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Silybum marianum seeds protected biochemical and oxidative stress markers in ethanol-induced toxicity of Wistar rat

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ABSTRACT

In the present study, an attempt was made to evaluate the potential activity of Silybum marianum seeds infused solution (SMI) against ethanol (Eth) toxicity of Wistar rat liver and kidneys. Thirty six males (control, SMI positive control (200 mg/kg b.w./day), the Eth1 (01 g/kg b.w./day), the Eth2 (02 g/kg b.w./day), the Eth1+SMI and the Eth2+SMI) were orally administered for six successive weeks to measure serum biochemical parameters as well as hepatic and renal malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPx) levels. According to the results, the Eth1+SMI and the Eth2+SMI alleviated ethanol-induced increased levels of ALT, AST, ALP, total cholesterol, triglycerides, LDL, urea, creatinine and MDA. Furthermore, the co-administration of SMI has maintained hepatic and renal GSH concentration and GPx activity close to that of the control. The SMI has alleviated ethanol-induced toxicity via the improvement of hepatic and renal markers.

***Keywords*:** Ethanol, kidney, liver, oxidative stress*, Silybum marianum.*

INTRODUCTION

Ethanol is a substance commonly abused in many communities and considered as one of the lifestyle habits around the world. According to the world health organization, alcohol consumption increased since 2000 in almost all regions of the world, in which African consumers drink an average of 40 g/day of pure alcohol (WHO, 2018). Acute and excessive abuse of ethanol has been shown to have toxic effects inducing many pathological disturbances affecting a number of organs. Due to its propriety of being a small molecule and soluble in both lipids and water, ethanol could penetrate to all tissues of the body and affects the most vital functions including liver and kidney (Lieber, 2000). Oxidative stress was believed to be the major responsible of alcohol-induced pathogenesis. Wherein the production of reactive oxygen species (ROS) is involved in the mechanism of ethanol inducing oxidative stress (Wang *et al*., 2018), typically leads to the peroxidation of lipids, oxidation of protein and DNA (Sugimoto and Takei, 2017), resulting in cell damage and organ dysfunction.

Currently, interest of medicinal plants have been growing faster in worldwide after realizing their benefits on health (Nandi and Ghosh, 2016). Wherein, modern pharmacopeia use about 25 % of drugs are derived from plants (Rahman and Fakir, 2015). The growing interest by the supplementations of plant products back to their antioxidant activity and free radical scavenging potential to prevent the diseases progression (Bouhalit and Kechrid, 2018). *Silybum marianum* or Milk thistle, known in Algeria as *choukahmar*, is one of the famous plants used in traditional medicine. It contains active compounds obtained mainly from the dried seeds where it is present in higher concentrations than in other parts of the plant and consist of four flavonolignans which are collectively known as silymarin (Onalan *et al*., 2016). Silymarin have shown multiple pharmacological activities including antioxidant, anti-inflammatory, antibacterial, antiallergic, antiviral, antimutagenic, antineoplastic, and antithrombotic agent (El-Demerdash *et al*., 2015). In addition, silymarin has been reported to regulate cellular and mitochondrial membrane permeability and increase membrane stability (Onalan *et al*., 2016). The activity of silymarin results from the direct scavenging of free radicals and other oxidizing intermediates, from the chelation of iron or copper ions, as well as from preventing free radical formation (El-Shennawy *et al*., 2016). In addition to reacting with ROS, silymarin could

increase cell physiological antioxidant power (Surai, 2015).

In this study, we sought to highlight the ameliorative role of *S. marianum* seeds infused solution against ethanol toxicity in rat via the evaluation of blood biochemical parameters and oxidative stress status of liver and kidneys.

MATERIALS AND METHODS

Chemicals

Absolute ethanol at 99.8% (Honeywell Laboratory, Germany) was diluted in distilled water to make the desired concentration of 40% (Eth).

Plant

Silybum marianum (Milk thistle) dry seeds were harvested from Ain-Berda, Annaba province, Algeria. Ten g of crushed seeds were poured over 100 ml of boiled distilled water and left to infuse for 30 minutes (Raskovic *et al*., 2002). The infused solution (SMI) was prepared daily during the six week experimental period and was given to animals at the dose of 200 mg/kg/day by gavage (Dose was 200 mg/kg; it means that we gave 200 mg of the plant to each kilogram of rat. We prepared the infusion by pouring 100 ml of water to 10g of crashed seeds. Then, we calculated how many milliliters in 200mg by applying the rule of three).

