Trop J Nat Prod Res, November 2021; 5(11):1927-1933 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Ameliorative Effects of *Saccharum officinarum* (Sugarcane) Peel Extract against Paracetamol-Induced Disorders in Hematological Indices and Splenic Changes in Adult Male Sprague Dawley Rats

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ARTICLE INFO

Article history:
Received 12 November 2020
Revised 17 March 2021
Accepted 14 November 2021
Published online 05 December 2021

ABSTRACT

In this era, global attention has been directed towards the use of natural antioxidants to protect against drug-related effects. The vital role of natural antioxidant agents is the protection against free radicals. This study was conducted to evaluate the impacts of sugarcane peel extract on splenic complications induced by paracetamol overdose. Forty male rats (150-180 g) were maintained under normal laboratory conditions. The rats were evenly divided into four groups (10 rats per group). Group I was given distilled water; Group II was administered with a single overdose of paracetamol (3 g/kg bwt) on the 13th day of the trial; Group III was pretreated with sugarcane peel extract at a dose of 2 mL for 13 days in addition to paracetamol (3 g/kg bwt) on the 13th day, 1 hr before the treatment; while Group IV received pretreatment with silymarin (0.3 g/kg bwt) for 13 days in addition to paracetamol on the 13th day, 1 hr before the treatment. At the end of the trial (the 14th day), some biochemical parameters, as well as spleen histopathology were evaluated. The results revealed that a single overdose of paracetamol caused a significant increase in malondialdehyde, red blood cells, hemoglobin, hematocrit, and neutrophils. However, there was a significant decrease in superoxide dismutase, catalase, glutathione, total leucocyte, and platelet counts. In contrast, the administration of the extract improved these changes. The findings of this study indicated that sugarcane peel extract has great impact and potential as a cure for splenic complications from paracetamol overdose.

Keywords: Hematological indices, Paracetamol, Silymarin, Spleen, Sugarcane peel extract.

Introduction

The global trend in natural antioxidants is aimed at mitigating drug-related side effects.[1]
In addition to the modulation of cell receptors and leucocyte expression, herbal medicine modulate numerous functions, including free radical production, protein and immunological gamma globulin secretion.[2-5] The spleen is vital in hematopoiesis because it allows senescent and atypical erythrocytes to pass through, as well as opsonized platelets, white blood cells (WBCs), and microbe exclusion.[6] Platelets are key mediators of hemostasis and thrombosis. They can be inhibited by a nonsteroidal anti-inflammatory drugs (NSAIDs).[7] Many healing plants and nutritional enhancers have been used since ancient times and they have been shown to have numerous biological activities such as antimicrobial and cytotoxic effects. Treatment of liver problems can be achieved by creating standardized and clinically proven selective prescriptions with a high safety profile.[8] Various health disorders are related to medications and xenobiotics that exist in the atmosphere.[9,10] *Saccharum officinarum* L. (sugarcane) species have been used in folk medicine as antidotes, antiseptic, anti-venom, bactericide, cardio-tonic, demulcent, intoxicant and as antiulcer.[11] Consequently, it is used as an antioxidant, anti-inflammatory,[12,13] and antitumor agent[11] due to its ability to inhibit free radicals.[14] Paracetamol is commonly used as an analgesic and antipyretic agent which produces acute liver damage at a higher dose.[15] The hepatotoxicity of paracetamol has been attributed to the formation of N-acetyl parabenzoquinone imine (NAPQI) that causes aerophilic stress and glutathione depletion.[16] It is a well-known antipyretic and analgesic agent that produces viscous mortification at a higher dose.[17] A key mechanism of toxicity is the hemoprotein P450-catalyzed metabolic activation of paracetamol (APAP), which generates the reactive substance, N-acetyl-p-benzoquinone imine (NAPQI), initiating toxicity in both rodents and humans.[18]
The aim of this study was to evaluate the ameliorative effects of *S. officinarum* (sugarcane) peel extract against paracetamol-induced disorders in hematological indices and splenic changes in adult male Sprague Dawley rats.

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Citation: Soliman NA, El Dahmy SI, Shalaby AA, Mohammed KA, Taha NM. Ameliorative Effects of *Saccharum officinarum* (Sugarcane) Peel Extract against Paracetamol-Induced Disorders in Hematological Indices and Splenic Changes in Adult Male Sprague Dawley Rats. Trop J Nat Prod Res. 2021; 5(11):1927-1933. doi.org/10.26538/tjnpr/v5i11.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Source of plant material
The current name of sugarcane is *Saccharum officinarum* L., 1753, and the taxonomic ID is 4547. Peeled sugarcane chopsticks were collected from a local center in Sharkia Governorate, Egypt in June 2017. The sugarcane chopsticks were cleaned, and the waste materials (peels) were dried in the open air under the shade for 7 days. Then, the dried peels were pulverized to powder using a mechanical grinder.

Sources and preparation of chemical agents
Acetaminophen (500 mg paracetamol per tablet) was obtained from El Nasr Pharmaceutical Chemical Company. It was prepared as a

suspension by using a metallic tube (oral gavage) for dosage. Silymarin Plus (140 mg/sachet) was purchased from Sedeco Pharmaceutical Company, Egypt. The powder was dissolved in distilled water and then administered orally to the experimental animals.

