P_3 > 0.05$
reduced glutathione (GSH)	33.65 $\pm$ 0.15	72.2 $\pm$ 0.35 $P < 0.01$	71.2 $\pm$ 0.32 $P_2 < 0.01$ $P_3 < 0.05$

P_1 = significance of difference of trimetazidine treated mice versus non treated (acetaminophen) mice.

P_2 = Significance of difference of Nacetyl cystein treated mice versus non treated (acetaminophine only treated) mice.

P_3 = Significance of difference of N-actyl cystein treated mice versus trimetazidine treated mice.

Table (4): Effect of N-acetylcysteine and trimetazidine on hepatic necrosis induced by acetaminophen.

Groups	No. of animals	Incidence of necrosis	Extent and distribution of necrosis					
			0	1	2	3	4	5
Control group	10	0	10					
Acetaminophen only treated group	10	10	0	0	2	4	4	0
TMZ treated group	10	2	8	1	1	0	0	0
N-acetylcysteine	10	1	9	1	0	0	0	0

0= Normal

1= Rare foci of necrotic cells in centrilobular zone (no more than 1-2 sites per section .

2 = Few necrotic foci. (less than half of centrilobular zones had sites of necrosis.

3= Many / diffuse centrilobular zones with necrosis.

4= Diffuse centrilobular to zonal necrosis

5= Diffuse submassive to massive necrosis.

Table (5): Effect of N-acetylcysteine and trimetazidine on hepatic inflammatory cellular infiltration induced by acetaminophen .

Groups	No. of animals	Incidence of necrosis	Extent of necrosis					
			0	1	2	3	4	5
Control group	10	0	10	0	0	0	0	0
Acetaminophen only treated group	10	10	0	0	2	5	3	0
TMZ treated group	10	5	5	3	1	1	0	0
N-acetylcysteine	10	5	5	4	1	0	0	0

0= No inflammatory cells

1= Rare inflammatory cells (no more than 1-3 cells in 1 or 2 centrilobular zones per section).

2 = Few inflammatory cells. 1-5 cells in less than 50% of centrilobular zones.

3= Moderate inflammatory cell infiltration (5-15 cells in most centrilobular zones).

4= Marked inflammatory cell infiltration (greater than 15 cells in most centrilobular to mid zonal areas).

5- Severe inflammatory cell infiltration (too numerous to count and infiltrating most of lobule).

Fig (1) : Effect of acetaminophen (500 mg/kg orally) on serum GPT level (Iu/L), serum MDA level (nmol/m/h) and serum reduced glutathione (GSH) nmol/mg.

Fig (2) : Effect of trimetazidine (20 mg/kg orally) N-acetylcysteine (200/kg orally) on serum GPT enzyme level (nmol/ml/h), and reduced glutathione (GSH) (nmol/mg patient in mice.

Fig (3) : Comparison between the effect of trimetazidine (20 mg/kg orally) and N- acetyl cysteine (200 mg/kg orally) on biochemical changes induced by acetaminophen (500 mg/kg orally).

Fig (4) : Hepatic lobule shows centrilobular necrosis extending to midzones (Grade 4) induced by acetaminophen.

Fig (5) : Hepatic lobule shows diffuse centrilobular to midzonal necrosis (Grade 4).

Fig (6) : Hepatic lobule shows centrilobular necrosis (Grade 3) induced by acetaminophin.

Fig (7) : Portal tract shows severe cellular infiltration .

Fig (8) : Hepatic lobule shows one focus of necrosis (Grade 1) after treatment with N-acetylcystien.

Fig (9) : Hepatic lobule shows one focus of necrosis (Grade 1) after treatment with trimetazidin.

Fig (10) : Normal Hepatic lobule shows only cloudy swelling.

DISCUSSION

This is the first study in which the effect of trimetazidine on acetaminophen induced hepatotoxicity is examined. Its effect is compared with N-acetyl cysteine, which is the recommended clinical treatment for patients in danger of acetaminophen overdose related hepatotoxicity.

In the present study, rats treated with acetaminophen showed a significant reduction in glutathione (50% reduction) level. This finding is in agree with the study of Lahouel et al., (41) who showed a downfall of hepatic glutathione (until 210% of reduction).