Animals and the experimental design

In this experiment, 36 male adult Albinos Wistar rats weighing between 200-220g were obtained from the Algiers Pasteur Institute (Algeria) were used. The experiment was carried outat the animal house under conventional conditions and were fed a standard diet (by GAC-ORAC, Bejaia, Algeria) (GAC-ORAC, is a national company produce Cattle and Rodents Feed) and given drinking water *ad libitum*. Animals were divided equally into 6 groups; the first represented the control, thesecond was the positive control SMI (200 mg *S. marianum*/ kgb.w./day) (using *S. marianumin* infusion as positive control to prove its safety for rats, in case the combined treatment provoked toxicity), the third and the fourth groups received Eth1 (1g ethanol/kg b.w./day and Eth2 (2g ethanol/kg b.w./ day), respectively. Rats of the fifth (Eth1+SMI) and the sixth (Eth2+SMI) groups were treated respectively with ethanol at 1g/kg b.w /day and 2g/ kg b.w /day, followed after one hour, by SMI at 200 mg/kg b.w /day. The treatment was performed orally using a gastric tube for six consecutive weeks.

Rats were sacrificed by decapitation under ether anesthesia after an overnight fast. Ether can be ethically used as an anesthetic in animals; it is being used by most researchers. According to While Animal Care and Use Committee, (2006) ether is safe to use as anesthetic for rodent. Blood was collected in dry tubes, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and stored at -20 °C to study the biochemical parameters. Liver and kidneys were dissected, cleaned from their adipose tissues, and then stored at -20 °C until used to study the oxidative stress status. Animals' treatments were authorized by the Ethical Committee of Animal Sciences at the University of Badji Mokhtar-Annaba, before starting the experimental work. Experiments were carried out in accordance with international guidelines for the care and use of laboratory animals.

Measurement of the biochemical parameters

In this study, alanine aminotransaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), total cholesterol (TC), triglycerides (TG) low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), creatinine (CREA) and urea were measured using an auto analyzer following the technical instructions of the commercial kits obtained from Spinreact (manufactured by SA - Santa Coloma, Spain).

Measurement of oxidative stress parameters

- Lipid peroxidase was measured as Malondialdehyde (MDA) concentration according to Ohkawa *et al*. (1979) method. This method is based on the formation between MDA and thiobarbituric acid (TBA) of a colored pigment absorbing at 530 nm and extractable by butanol in an acidic hot medium.
- Reduced Glutathione (GSH) levels was measured according to Weckbercker and Cory (1988), which is based on the measurement of the optical absorbance of 2-nitro-5-mercapturic acid that it results from the reduction of 5,5'-dithio-bis-

Table 1: Plasma biochemical parameters of control and the treated groups after 6 weeks of treatment

	Control	SMI	Eth1	Eth2	Eth1+SMI	Eth2+SMI
AST (UI/L)	190.47±3.9^{b}	175.62±8.32^{b}	207.49±7.87^{a}	212.04±6.09^{a}	182.63±17.37^{b}	86.25±3.09^{b}
ALT (UI/L)	34.60±7.75^{b}	29.50±0.43^{b}	41.23±1.49 ^{a}	41.71±2.08^{a}	30.81±1.60^{b}	32.23±1.66^{b}
PAL(UI/L)	105.67±13.05^{b}	91.83±10.36^{b}	144.00±4.52^{a}	153.50±5.92^{a}	97.92±6.14^{b}	01.00±4.90^{b}
TC (g/l)	0.59±0.06 ^{b}	0.58±0.02 ^{b}	0.69±0.04^{a}	0.77±0.05 ^{a}	0.59±0.05 ^{b}	0.60±0.05 ^{b}
TG (g/l)	0.86±0.027^{bc}	0.78±0.073 ^{c}	0.94±0.029 ^{ab}	1.04±0.060 ^{a}	0.79±0.072 ^{c}	0.80±0.061 ^{c}
LDL-C (g/l)	0.10±0.018^{bc}	0.09±0.020^{c}	0.14±0.018 ^{ab}	0.15±0.023 ^{a}	0.11±0.018 ^{bc}	0.11±0.017 ^{bc}
HDL-C (g/l)	0.37±0.019^{ab}	0.39±0.013^{a}	0.26±0.035^{c}	0.26±0.040^{c}	0.32±0.031$_{b}$	0.32±0.031^{b}
Crea(mg/l)	4.43±0.35^{b}	4.14±0.11^{c}	4.66±0.18 ^{ab}	4.88±0.19^{a}	4.33±0.22 ^{bc}	4.42±0.29 ^{bc}
Urea (g/l)	0.48±0.03^{b}	0.41±0.01^{c}	0.58±0.05^{a}	0.63±0.02^{a}	0.48±0.04^{bc}	0.49±0.03^{b}

