Renoprotective effects of *Silybum marianum* (L.) *Gaertn* (Silymarin) on thioacetamide-induced renal injury: Biochemical and histopathological approach

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Abstract: Thioacetamide (TAA), recognized as an experimental toxin, mainly causes acute liver damage through the production of free radicals. TAA as well induces renal dysfunction hence; renal failure is often related with the end-stage of the hepatic damage. The aim of the current study was to examine the protective effects of Silymarin (Sil) against TAA-induced kidney damage in this current study. The twenty eight rats were separated into four groups. Group 1 was performed as control (saline 0,5 mL intraperitonally i.p.). Group 2 was given to 50 mg/kg TAA (i.p.). Group 3 was administrated with TAA just after 50 mg/kg Sil (per os (p.o.)). Group 4 was treated to TAA just after 100 mg/kg Sil. In end (fourteenth days) of study, tissue and blood samples of animals were collected for morphological and biochemical assessment. Our results show that Sil treatment apart from the TAA administration profitably changed the poisonous effects on the rats. In addition, 100 mg/kg Sil was more protective than 50 mg/kg Sil treatment indicated by histopathological, and biochemical values. In conclusion, Sil therapy before TAA could guard kidney tissues against TAA induced nephrotoxicity.

Keywords: Silymarin, renoprotectivity, thioacetamide, nephrotoxicity.

INTRODUCTION

Kidney dysfunction is a widespread problem in the majority of patients with acute hepatic failure and in the advanced stages of their illness (Ring-Larsen and Palazzo, 1981). Hepatotoxic substances such as acetaminophen (Pritchard and Butler, 1989; Bourdi *et al*., 2002), carbon tetrachloride, lipopolysaccharides (Shi *et al*., 1998; Morio *et al*., 2001), TAA, D-galactosamine, and tumor necrosis factor alpha have widely engaged in giving experimentally induced hepatic damage cases (Bradham *et al*., 1998; Cengiz *et al*., 2016). TAA is a hepatotoxin compound which is commonly used to generate acute liver damage in experimental studies (Sarkar and Sil, 2007). Furthermore, it is known that the harmful effects of TAA are not only limited in the hepatic but cause morphological damage in organs such as kidney and brain (Al-Bader *et al*., 2000; Liu *et al*., 2000; Caballero *et al*., 2001). Ledda-Columbano *et al*. (1991) emphasized that TAA induced apoptosis in the rat. The harmful effects of TAA may also vary depending on such factors as concentration, number of doses, duration of administration and method of administration. *Silybum marianum* is a vegetable medicine including Sil, consist of flavano-lignans such as silybin silychristine and silydianin (Valenzuela and Garrido 1994). Various procedures have been applied for the bioavalibility of Sil including anti-oxidation, anti-lipid peroxidation and anti-inflammatory effects (Wen *et al*., 2008). Because it is safe, inexpensive and easy to access, many experiments on different diseases have been made. For instance, Sil has been used in a diversity of diseases, such as hepatitis, alcoholic hepatitis, kidney damage, lung injury and fatty liver. Renoprotective feature of Sil may be owing to various mechanism like inhibition of lipid peroxidation, anti-inflammation, raise of detoxification and antioxidation (Flora *et al*., 1998). Renoprotective effect of Sil was considered chiefly in rats. The goal of our study was to evaluate the defensive effects of Sil on TAA nephrotoxicity in rats.

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MATERIALS AND METHODS

Chemicals

Both Sil and TAA were purchased by Sigma Aldrich (St. Louis, MO, USA).

Animals and treatments

Male albino Wistar rats weighing 220±6.5g (mean ± S.D, n = 28) were used in our study. The animals were housed in polypropylene cages, given standard rat chow and drinking water, and maintained under controlled temperature (26ºC), with a 12 h light/12 h dark cycle. The animals were haphazard categorized into 4 groups, each containing 7 rats. All procedures concerning the animals in this study were approved by the ESOGU Animal Welfare Committee (Ethical Commite Number; 2018-666-1).