Furthermore it is in consistent with the study of Sener et al., (34). They found that glutathione levels were reduced following acetaminophen administration. Also, it is as the study of Ahmed & Khater (42), who found that treatment of rats with acetaminophen led to a significant reduction of hepatic reduced glutathione (65%). Also, it is similar to other study that showed depletion of liver reduced glutathione occurred in animals receiving toxic acetaminophen dose. (43). More over, this result is in agree with the study of Joya et al., (45), who showed that paracetamol treatment caused decrease in glutathione level in the nor-

mal and ethanol treated rats. Reduction of glutathione can be explained by formation of a large amount of a highly reactive and cytotoxic intermediate during the metabolism of acetaminophen in the liver through glucuronidation and sulfation. This intermediate is called N-acetyl-p-benzo-quinoneimine (NAPQI) is formed in a small portion only at the therapeutic doses, which is quickly conjugated by hepatic glutathione to yield a harmless water soluble product. However, after acetaminophen overdose, the capacity for glucuronidation and sulfation is exceeded and a large amount of NAPQI is formed. After depletion of glutathione NAPQI binds covalently to hepatic parenchymal cell proteins and DNA with resultant liver injury. (12&14).

In the present study, liver injury is monitored by assay of serum GPT. There was a significant increase in SGPT in rats treated with acetaminophen. This finding is in agreement with that which suggested that all patients who developed hepatotoxicity presented with aspartate aminotransferase about 50IU. (45,46).

Furthermore, Sener et al., (34) found that aspartate aminotransferase

in blood were increased significantly following acetaminophen treatment. Moreover, it is in consistent with that of Dimova et al., (43); Janbaz et al., (47); Stefano Fiorucci et al., (48) and Dass & Shah (49)

Increase in SGPT is a marker of liver cell injury which is a consequence of many factors, one of them is loss of intracellular glutathione, which have a harmful effect on hepatocyte viability. Most specifically, once the hepatic parenchymal (intracellular) glutathione level is reduced to only 30% of normal, cellular death is a more likely event as intracellular proteins are bound, altered and destroyed. This is a direct result of toxic intermediary (NAPQI) that accumulate due to acetaminophen overdose

Furthermore, in the present study it was found that, treatment of rats with acetaminophen led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This finding is in consists with the study of Ahmed & Khater⁽⁴²⁾ and Jaya et al., (44). The previous results induced by acetaminophen treatments (depletion of GSH, increase in SGPT and MDA), indicating liver cell

injury, were confirmed with the histopathological examination of livers of rats after administration of acetaminophen. This appeared in the form of areas of necrosis mainly in centribular zones associated with inflammatory cells. The necro inflammatory lesions extended to mid zones in some cases.

In the present study it was found that NAC decreased the lesion induced by acetaminophen. This is evidenced by a significant reduction in serum GPT, significant increase in glutathione level and significant decrease in MDA. These findings are in consistant with that of Bajt et al., (50). they showed that pretreatment with N-acetylcystein before acetaminophen decreased the acetaminophen -induced cell death. Also, it is consistent with that of Sener et al., (34).

A treatment with N-acetylcystien 1 h before acetaminophen, prevented the acetaminophen- induced decrease of GSH levels and reduced hepatotoxicity.

NAC is a weak free radical scavenger but it also affects cellular function by supplying cysteine for the syn-

thesis of intracellular GSH. GSH in turn is a powerful antioxidant. It can work in several ways to combat the effects of free radicals. This protein serves as an alternative substrate for direct reaction with free radicals; it degrades H_2O_2 via a glutathione peroxidase catalysed reaction, it reacts with lipid peroxides, restoring lipid structure; and it reacts by a mixed disulfide reaction with protein disulfide reconstituting sulfhydryl groups. Other mechanisms include that NAC increase hepatic oxygen consumption or utilization. This is due to it promotes the flow of blood through small hepatic capillaries and hepatic micro circulation. NAC may alter the hepatic vascular endothelium and subsequently, hepatic micro vascular tone. (8,50)

In the present study, it was found that TMZ ameliorates the acetaminophen-induced lesions which is evidenced by a significant reduction in SGPT.

