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Examining *Silybum marianum* as a natural countermeasure to 1,4-dioxane induced hemato-, hepato- and nephrotoxicity in male Sprague-Dawley rats

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Abstract: *Silybum marianum* (*S. marianum*) is famous for its nutritional value and medicinal benefits; while 1,4-dioxane is extensively used at the industrial level and in daily routine, but with all its uses, it is also becoming a major water contaminant with hazardous impacts on human health. The current study assessed the therapeutic potential of the ethanolic extract of *S. marianum* leaves against 1,4-dioxane induced hemato- hepato- nephrotoxicity in male Sprague-Dawley (SD) rats by involving 40 male SD rats, distributed into eight groups viz., control group (C), *S. marianum* extract groups (S1, S2 and S3 at 85, 165 and 247 mg/kg, respectively), positive control group (G1): treated with 1,4-dioxane at 3000 ppm, and three co-treated groups (G2, G3, G4: 1,4-dioxane at 3000 ppm + *S. marianum* at 85, 165 and 247 mg/kg, respectively). After the completion of the trial (60 days), significant (P<0.05) improvements in body weight, hepatic-, renal- and lipid profile as well as histo-architecture of liver and kidney were observed in co-treated groups in a dose-dependent manner. While 1,4-dioxane at 3000 ppm severely altered the selected parameters in SD rats. Conclusively, *S. marianum*, due to its therapeutic potential at 247 mg/kg, countermeasured the 1,4-dioxane induced hemato-, hepato- and nephrotoxicity in male SD rats.

Keywords: 1,4-dioxane, *Silybum marianum*, hematology, hepatotoxicity, nephrotoxicity

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INTRODUCTION

1,4-Dioxane (1,4-DX) is a synthetic compound, heterocyclic ether widely utilized as a stabilizing agent in chlorinated solvents and as a solvent in the manufacture of diverse commercial and industrial formulations, including dyes, varnishes, greases, waxes, detergents, cosmetics, and personal care products (USEPA, 2017). Its extensive use and improper disposal practices have led to its frequent release into domestic wastewater streams, thereby posing a persistent threat to aquatic systems. The environmental concern surrounding 1,4-DX is further intensified by its high mobility, low volatility, and extended half-life in groundwater, which collectively hinder its natural attenuation and render conventional remediation approaches largely ineffective. In response to growing evidence of its toxicity and widespread occurrence, regulatory bodies in states such as California and New York have initiated proactive measures to restrict its inclusion in consumer products, acknowledging the urgent need for resource control and advanced treatment strategies (Doherty *et al*., 2023).

Numerous studies have revealed that 1,4-DX can induce carcinogenicity and hepatic toxicity in mice and humans (Carrera *et al.*, 2017). In male and female Crj: BDF-1 mice, single-cell necrosis and centrilobular hepatocytes swelling were observed on ≥4000 ppm 1,4-DX exposure in drinking water for 13 weeks (Kano *et al*., 2008). Literature has reported that kidney injuries can also be caused by 1,4-DX at high exposure (Kano *et al*., 2009). Renal tissue damage, metabolic pathways' disruption, and stimulated oxidative defense systems were observed at 500 mg/L dioxane exposure (Qiu *et al*., 2019).

Medicinal plants nowadays have been used to improve liver and kidney dysfunction via their powerful antioxidant potentials (Abdel-Daim *et al*., 2020). *Silybum* (*S*.) *marianum,* being a medicinal plant, has a vast antiquity in traditional medicine against liver ailments, kidney disorders, gastronomic abnormalities, cardiac diseases, fever, and rheumatism (Peschel, 2014). It contains silymarin flavonolignans, which possess several unique biological activities such as anti-inflammatory, antioxidant, immuno-modulatory, and liver regenerating functions (Abenavoli *et al*., 2018).

A growing number of experimental and clinical research have established *Silybum marianum* as a notable hepatoprotective plant, primarily due to its potent antioxidant activity and multifaceted liver-protective properties (Shaker *et al*., 2010; Bahmani *et al*., 2015; Jiang *et al*., 2022). The principal bioactive compound, silymarin, has been extensively recognized for its therapeutic potential in managing a variety of liver disorders, particularly those involving hepatic dysfunction or degenerative changes such as necrosis (Bahmani *et al*., 2015; Jiang *et al*., 2022). Although the precise molecular mechanisms remain under investigation, current evidence suggests that silymarin exerts its protective effects through multiple pathways. These include scavenging reactive oxygen species, modulating inflammatory responses, enhancing hepatocyte membrane stability, regulating cellular permeability, promoting hepatic

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regeneration, and inhibiting fibrogenesis by preventing excess collagen deposition, a precursor to cirrhosis. Both *S. marianum* and silymarin have demonstrated efficacy and safety as supportive agents in the management of hepatotoxicity (Rajappa *et al*., 2024).