50 mg of TAA was dissolved in 0.9% NaCl and brought ready for injection (Singh and Trigun, 2014). TAA injection was i.p., and Sil was administered as p.o. The both doses of Sil were solved in nontoxic doses of

dimethyl sulfoxide (DMSO). Sil administration started 14 days before TAA injection and then TAA was given for 14 days. One day after the last injection, the animals were anesthetized and blood, kidneys were removed.
Group 1: Control rats (0,5 mL saline only)
Group 2: TAA (50 mg/kg, i.p.)
Group 3: Sil (50 mg/kg, p.o.) + TAA (50 mg/kg)
Group 4: Sil (100 mg/kg) + TAA (50 mg/kg)

Histopathological investigations
Sections were taken from the kidneys and routine histopathological staining was performed (Cengiz *et al*., 2016).

Biochemical assays
Blood urea nitrogen (BUN) and uric acid values were measured from the obtained serum using an auto-analyzer (HITACHI-917).

STATISTICAL ANALYSIS

SPSS 12.0 for Windows was used to assess the data obtained in this study. The difference observed for serum BUN and uric acid levels in the groups were assessed via one-way ANOVA. The numerical value (P) for the difference was significant at $p<0.05$.

RESULTS

Hematoxylin-eosin stained specimens were observed under light microscope. Renal tissue from the control group (Group 1) was normal. However, renal structures (Group 2) given 50 mg / kg TAA were quite damaged (fig. 1 and table 1). The rats given 50 mg / kg Sil + TAA had a normal renal cortex and reduced tubular damage. At the same time, both Bowman capsular vasoconstriction and narrowing were observed to be much lower than in Group 3. In the sections of the 100 mg/kg Sil +TAA treated group, the tube damage was reduced and there was a renal cortex close to normal and the damage was less when compared to a Group 3 (fig. 1and table 1).

Biochemical results
Serum BUN and uric acid levels of Group 2 were significantly increased compared to the control group. In the experimental groups of Groups 3 and 4, BUN and uric acid levels were significantly lower when compared to Group 2. In addition, serum BUN and uric acid values of Group 4 were found to be close to control when compared with Group 3. Our biochemical results show that the two doses of Sil provide protection against TAA-induced injury. However, 100mg/kg Sil has been shown to provide better protection than 50 mg / kg Sil (table 2).

DISCUSSION

TAA-induced fulminant hepatic failure is the result of high free radical production leading to oxidative stress. TAA has also been shown to produce hepatic toxicity, increase Bax and caspase-3 expression, and significantly reduce Bcl-2 expression (David *et al*., 2011). Experimental studies have reported that liver damage is connected with the development of nephrotoxicity (Ring-Larsen and Palazzo, 1981). Moore *et al*. (1991) suggested that approximately 50% of patients with severe hepatocyte damage or massive hepatic necrosis were accompanied by renal failure (Moore *et al*. 1991). A person with impaired liver function has also been shown to have a decrease in renal blood flow in the kidney as shown fig. 2 (Guarner *et al*., 1987).

Atef *et al*. (2017) found that there are some changes in kidney structures, including necrosis and high degeneration of glomerular and Bowman capsules in mice treated with thioacetamide only and in contrast to control data, serum creatinine (38.5%), BUN (26.3%) and uric acid (24.0%) levels were increased in mice given TAA. In another study, serum BUN and uric acid levels in the 200 mg/kg thioacetamide-administered group were found to decrease significantly when compared with the control group (Moghadamnia *et al*., 2016). The end result of TAA administration is the evidence for the failure of renal function to increase the levels of urea, BUN and creatinine (Ozen *et al*., 2004). Similarly, Kadir *et al*. (2013) emphasized that renal morphology is impaired with severe and generalized tubular epithelium in the histopathological examination of sections from rat kidneys treated with TAA. In addition, biochemical evaluations indicated that serum BUN and cre levels were significantly increased in the group given TAA compared to the control group (Kadir *et al*., 2013). In our histological and biochemical findings, kidney tissue was damaged in the sections of the experimental group administered TAA. As a matter of fact, damage to the kidney tubules, narrowing of the bowman gap and vacuolization were observed. In parallel with this, tissue damage markers BUN and uric acid levels were found to be significantly higher than control group. The results of the experimental groups given TAA showed similarities to the above-mentioned results (fig. 1 and table 1-2).