This is confirmed with the results of Elemadi et al., (51) who found the pretreatment of rats with a potent derivatives (S-15176) alleviated the deleterious ischemia - reperfusion effects at both cellular and mitochon-

drial levels especially leakage of alanine amino transferase & aspartate aminotransferase. Also, it is in agreement with that of setaff et al., (52). They found that pretreatment with TMZ analogue reduced amniotransferase leakage from Hepatocytes. This is, also confirmed with the results of Setaff et al., (28) & Zeid et al., (30). They found that TMZ lowered the increase SGPT enzyme level due to ischaemia reperfusion.

Furthermore, the decrease of acetaminophen induced hepatic lesion by TMZ is evidenced by a significant reduction in MDA. This is consistent with that of singhe & chopra, (53), who found that pretreatment of animals of renal ischemia with TMZ markedly reduced elevated thiobarbituric acid(MDA) levels. They also found that TMZ exert protective effect probably by radical scavenging activity. Also it was found that TMZ had preferential action on the oxidative system which could decrease oxygen free radical production and increase mitochondrial integrity. (54) TMZ produced an increase in mitochondrial turnover, there were no differences in the myocardium subjected to ischemia in both series in terms of observable mitochondrial

damage. (55).

Furthermore, pretreatment of animals with acute tubular necrosis with TMZ 30 min before Fe-NTA administration markedly reduced elevated TBARS and restored the depleted renal antioxidant enzymes (56). Also, mitochondrial integrity was improved by TMZ (57).

Moreover, TMZ markedly reduced elevated level of TBARS and significant attenuates renal dysfunction and morphological changes in rats subjected to renal ischemia reperfusion. These results clearly demonstrate the in vivo antioxidant effect of TMZ (58). Also, Sucu et al., (59) found that TMZ significantly reduced malondialdehyde level (60).

Zeid et al., (30) & Baumert et al., (61) suggested that TMZ can attenuate MDA production related to ischemia reperfusion injury. It is considered as a free radical scavenger in ischemia reperfusion (25).

Also, in the present study reduction of acetaminophen induced lesion is evidenced by restoration in the level of glutathione to normal. This is consistent with that of Chaunder et al.,

al., (60). They found that TMZ improved oxidative stress indicated by glutathione level, malondialdehyde and activities of catalase reductase & superoxide dismutase. Also, Zharova et al., (62). Found that the activity of glutathione peroxidase utilizing lipid peroxides in plasma markedly increased during TMZ therapy (26). Moreover the pretreatment with TMZ analogue increased plasma glutathione level (53)

Comparing the effect of trimetazidine to N-acetyl cysteine. It was found that TMZ ameliorates the acetaminophen induced lesion to a similar extent by N-acetyl cysteine.

In spite that there is a difference in their effect on GSH, TMZ has an additional effect on mitochondrial function (56) & its anti-inflammatory active (63). Furthermore, its effect on Ca^{++} can abolish one of pathophysiological factors in acetaminophen induced hepatotoxicity. However. Manov et al., (64,65). Concluded that in spite of NAC can prevent the oxidative damage in HepG₂ cultured cells, it cant prevent acetaminophen induced plasma membrane asymmetry, alteration of Ca^{++} homeostasis and ultra structural changes.

CONCLUSION

The present study compared the effect of TMZ with that of N-acetyl cysteine on acetaminophen induced lesion.

It was found that TMZ ameliorates the acetaminophen induced lesion to a similar extent by N-acetyl cysteine.

On the light of this study, it could be concluded that TMZ and N-acetyl cystein have a comparable beneficial effect hepatotoxicity.

In spite that there is a difference in their effect on GSH, TMZ has an additional effect on mitochondrial function (56) & its anti-inflammatory active (63). Furthermore, its effect on Ca^{++} can abolish one of pathophysiological factors in acetaminophen induced hepatotoxicity. However. Manov et al., (64,65). Concluded that inspite of NAC can prevent the oxidative damage in HepG₂ cultured cells, it can't prevent acetaminophen induced plasma membrane asymmetry, alteration of Ca^{++} homeostasis and ultra structural changes.

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