On a molecular level, silymarin exerts its anti-inflammatory action by inhibiting the activation of the NF-κB pathway, resulting in the downregulation of pro-inflammatory cytokines such as TNF-α and IL-6 (Altındağ, 2022; Mi *et al*., 2022). It also activates the Nrf2 signaling pathway, which upregulates genes involved in antioxidant defense and detoxification. Additionally, silymarin downregulates fibrotic markers like TGF-β1, reducing the risk of long-term tissue scarring and organ dysfunction (Wadhwa *et al*., 2022; Bai *et al*., 2023). The combined effects of these mechanisms explain the observed normalization of liver and kidney function tests, improvement in hematological profiles, and restoration of tissue architecture in *S. marianum*-treated groups. Thus, the findings of this study support the therapeutic potential of *S. marianum* as a natural intervention against 1,4-dioxane-induced multi-organ toxicity. Keeping in view the toxic potential of 1,4-dioxane and the protective effects of *S. marianum* extract as evident from the previous literature, it was hypothesized that *S. marianum* counteracts the 1,4 Dioxane-induced toxicity in male Sprague-Dawley rats by reducing oxidative stress and inflammation, physically inhibiting its entry into cells, promoting the healing of damaged organs, and altering its metabolism. Therefore, the current study examined the hemato- hepato-nephro-protective effects of *S. marianum* leaves ethanolic extract against 1,4-dioxane induced toxicity in male Sprague-Dawley (SD) rats. In the current study, rats in different groups were orally gavaged with 1,4-dioxane and different doses of *S. marianum* extract alone or in combination for sixty days. Statistical analyses were applied to examine the metabolic changes. This study suggested the *S. marianum* efficacy for the protection of organs in model organisms against 1,4-dioxane induced toxicity. The study will be beneficial to assess the potential risk of 1,4-Dioxane and its safety in guiding its future applications and other alternatives.

MATERIALS AND METHODS

Plant material

The leaves of *S. marianum* were collected from different fields in Karachi, Pakistan in March 2021. Leaves were utilized in this experiment. The identification was verified by contemplating the authentic samples at University of Agriculture, Faisalabad, Pakistan (Specimen Voucher No. 315/21/02).

Plant extract preparation

The leaves were washed thoroughly to remove any dust particles or any impurity. Then they were shade-dried and ground through a grinder (Panasonic-MX-AC400) to derive the fine powder. Then this fine powder was utilized to determine the physicochemical parameters of the selected medicinal plants. Then 95% ethanolic extract was acquired by using Soxhlet's apparatus through crushed materials. Evaporation was done through a rotary evaporator under reduced pressure at 40 °C to get semi-solid ethanol-free mass which was further dried and utilized for different analyses. In this study, the percentage yield of ethanolic extract of *S. marianum* leaves was reported as11.67%. After extraction, extract was stored at 4°C for further study.

Procurement and husbandry of model organisms

The study was carried out by procuring male Sprague-Dawley rats from the animal house of Government College University Faisalabad, Pakistan after the approval of the ethical committee of Government College University on animal experimentation. The rats were weighed (200-220g). Animals were kept at 25°C and 45-60% humidity in a pathogen-free environment. Commercial rodent feed and water *ad labium* were given to rats. Animals were acclimatized for 7 days prior to trial commencement.

Dose selection

In the pilot experiment, LD_{50} was evaluated according to standard protocol (APHA, 1998). The sub-lethal dose of 1,4-dioxne (3000 mg/kg BW of rat) was selected at $1/30^{th}$ of 1,4-dioxane LD_{50}. The LD50 was 1,050 and 970 mg/kg b.w. in male and female mice, respectively (Radko and Cybulski, 2007). The safe doses of *S. marianum* were selected based on previously published literature (Soleimani *et al*., 2019; Fanoudi *et al*., 2020). The rats were gavaged on alternative days for 60 days with free access to food and water. All treatments were carried out corresponding to the entrenched protocols by the ethical committee of animal care and maintenance. Briefly, 40 mature male Sprague-Dawley rats of 215-220g were distributed into eight groups, viz., C: control group without any treatment, only *S. marianum* extract treated groups (S1, S2 and S3 treated at 85, 165 and 247 mg/kg, respectively), only 1,4-dioxane treated group G1 (positive control treated at 3000 ppm 1,4-dioxane), and Co-treated groups G2, G3 and G4 groups (co-treated at3000 ppm 1,4-dioxane+85 mg/kg *S. marianum* Ethanolic Leave Extract (SLE), G3: co-treated at3000 ppm 1,4-dioxane+165 mg/kg SLE and G4 group: co-treated at 3000 ppm 1,4-dioxane+247 mg/kg SLE, respectively (table 1). All groups were supplied with distilled water *ad libitum* and commercial rodent feed having 19% crude protein. Rats were given the defined doses orally gavage for 60 days on an alternative day. Appearance and behavioral alterations, and animal mortality were noted twice a day. At the end of the experimental period (day 61), the animals were fasted overnight, anesthetized using ketamine hydrochloride (30 mg/kg body weight), and subsequently euthanized for sample collection (Latif *et al*., 2019). Approximately 10 ml fresh blood sample was

collected to analyze hematological and serum profile through midray hematology analyzer bc-10. During dissection, liver and kidneys were removed and stored in 10% neutral buffered formalin for further histological investigations.

Hematology and blood profile

Blood and serum profiles were analyzed by mindray hematology analyzer bc-10 according to manufacturer's guidelines.