$^{a-c,}$ Means do not share the same letter are significantly different (P < 0.05),According to one-way by Tukey test. SMI: *Silybum marianum* seeds infused solution; Eth1:01 g /kg b.w./day; Eth2: 02 g/kg b.w./day; TC: Total cholesterol; TG:triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; Crea: Creatinine.

2-nitrobenzoique acid (DTNB) by the groups (-SH) of glutathione. For this, deproteinization of the homogenate is essential in order to keep only the specific thiol groups of glutathione.

● The enzymatic activity of glutathione peroxidase (GPx) were evaluated by the method of Flohe and Günzler (1984). The method is based on the reduction of hydrogen peroxide (H2O2) in the presence of reduced glutathione (GSH); the latter is transformed into (GSSG) under the influence of GPx. The results of GSH and GPx were expressed in GSH (nmol/mg proteins) and µmol of GSH/mg proteins, respectively. Thus, liver and kidneys total protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin as a standard.

Statistical analysis

The differences between the groups were tested for statistical significance by one-way analysis of variance (ANOVA), followed by Tukey test for multiple comparisons, where Minitab 18 Software application was used. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Biochemical parameters

As shown in table 1, a significant increase in the enzymatic activity of ALT, AST, and ALP was observed in both groups exposed to ethanol compared to the rest of experimental groups. So far, the measurement of serum AST, ALT, and ALP has been commonly used as reliable markers for assessment of hepatotoxicity (Sun *et al*., 2014). Results harmonize with Wang *et al*. (2018) who matched that alcohol intake successfully induce liver damage as remarked by the elevations of serum activity of AST, ALT and ALP. The aminotransferases can be elevated in cases where the integrity of the liver cells is affected (Nallagangula *et al*., 2017). However, ALP can be released in the blood stream when the parenchymal liver cells were injured (Carl *et al*., 2012).

Conversely, the co-administrated groups showed a lowered serum activities of ALT, AST and ALP compared to ethanol treated groups (Table 1). These results are in harmony with previous investigations that documented the ability of *S. marianum* or one of its compounds to restore the elevated serum activities of ALT, AST and ALP induced by different xenobiotics (Heidarian *et al*., 2019; Zaki *et al*., 2019). Our results might indicate that SMI prevented the leakage of these enzymes into the serum. Accordingly, silymarin regulates membrane permeability and increases its stability when exposed to xenobiotic damage (Abenavoli *et al*., 2010). Additionally, silymarin was found to stimulate RNA polymerase enzyme in the nucleus of liver cells leading to an elevation of ribosomal protein synthesis and regenerates hepatocytes (Bahmani *et al*., 2015).

Lipid profile is important marker used for monitoring liver damage. In the current study, the gavage of ethanol caused lipid profile disorder

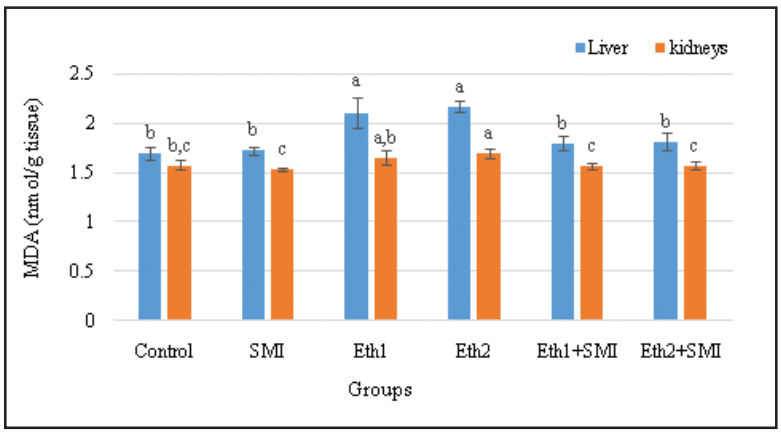

Fig. 1: **Variation of hepatic and renal MDA concentration (mean ± SD) of the control and the treated groups with Ethanol and *S. marianum* after 6 weeks of experiment. Means that do not share the same letter are significantly different at p<0.05.**