Sil is a polyphenolic flavonoid from plants. It is widely used in the treatment of drug-induced liver disease. Many studies have shown that Sil has antioxidant properties and inhibits inflammation and apoptosis. It has been shown emphasized that the kidney damage induced by 700 mg / kg of D-GalN and 15µg/kg of TNF-α significantly reduced the groups given 100mg/kg of Sil (Cengiz *et al*., 2016). In addition, elevation of BUN and Cre levels due to renal injury reversed significantly. In one study, they noted that 50mg/kg of Sil alters the increase in BUN and Cre levels caused by cisplatin (Karimi *et al*., 2005). In another study, single dose adriamycin (10mg/kg) showed nephrotoxicity and protection against nephrotoxicity due to adriamycin after a treatment of 50 mg / kg Sil (El-

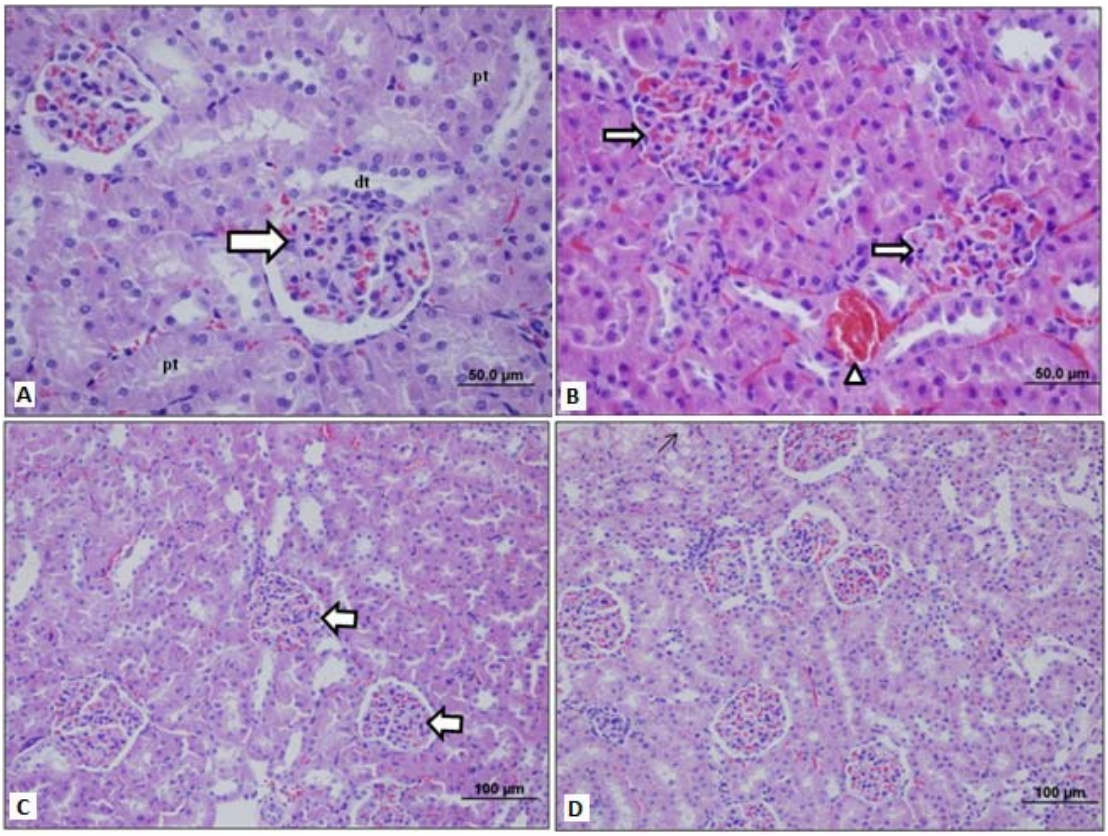

Fig. 1: Kidney with normal histological structures (group 1) (A) (Glomerulus (Open arrow), proximal tubule (PT), distal tubule (DT), kidney with damaged histological structures by TAA group 2 (B) (Tubular damage, narrowing, Bowman's space (right arrow) and vacuolization (triangle). A near-normal kidney cortex, along with tubular damage, is shown in group 3 (C). A near-normal kidney cortex, along with less tubular damage, is observed in group 4 (D), although the damage did not totally disappear compared to group 3. Partial vacuolization in some tubules (right arrow) (D) and partial glomerular damage in the Malpighian corpuscles.