Histological studies

For histological studies, small tissues of the liver and kidney (2-4mm) were fixed in sera (ethanol: 60ml, formaldehyde: 30ml and glacial acetic acid: 10ml). Samples were dehydrated according to standard protocols. Then tissues were embedded in cedar wood oil till they became transparent/ clear at 25°C. After dehydration, embedding was done in various steps according to the standard protocol. Then tissues were kept in molds for block formation, and then 5µm sections were cut by microtome (Histoline MR-2258). Slides were stained with hematoxylin and eosin and studied under the microscope and photographed for further analysis (Vasantharaja and Ramalingam, 2018). Histological changes were assessed using a semi-quantitative five-point grading scale: (-) indicating normal histological architecture; (+/-) representing mild alterations; (+) for moderate changes; (+/+) for severe alterations; and (+/+/+) denoting very severe histopathological damage (Noureen *et al*., 2018).

STATISTICAL ANALYSIS

The data were statistically analysed using Minitab 17 software using the General Liner Model (ANOVA) to compare the main effect of either 1,4-Dioxane (T), *S. marianum* extract (S) and their TxS interaction on the body weight, hematological, liver, kidney and lipid profiles of rats. These effects were declared significant if $P < 0.05$ and highly significant if $P < 0.01$. The Tukey's test was used if there were more than two means to compare at $P < 0.05$. Quantitative data were explicated as mean ± SD.

RESULTS

Body weight

Table 2 shows a comparison of body weight and ANOVA of male Sprague-Dawley rats on different days among different groups. It was observed that the body weight of rats in the G1 group started severely declining due to 1,4-dioxane administration from the 15th day of exposure (204.8±1.09) and continued throughout the experiment (146±1.41) as compared to the control group. On the other hand, *S. marianum* treated groups showed improvement in body weight of rats in a dose-dependent manner. In co-treated groups, the highest improvement in body weight among treatment groups was recorded in G4 (288.2±3.96) treated at 247 mg/kg dose of *S. marianum*. There was a highly significant difference in the body weight of male Sprague-Dawley rats after 15 days of treatment among groups.

Blood profile

Table 3 shows significant reduction in values of RBCs (5.16±0.06), HGB (9.77±0.08), HCT (28.22±1.06), MCV (34.87±0.8), MCHC (31.37±1.40), LYM (7.03±0.26), MID (0.52±0.03) and GRA (0.89±0.02), while higher values of WBC (18.14±0.85), MCH (18.10±0.38), and PLT (797.7±8.43) were observed in G1 (treated (at 1,4-dioxane 3000 ppm) in contrary to other groups ($p<0.0.5$). A dose-dependent improvement in the hematological profile of rats was observed in groups treated with only *S. marianum* extract. It was observed that *S. marianum* extract administration at 247 mg/kg in co-treated groups significantly increased RBCs (7.59±0.14), HGB (11.19±0.2), HCT (41.06±1.04), MCV (50.32±1.2), MCHC (45.53±0.74), LYM (9.11±0.16), MID (1.26±0.03) and GRA (1.75±0.11) while decreased WBC (11.29±0.42), MCH (15.42±0.27) and PLT (599±4.81) values ($p<0.0.5$). ANOVA shows highly significant variations in 1,4-dioxane, and *S. marianum* treatments and their interactions ($P<0.01$, table 3).

Hepatotoxicity

Table 4 shows the liver profile of Spague-Dawley rats administered with 1,4-dioxane and *S. marianum* leaves extract. It was observed that ALT (26.68±1.15) was decreased in the G1 group, while AST (144.59±2.04), ALP (337.11±2.39) and Bilirubin (0.74±0.02) were increased in G1 group versus other groups. Only *S. marianum* treated groups showed an improvement in liver profile in a dose-dependent manner. It was observed that *S. marianum* combined dose at 247mg/kg significantly ($P<0.05$) increased ALT (43.08±0.89) and decreased AST (119.64±2.50), ALP (293.08±5.05) and Bilirubin levels (0.49±0.02) according to normal values. In this study, main effects and their interactions showed highly significant variations in all parameters of liver profile ($P<0.01$).

Nephrotoxicity

Table 5 shows the kidney profile of Sprague-Dawley rats administered with 1,4-dioxane and *S. marianum* leaves extract alone or in combination. It was observed that 1,4-dioxane induced nephrotoxicity in treated groups such as urea (40.08±1.30) and creatinine concentrations (98.70±1.08) were increased in the G1 group after receiving 3000 ppm 1,4-dioxane. While, *S. marianum* leaves extract at 247mg/kg significantly decreased urea (25.87±0.73) and creatinine concentrations (68.38±1.42) in G4 by recovering their normal concentrations. In this study, *S. marianum* leave extract showed no adverse effects on the kidney. In this study, highly significant variations were observed with respect to 1,4-Dioxane and *S. marianum* treatment and their interactions in the kidney profile of male Sprague-Dawley rats (table 5, $P<0.01$).

Table 1: Distribution of male Sprague-Dawley rats into different groups according to treatments.