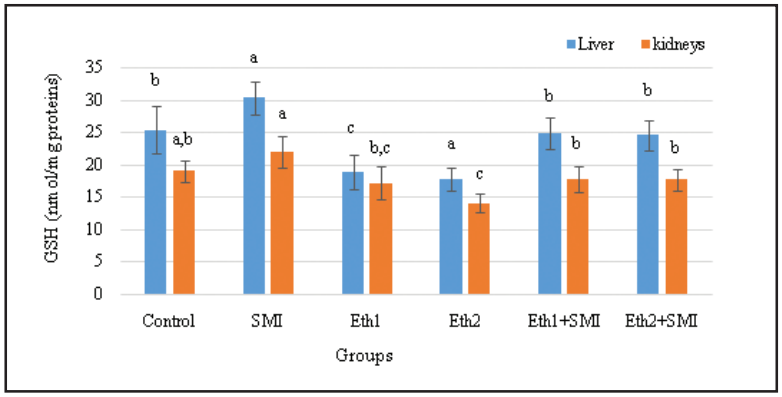

Fig. 2: **Variation of hepatic and renal GSH (mean ± SD) of the control and the treated groups with Ethanol and *S. marianum* after 6 weeks of experiment. Means that do not share the same letter are significantly different at p<0.05.**

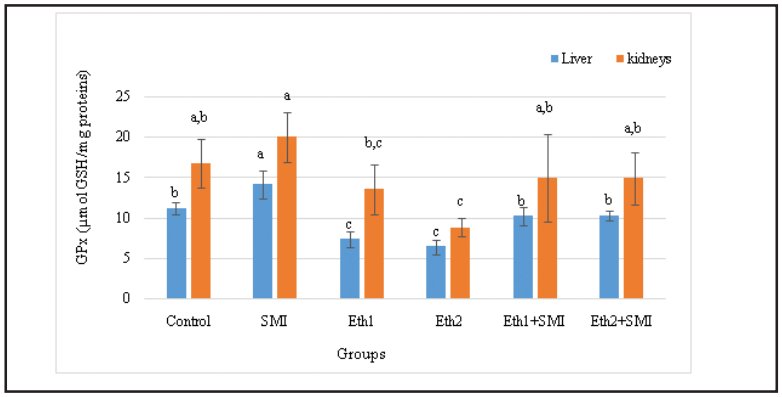

Fig. 3: **Variation of hepatic and renal GPx activity (mean ± SD) of the control and the treated groups with Ethanol and *S. marianum* after 6 weeks of experiment. Means that do not share the same letter are significantly different at p<0.05.**

characterized by, an increase in serum total cholesterol, triglycerides and low-density lipoprotein cholesterol levels with a decrease in high-density lipoprotein cholesterol levels (Table 1). The results obtained are consistent with several other studies including that of Kamoun *et al.* (2017). According to You and Arteel, (2019), ethanol was found to be the unique among toxins, which disrupts almost all aspect of liver lipid metabolism.

Contrariwise, as appeared for data of table 1, rats treated concomitantly with ethanol and SMI (Eth1+SMI and Eth2+SMI) kept the normal level of the cited parameters like that of the control. The observed results could be related to the action of silybin or silibinin, one of the structural isomers of silymarin. Wherein, silybin was found to have an inhibitor of lipid accumulation by down regulation of LPIN1 (also known as LIPIN1), a major enzyme in the triglycerides synthesis pathway, and FASN, an important enzyme in fatty acid synthesis involved in triglycerides and fatty acids synthesis (Suh *et al*., 2015). More interestingly, it was reported that silibinin have an anti-adipogenic effect, which associated with upregulation of INSIG proteins, the transcriptional inhibitors of Sterol regulatory element-binding protein-1c (SREBP1-c) (Ka *et al*., 2009). SREBP-1c is an essential transcription factor that stimulates the expression of lipid synthesis genes, including fatty acid synthase (FAS), stearoyl CoA desaturase 1 (SCD1) (Raghow *et al*., 2008), stimulated by alcohol exposure leading to hepatic steatosis (You *et al*., 2002). Those circumstances, could explain the mechanism by which SMI restored the normal level of the lipid profile in this study.