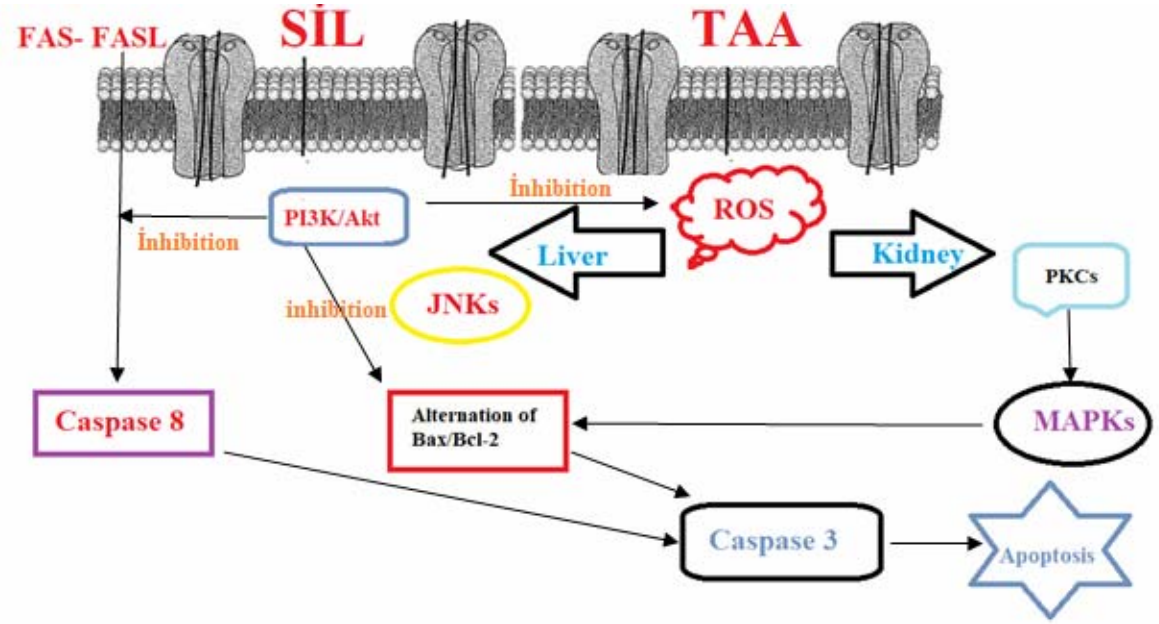

Fig. 2: Liver and kidney damage caused by TAA and the protective mechanism of Sil (Shatadal *et al*., 2016).

Table 1: Histological scores of the study groups

Groups	Narrow in Bowman	Tubular damage	Inflammation	Glomerular damage	Congestion	Total Score
Group 1	0	0	0	0	0	0
Group 2	3	3	2	3	3	14*
Group 3	1	1	1	1	1	5
Group 4	0	1	0	1	0	2

0: no damage 1: little damage 2: medium damage 3: intense damage

Table 2: Serum BUN and Uric acid levels of the samples taken from rats

	BUN (mg/dL)	Uric acid (mg/dL)
Group	Mean ± SD	Mean ± SD
1	15,19 ± 1,73	110,79 ±15,96
2	18,42 ± 0,71[a]	138,24 ± 10,39[a]
3	17,09 ± 1,21[a]	125,95 ± 7,175
4	15,89 ± 1,32	116,35 ± 17,53
P	*p<0.05*	*p<0.05*

All values are the mean ± SD (n =7). [a]*p<0.05* significant difference compared to control.

Shitany *et al*., 2008). Ramachandran *et al*. (2010) revealed that milk thistle effects on nephrotoxicity induced by a single IP dose of 750 mg/kg acetaminophen in male Wistar albino rats. In study, Sil (50 mg/kg) significantly improved kidney function indicator like plasma urea, creatinine and uric acid levels. Our work gave similar results to the above mentioned studies; kidney damage decreased in the groups given Sil, and there was a decrease in serum BUN and uric acid levels. Histopathological notes further confirm the membrane stabilizing effect of Sil in TAA challenged rats. 100 mg/kg Sil was found to be more effective in reducing kidney damage compare to 50 mg/kg Sil pretreated group (fig. 1 and table 2). In conclusion, our results suggest that TAA induces oxidative stress that causes to systematic cell deaths within both the liver and kidney (fig. 2). Sil, known as a strength antioxidant, may guard those organs by enriching the antioxidant enzyme activities and scavenging reactive oxygen specied as evidenced from biochemical results. But, the accurate mechanism of action of Sil requires more research.

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