Sr. No.	Groups	Treatment	Dose (mg/kg)
1	C	Without any treatment (Control)	0
2	S1	*S. marianum*	85
3	S2	*S. marianum*	165
4	S3	*S. marianum*	247
5	G1	1,4-dioxane (Positive control)	3000 ppm
6	G2	1,4-dioxane +*S. marianum*	3000+85
7	G3	1,4-dioxane+ *S. marianum*	3000+165
8	G4	1,4-dioxane + *S. marianum*	3000+247

Table 2: Comparison of mean body weight (g) of male Sprague-Dawley rats among different groups and ANOVA showing significance of main effects and interactions

Groups		Mean ± SD of body weight (BW) at selected days (D)				
		0	15	30	45	60
C		217.4±4.15^{a}	233±2.23^{c}	249.4±3.13^{b}	271.6±3.20^{c}	293.6±2.40^{c}
S1		216.8±3.34^{a}	236±1.22^{c}	251±1.58^{b}	273.6±1.14^{c}	301.4±2.07^{b}
S2		216.8±3.42^{a}	241.8±2.16^{b}	257.4±1.51^{a}	280.2±1.92^{b}	309.2±1.48^{a}
S3		218±2.34^{a}	248.8±1.30^{a}	262±1.58^{a}	287±1.58^{a}	315.2±1.92^{a}
G1		216.8±4.20^{a}	204.8±1.09^{e}	180.6±3.57^{e}	169.8±1.92^{f}	146±1.41^{f}
G2		217.3±3.63^{a}	208.8±0.83^{e}	228.6±2.07^{d}	237.8±4.76^{e}	246.6±6.30^{e}
G3		216.8±3.42^{a}	220.8±1.92^{d}	238.4±2.19^{c}	250.8±3.34^{d}	261±3.93^{d}
G4		218±2.34^{a}	221±3.67^{d}	249.4±3.20^{b}	270.2±3.89^{c}	288.2±3.96^{c}
ANOVA	Df	BW (0D)	BW (15D)	BW (30D)	BW (45D)	BW (60D)
1,4-Dioxane (T)	1	ns	***	***	***	***
S. marianum (S)	3	ns	***	***	***	***
T × S	3	ns	***	***	***	***

Mean values with different small letters in columns varies significantly among different groups (P<0.05). ns= non-significant, ***= highly significant.

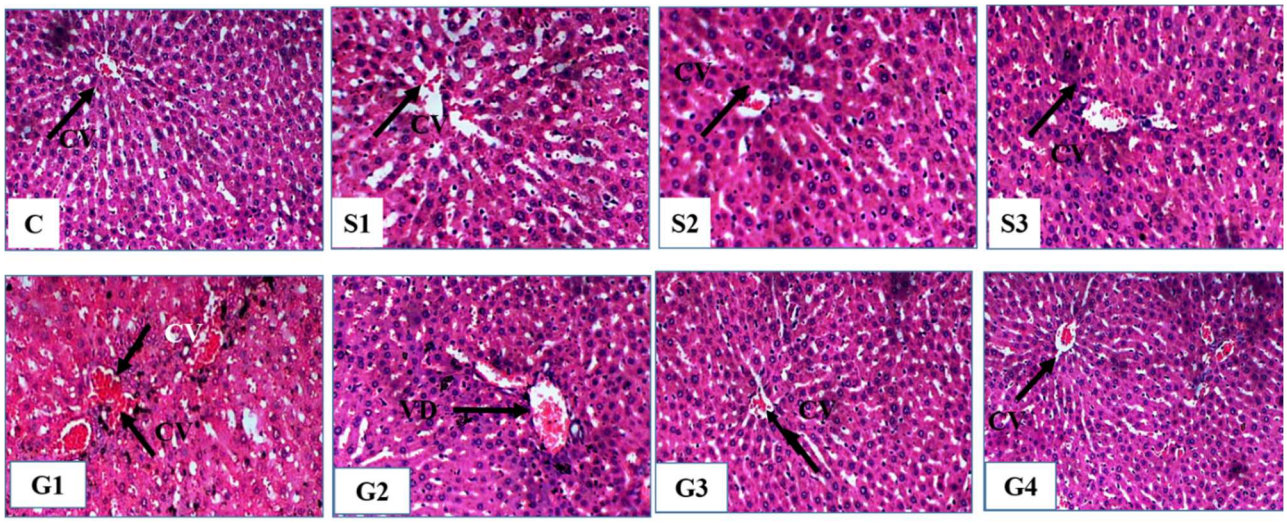

Fig. 1: Photomicrograph of rat liver tissue (H&E staining, magnification 40X). C) Control group and *S. marianum* groups (S1, S2, and S3) liver sections depicting normal liver morphology, normal hepatocytes (H) and sinusoids (S). G1 depicting 1,4-dioxane (3000ppm) treated group with visible vascular dilation (VD) .G2 depicting *S. marianum* 85 mg/kg + 1,4-dioxane 3000 ppm treated group with stabilizing central vein (CV), G3 depicting *S. marianum* 165 mg/kg + 1,4-dioxane 3000 ppm treated group showing more improvement in central vein (CV). G4 showing *S. marianum* 247 mg/kg + 1,4-dioxane 3000 ppm treated group with highly improved central vein (CV).