Urea and creatinine are good index for testing the normal functioning of kidney, in which the increase of these substances in the serum is an indicator of kidney dysfunction (Kamal, 2014). In the actual investigation, as illustrated in Table 1 rats exposed to ethanol (Eth1 and Eth2) had remarkably raised urea levels. Nevertheless, creatinine level was increased only in the higher ethanol dose. The marked elevation of serum urea and creatinine levels after ethanol administration in agreement with the results obtained by other researchers used different doses of ethanol (Maralla *et al*., 2012; Bulle *et al*., 2016). Thus, several potential mechanisms by which alcohol may directly or indirectly affect the kidneys functions was suggested (Varga *et al*., 2017). However, the increases of urea and creatinine concentrations could be an index of failure in renal filtration function. According to Latchoumycandane et al. (2014), both kidney architecture and its functional filtration were altered after ethanol consumption. Similarly, Bulle *et al*. (2016) in their study have remarked that renal filtration was reduced after ethanol consumption accompanied with increases in circulation of both urea and creatinine. Moreover, Ethanol-induced oxidative stress was suggested as one of the mechanisms associated with alcoholic kidney injury (Varga *et al*., 2017). It was stated that, the abundance of long chain polyunsaturated fatty acids in renal lipids makes membrane of renal tissue defenseless against reactive oxygen species induced by oxidative stress (Rodrigo and Rivera, 2002).

On the other hand, rats treated concomitantly with ethanol and SMI have the same level of urea and creatinine as that of the control, while in the positive control (SMI), urea and creatinine levels were the lowest (table 1). Salama *et al*. (2015) reported that feeding *Silybum marianum* extraction to rat treated with ethanol succeeded in maintaining the levels of the sited parameters to the normal range. The observed results could be related to the effect of the main S. marianum compound, the silymarin. Silymarin may have therapeutic potential for the treatment of nephropathy by increasing the activity of antioxidant enzymes in kidneys and repairing renal morphology (Hamza *et al*., 2015).

Oxidative stress parameters

Keeping in mind that ethanol-induced oxidative stress is one of the main pathologic mechanisms in liver (Kim *et al*., 2014; Li *et al*., 2015) as in kidneys (Varga *et al*., 2017; Cikler-Dulger and Sogut, 2020) and in the light of the strong antioxidant properties of *S. marianum* extract seed compounds (Surai, 2015), this study was extended to oxidative stress parameters in both liver and kidneys. Consistently with the previous investigations (Dogan and Anuk, 2019; Goc *et al*., 2020), the actual data showed results show a significant rise of hepatic MDA levels in Eth1 and

Eth2 in comparison to control and other treatments groups. In other hand, results indicate an increase of renal MDA level of Eth2 group compared to the other groups, while renal MDA level of Eth1 group remains not changed in comparison with other treatments groups. In addition, results demonstrate a significant decrease in the levels of hepatic GSH and GPx of Eth1 and Eth2 groups compared to other groups. Regarding the kidneys, the levels of GSH and GPx decreased only in Eth2 group compared to the other groups (Figure 1-3).

Conversely, when it administrated concomitantly with ethanol, SMI has Kept MDA, GSH and GPx levels to the normal level (Figure 1-3). Results are in good agreement with other researches that demonstrated the advantage of using *S. marianum* extract or one of its active compounds to neutralize oxidative stress induced by various chemicals in liver and kidneys (Salama *et al*., 2015; El-Shennawy *et al*., 2016). Such effect could be reasonably assumed to the ability of *S. marianum* to participate as an antioxidant defender by preventing free radical formation thought the inhibition of specific enzymes responsible for the formation of free radicals (Surai, 2015). More interestingly, silymarin might promote protein synthesis such as glutathione causing an elevation of GPx activity (Fatehi *et al*., 2018). In the same manner, silymarin could preserve GSH concentration by increasing the levels of methionine, S-adenosylmethionine, and cysteine metabolites that are needed to synthesize GSH (Kim *et al*., 2016).

CONCLUSION

In this experiment, ethanol intake disrupted most parameters related to hepatic and renal functions. On the other hand, the co-administration of S. marianum infused solution together with ethanol succeeded in maintaining the levels of the majority of parameters cited above at normal ranges. The actual investigation is an add to a growing corpus of researches that discussed the negative effects of ethanol on hepatic and renal functions and the benefit of using *S. marianum* to neutralize such toxicity.

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