Lipid profile

Table 6 shows the lipid profile of male Sprague-Dawley rats administered with 1,4-dioxane and *S. marianum* leaves extract alone or in combination. It was observed that cholesterol (180.75±2.21), LDL (96.11±1.88), and triglycerides (169.37±1.73) were increased, while HDL (12.31±0.30) was decreased in G1 after 3000 ppm 1,4-dioxane treatment versus other groups. It was notable that *S. marianum* combined high dose of 247 mg/kg significantly ($p<0.05$) normalized the lipid profile compared to other treated groups. Male Sprague-Dawley rats treated with only *S. marianum* extract showed improvement in lipid profile in a dose-dependent manner. ANOVA showed significant variations in all treated groups ($P<0.01$).

Histology

Fig. 1 shows photomicrograph of rat liver tissue in different groups, where liver sections in the control group and *S. marianum* treated groups showsed normal structure, while positive control showed fat deposition, inflammation, and severe injuries. Rats treated at 85, 165, and 247 mg/kg provide protection against toxicant in a dose-dependent manner and the liver histoarchitecture was improved in co-treated rats treated with high doses of *S. marianum*. The intensity of histological changes in liver tissue among different treatment groups is shown in table 7.

Fig. 2 shows the photomicrograph of rat kidney in different groups. The histological study of the kidney of control and *S. marianum* treated groups exhibited regular structure of glomerulus and renal tubes, while those in G1 (3000 ppm 1,4-dioxane) showed hemorage, tubular degradation with visible pyknotic nucleus, vascular dilation and glomerulosclerosis (GS). Co-treated groups at 1,4-dioxane (3000 ppm) and *S. marianum* from low to high (85, 165 and 247 mg/kg) doses showed improvement of structure in the lining and of epithelial cells of renal tubes in a dose-dependent manner. The G4 group co-treated with 1,4-dioxane 3000 ppm + *S. marianum* 247 mg/kg showed a highly improved structure of the glomerulus and collecting ducts. Table 8 shows the intensity of histological changes in kidney tissue among different treatment groups.

DISCUSSION

1,4-Dioxane has historically been used to stabilize chlorinated solvents and more recently has been found as a contaminant of numerous consumer and food products (Pollitt *et al*., 2019). Medicinal plants are extensively comprised of natural antioxidants such as carotenoids and polyphenols, which display an extensive range of biological characteristics including anti-aging, anti-inflammatory, anticancer and anti-atherosclerosis (Xu *et al*., 2017). *Silybum marianum* is beneficial for liver ailments like hepatitis, cirrhosis and gallbladder disorders. Silymarin, being the main active compound of *S. marianum,* is commonly utilized as a herbal supplement in the US (Marmouzi *et al*., 2021). Positive impacts of silymarin in ischemia reperfusion and nephrotoxicity in rats have been reported by previous studies (Tan *et al*. 2015, Soodvilai *et al*. 2019; Elhassaneen *et al*., 2024).

In the current study, administration of 1,4- dioxane at 3000 ppm significantly reduced the body weights of rats in the G1 (204.8±1.09 g) than the control group (233.0±2.23 g) on the 15th day of exposure, which further declined to 146.0±1.41 at 60th day of exposure. The current study reported the deleterious effects of 1,4-dioxane on the body weight of rats, while in co-treated groups improvement in body weight was observed in a dose-dependent manner. Our study is supported by Lieshchova and Brygadyenko (2023), who investigated that the addition of *S. marianum* at the dose of 155 mg/day significantly improved body weight of rats ($p<0.05$). Current findings are also validated by Maaliah *et al*., 2024, who investigated that the doses of 100 and 300mg/kg of *S. marianum* substantially increased the body weight of rats than diabetic rats.

Significantly decreased RBCs, HB, HCT, MCV, MCHC, LYM, MID, and GRA and increased WBC, MCH, and PLT were observed in the G1 group treated with 1,4-dioxane at 3000 ppm. Due to a decline in RBCs, HCT and HB, chronic anemia can be induced in the body. WBCs can indicate high chances of infection in the body. The current findings are in agreement with the study by Maakaron, 2025, who revealed that high doses of ether caused toxicity similar to dioxane, which caused a decrease in HB, RBCs, and HCT concentrations and insufficient production and pigmentation in blood than the control group. Zivot *et al*., 2018 also investigated that the groups treated with diethyl ether can cause reduction of RBCs and bone marrow functions, which agrees with the current study. Bouhalit *et al.,* 2017 depicted that the 100 mg/kg dose of Silymarin significantly increased red blood cell count (RBC), hemoglobin (Hb) concentration, platelet counts (Plt), hematocrit and decrease in white blood cell counts (WBC) in Wister albino rats which strongly supported current study, where supplementation of *S. marianum* at 247 mg/kg significantly improved the blood profile in Sprague-Dawley rats. Eid *et al.,* 2022 stated that silymarin at1000 mg/kg significantly improved total erythrocyte count, leukocytes count, PCV and Hb, which validated our findings that the higher dose of *S. marianum* significantly improved blood profile in rats.

In the current study 1,4-dioxane adversely affected the liver biochemistry and it's functioning. Adverse decline in ALT (26.68±1.15) and increase in AST (144.5±2.04) and ALP (337.1±2.39) were clear indications of cytotoxicity induced by 1,4-dioxane at 3000 ppm in positive control group (G1). Lower levels of ALT and higher levels of AST and ALP can induce several chronic diseases related to the liver, kidney and other vital organs.

Table 3: Hematological profile of male Sprague-Dawley rats among different groups and ANOVA showing significance of main effects and their interactions

Groups		RBC (10^{12}/l)	WBC (10^9/l)	HGB (g/dl)	MCH (pg)	PLT (10^9/l)	HCT (%)	MCV (fl)	MCHC (g/dl)	LYM (10^9/l)	MID (10^9/l)	GRA (%)
Control		8.28±0.04^{a}	10.18±0.07^{e}	12.83±0.10^{a}	14.86±0.08^{e}	553.5±2.43^{d}	49.33±1.20^{d}	55.78±0.93^{e}	48.00±1.05^{e}	12.08±0.22^{e}	1.75±0.01^{b}	3.72±0.02^{ab}
S1		8.33±0.02^{a}	10.16±0.01^{e}	12.76±0.01^{a}	14.83±0.01^{e}	549.7±0.49^{e}	51.90±0.14^{c}	57.18±0.04^{bc}	49.12±0.03^{e}	12.85±0.02^{b}	1.76±0.01^{b}	3.69±0.02^{b}
S2		8.38±0.02^{a}	10.12±0.03^{e}	12.81±0.01^{a}	14.80±0.01^{e}	545.8±4.83^{de}	55.01±0.03^{b}	58.64±0.03^{ab}	51.95±0.11^{b}	13.58±0.01^{a}	1.82±0.01^{a}	3.72±0.02^{ab}
S3		8.41±0.01^{a}	10.10±0.01^{e}	12.88±0.02^{a}	14.77±0.00^{e}	542.2±1.59^{de}	58.01±0.12^{a}	59.33±0.03^{a}	55.05±0.04^{a}	13.91±0.03^{a}	1.86±0.01^{a}	3.80±0.01^{a}
G1		5.16±0.06^{e}	18.14±0.85^{a}	9.77±0.08^{d}	18.10±0.38^{a}	797.7±8.43^{a}	28.22±1.1^{h}	34.87±0.89^{g}	31.37±1.4^{g}	7.03±0.26^{g}	0.52±0.03^{f}	0.89±0.02^{f}
G2		6.21±0.02^{d}	15.26±0.23^{b}	9.90±0.11^{d}	17.18±0.23^{b}	706.8±5.19^{a}	31.05±1.44^{g}	40.49±1.28^{f}	35.8±0.92^{f}	7.49±0.33^{f}	0.62±0.03^{e}	1.18±0.05^{e}
G3		6.47±0.06^{e}	13.09±0.29^{c}	10.61±0.12^{c}	16.28±0.17^{c}	666.8±7.99^{b}	35.44±1.09^{f}	45.08±1.15^{e}	41.29±1.11^{e}	8.26±0.17^{e}	0.88±0.01^{d}	1.33±0.02^{d}
G4		7.59±0.14^{b}	11.29±0.42^{d}	11.19±0.23^{b}	15.42±0.27^{d}	599±4.81^{c}	41.06±1.04^{e}	50.32±1.24^{d}	45.53±0.74^{d}	9.11±0.16^{d}	1.26±0.03^{c}	1.75±0.11^{c}
ANOVA	Df	RBC	WBC	HGB	MCH	PLT	HCT	MCV	MCHC	LYM	MID	GRA
1,4-Dioxane (T)	1	***	***	***	***	***	***	***	***	***	***	***
S. marianum (S)	3	***	***	***	***	***	***	***	***	***	***	***
TxS	3	***	***	***	***	***	***	***	***	***	***	***

Mean values with different small letters in columns varies significantly among different groups (P<0.05). ***= highly significant

The current study showed a similar pattern of liver profiles investigated by Stickney *et al*. (2003), who observed an increase in hepatocyte cell proliferation through 1,4-dioxane exposure, and it also promoted tumor formation in the liver of rats. Chen *et al*. (2022) described liver toxicity due to sub-chronic exposure to 1,4-dioxane at 5000 ppm in mice. Eid *et al*., 2022 stated that the serum total proteins, amino-transferase (AST) and alanine aminotransferase (ALT), urea and creatinine significantly improved at the dose of 1000 mg/kg of silymarin than Ochratoxin-A treated group which validated current study, where 247 mg/kg dose of *S. marianum* significantly improvement liver profile. Sherif and Al-Gayyar (2013) treated the sodium nitrite group with silymarin at 25 mg/kg daily for 12 weeks resulted in significant, dose-dependent improvements in liver function markers, also supporting the current study. Khazaei *et al*., 2022 stated that the ingestion (0.5% and 1%) of milk thistle powder significantly increased feed intake, body weight, improved carcass components, blood parameters and lipid profile, which supported current findings where *S. marianum* improved all selected parameters. In this study, 1,4-dioxane induced histological alterations in targeted organs**.** 1,4-dioxane at 3000 ppm treated group showed visible vascular dilation, proliferating the portal vein and destructing the central vein. Kano *et al*. (2008) also observed degenerative alterations like vacuolic alterations in hepatocytes, cell necrosis and cell infiltration in the liver of male and female rats exposed to 1,4-dioxane. Kasai *et al*. (2008) also observed lesions, cell necrosis, and swelling of hepatocytes in the liver of rats exposed to 1,4-dioxane at 3200 ppm. Elhassaneen *et al*., 2024 stated that supplementation of the rat diets with SME (200 to 800 mg/kg bw/d), a corrective effect on liver structure was observed against Benzo[a]pyrene, which validated current findings, where 247 mg/kg dose of *S. marianum* normalized the histological architecture of the liver tissue. In the current study, 1,4-dioxane also induced histological alterations like pyknotic nucleus, vascular dilation and glomerulosclerosis in the kidney of the positive control group (G1). Qiu *et al*. (2019) also observed glomerular cell proliferation, hyperemia and little inflammation in the 500 mg/L dioxane treated group. The current study is in good agreement with Karakuş *et al*., 2017, who investigated that 100 mg/kg/day *S. marianum* extract by gavage showed a corrective effect against histopathological changes in the kidney of rats caused by carbon tetrachloride.

CONCLUSION

This study has highlighted the importance of medicinal plants in treating chemical-induced toxicity. Study revealed that *S. marianum* leaves extract has great potential to improve the hematological profile as well as liver and kidney damage in SD rats caused by 1,4-dioxane.

Table 4: Liver profile of Spague-Dawley rats among different groups and ANOVA showing significance of main effects and their interactions

Groups		ALT (U/ml)	AST (U/ml)	ALP (U/l)	BIlirubin (mg/dl)
C		45.9±0.81^{a}	112.36±1.42^{e}	285.57±0.91^{e}	0.48±0.01^{de}
S1		44.8±0.02^{ab}	109.6±0.52^{ef}	283.4±1.56^{e}	0.47±0.00^{de}
S2		44.5±0.01^{abc}	107.2±0.10^{fg}	281.5±2.05^{e}	0.46±0.00^{ef}
S3		43.9±0.46^{bc}	105.5±0.10^{d}	278.1±1.06^{f}	0.43±0.01^{f}
G1		26.68±1.15^{f}	144.5±2.04^{a}	337.1±2.39^{a}	0.74±0.02^{a}
G2		31.5±0.91^{e}	135.26±2.42^{b}	327.4±1.52^{b}	0.61±0.01^{b}
G3		38.01±0.68^{d}	130.66±2.87^{c}	311.16±3.08^{c}	0.54±0.02^{c}
G4		43.08±0.89^{c}	119.64±2.50^{d}	293.08±5.05^{d}	0.49±0.02^{d}
ANOVA	Df	ALT	AST	ALP	Bilirubin
1,4-Dioxane (T)	1	***	***	***	***
S. marianum (S)	3	***	***	***	***
T × S	3	***	***	***	***

Means values with different small letters in columns varies significantly among different groups (P<0.05). ***= highly significant

Table 5: Kidney profile of Spague-Dawley rats among different groups and ANOVA showing significance of main effects and their interactions

Groups		Urea (mg/dl)	Creatinine (µmol/l)
C		24.3±0.78^{d}	74.18±0.74^{d}
S1		22.1±0.02^{e}	74.0±0.02^{d}
S2		21.3±0.03^{e}	72.4±0.03^{de}
S3		20.6±0.03^{e}	70.2±0.02^{ef}
G1		40.08±1.30^{a}	98.7±1.08^{a}
G2		35.98±0.98^{b}	92.93±1.60^{b}
G3		30.52±0.92^{c}	85.84±1.73^{c}
G4		25.87±0.73^{d}	68.3±1.42^{f}
ANOVA	Df	Urea	Creatinine
1,4-Dioxane (T)	1	***	***
S. marianum (S)	3	***	***
T × S	3	***	***

Means values with different small letters in columns varies significantly among different groups (P<0.05). ***= highly significant.

Table 6: Lipid profile of male Sprague-Dawley rats among different groups and ANOVA showing significance of main effects and their interactions

Groups		Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)
C		134.22±1.09^{e}	24.6±0.88^{a}	64.43±1.71^{d}	132.6±2.58^{e}
S1		132.7±0.06^{ef}	24.8±0.05^{a}	61.7±0.05^{e}	132.6±1.12^{e}
S2		131.7±0.04^{ef}	25.3±0.04^{a}	60.5±0.03^{ef}	132.5±0.04^{e}
S3		130.8±0.09^{f}	25.9±0.05^{a}	59.2±0.02^{f}	131.5±0.03^{e}
G1		180.75±2.21^{a}	12.3±0.30^{e}	96.11±1.88^{a}	169.37±1.73^{a}
G2		169.9±1.59^{b}	16.79±0.76^{d}	85.04±0.93^{b}	155.21±1.17^{b}
G3		149.98±1.99^{c}	20.07±1.25^{c}	73.9±1.30^{c}	141.4±1.44^{c}
G4		140.47±1.39^{d}	22.73±1.01^{b}	66.54±1.09^{d}	136.63±0.94^{d}
ANOVA	Df	Cholesterol	HDL	LDL	Triglycerides
1,4-Dioxane (T)	1	***	***	***	***
S. marianum (S)	3	***	***	***	***
T × S	3	***	***	***	***

Means values with different small letters in columns varies significantly among different groups (P<0.05). ***= highly significant.

Table 7: Histological changes in the liver of male Sprague-Dawley rats among different groups.

Groups	Inflammation	Vascular dilation	Hemorrhage
Control	-	-	-
S1	-	-	-
S2	-	-	-
S3	-	-	-
G1	+++	++	+++
G2	++	++	++
G3	+	+	-/+
G4	-/+	-	-/+

[(−) no histological alterations (normal histological structure); (+/-) mild histological alterations; (+) moderate histological alterations; (++) severe histological alterations; and (+++) very severe histological alterations]

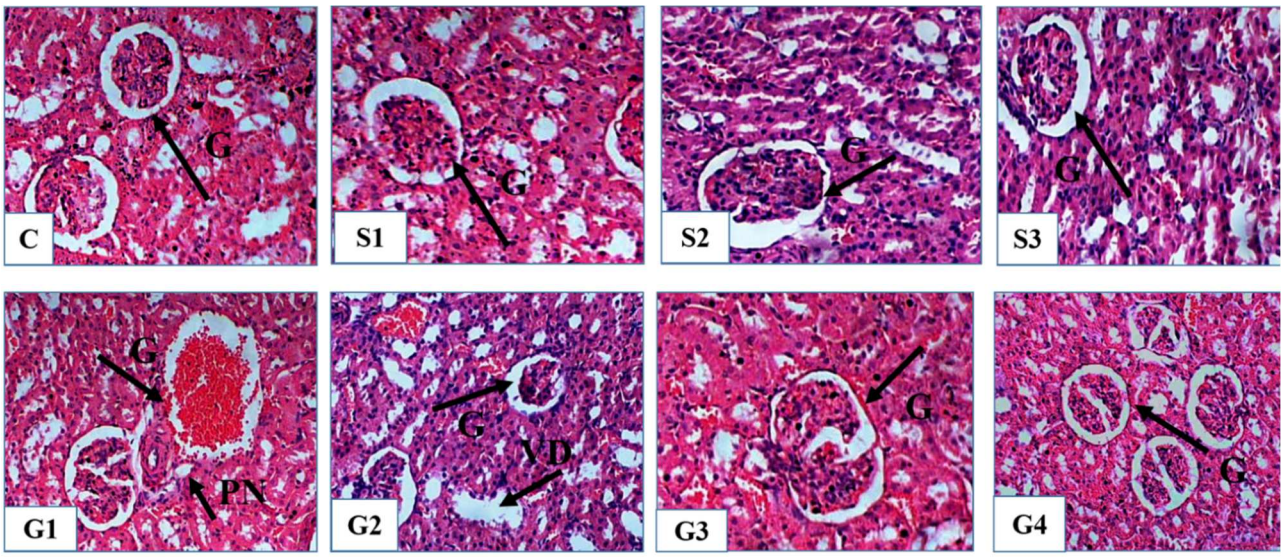

Fig. 2: Photomicrograph of rat kidney (H&E staining, magnification 40X) in different groups. C) kidney section from control group, S1, S2 and S3 depicting normal kidney morphology displaying normal glomerulus (G). G1) depicting 1,4-dioxane (3000 ppm) treated group with visible pyknotic nucleus (PN), vascular dilation (VD) and glomerulosclerosis (GS), G2) showing *S. marianum* 85 mg/kg + 1,4-dioxane 3000 ppm treated group with improved glomerulus (G) and vascular dilation (VD), G3) depicting *S. marianum* 165 mg/kg + 1,4-dioxane 3000 ppm treated group showing more improvement in glomerulus (G). G4) 1,4-dioxane 3000 ppm+ *S. marianum* 247 mg/kg treated group showed highly improved glomerulus (G) and collecting ducts (CD).

Table 8: Histological changes in the kidney of male Sprague-Dawley rats among different groups

Groups	Pyknotic Nucleus	Inflammation	Tubular Dilation	Glomerular dystrophy
Control	-	-	-	-
S1	-	-	-	-
S2	-	-	-	-
S3	-	-	-	-
G1	+++	+++	++	+++
G2	++	+++	++	++
G3	+	++	+	-/+
G4	_	-/+	-	-/+

[(−) no histological alterations (normal histological structure); (+/-) mild histological alterations; (+) moderate histological alterations;(++) severe histological alterations; and (+++) very severe histological alterations]

This is a novel study as *S. marianum* has never been used previously against this very common contaminant. *S. marianum* has proven its therapeutic potential against 1,4-dioxane induced toxicity. Therefore, it is concluded that *S. marianum* may be considered as a crucial ethnomedicine in treating many health issues caused by 1,4-dioxane and other related contaminants.

Ethical approval

The study was approved by the Ethical Review Committee (ERC) of Government College University Faisalabad on animal experimentation with Ref. No. GCUF/ERC/21/01A.

Author's contribution

Farhat Jabeen designed and supervised the study and reviewed the manuscript. Abdul Rauf has carried out all *in vivo* studies, phytochemical analysis, bioassays, statistical analysis and prepared the initial draft of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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