Source of experimental animals
In this study, 40 healthy adult male rats weighing 150-180 g were used. The animals were obtained from the animal house of the Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt. The rats were maintained under standard laboratory conditions of aeration and room temperature. They were allowed free access to a standard diet (the commercial rodent chow) and water *ad libitum*.

Ethical approval
Protocol No. 1-1-2018 which was used in this study was approved by the Scientific and Medical Research Center (ZSMRC), Faculty of Medicine, Zagazig University, Sharkia, Egypt. Also, the guideline of the institutional Animal Ethics Committee was followed according to the regulation of the committee for experiments on animals (Protocol No.: 902 dated: 26/09/2009).

Preparation of sugarcane peel extract
Sugarcane powder (650 g) was extracted with 20 L of absolute ethanol by maceration at room temperature, three times in 6 days. The extract was concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland), and the concentrated extract was fractionated with a separating funnel (n-hexane 40-60). The n-hexane solvent was evaporated with a rotary evaporator at low pressure to give 250 g of the dried residue. Acacia gum (100 g) was added to 100 g of the dried extract and 100 mL of hot distilled water at 60°C. The mixture was mixed thoroughly and then distilled water was added to make up to 1 L. The suspension (2 mL) was used as a daily dose.

Experimental animal grouping and treatment
In this study, the 40 experimental Sprague Dawley male rats were randomly and equally divided into 4 groups (10 rats per group). The rats were kept in metal cages (5 rats/cage) and sustained under the laboratory conditions of aeration and temperature in the Scientific and Medical Research Center (ZSMRC), Faculty of Medicine, Zagazig University (No P1-1-2018). The animals were allowed free access to a standard diet (the commercial rodent chow) and water *ad libitum*. All animals were kept under observation and acclimation to the environment for two weeks before starting the experiment. Group I (control group), was orally given distilled water. Group II was administered with a single overdose of acetaminophen (3 g/kg bwt) from the prepared suspension on the 13th day of the trial to induce spleen injury (toxic control).[19,20] Group III was pre-treated with *Saccharum officinarum* peel extract daily at a dose of 2 mL for 13 days, in addition to acetaminophen (3 g/kg bwt) on the 13th day, 1 h before the treatment. Group IV received pretreatment with silymarin (0.3 g/kg body weight)[21] for 13 days in addition to acetaminophen (3 g/kg bwt) on the 13th day, 1 h before the treatment. The spleen was removed from each rat in all the groups and was rinsed with cold normal saline, dried on a filter paper, and kept in a 10% formalin solution for histological examinations.

Laboratory analysis
The obtained blood serum was kept at -20°C to estimate MDA, superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) serum levels. Blood serum GSH levels were determined using kits (GSH CAT NO GR2511) of Biodiagnostic Company, Egypt. Blood serum SOD activities were measured by using SOD kit NO. SD2521 as described by Nishikimi *et al*.[22] The blood serum CAT levels were determined by the kit (CAT.NO CA2517) of Biodiagnostic Company, Egypt. Serum MDA was determined using the biodiagnostic kit according to the procedure of Satoh.[23] Hematological parameters were determined in EDTA-blood by using a fully automated analyzer, HeCo C SEAC S.R.L.Via di Prato, 72/74 - Calenzano – Firenze - Italian Republic.

Statistical analysis
Numerical data were expressed as mean ± SD. The number of markers was analyzed by ANOVA through analysis of variance between groups.[24] Significant level was set at $P < 0.05$.

Results and Discussion

Effects of Saccharum officinarum peel *extract **on** oxidative stress*
The results presented in Tables 1-4 revealed that in acetaminophen (Group II), there was a significant increase ($P < 0.0001$) in the level of serum MDA (14.86 ± 2.88 nmol/g) and a significant decrease ($P < 0.0001$) in serum GSH (0.72±0.05 mmol/g), SOD (1.61 ± 0.42 U/g), and CAT (284.4 ± 4.35 U/g) compared to the control group. Rather than using silymarin plus, pretreatment with *Saccharum officinarum* peel extract (SOPE) resulted in a significant decrease ($P < 0.0001$) in serum MDA levels in Group III (Acetaminophen+ Sugarcane peel extract) at 6.971.76 nmol/g and a significant increase ($P < 0.0001$) in serum CAT up to the value of the normal control group. When compared to Groups II and IV, GSH and SOD levels in Group III increased (1.100.13 mmol/g and 3.0970.72U/g, respectively).

*Impact of Saccharum officinarum peel extract **on** vital hematological indices*
There was a significant increase ($P < 0.05$) in the hemoglobin value of Group II (16.57 ± 1.75 g/dL) and a significant increase ($P < 0.0001$) in the RBC count in Group II (8.51±0.79 x 10^6/µL) as shown in Figure 1. Also, it was observed that there was a significant decrease ($P < 0.001$) in platelet count for Group II (605.9±161.95 x10^3/µL) when compared to the negative control (Group I) which ranged from 5.40 - 8.80 x10^3/µL to 6.66 ± 1.23 x10^3/µL. This decrease was found to be significant ($P < 0.001$ and $P < 0.05$) when compared with Groups III and IV. The sugarcane peel extract showed an increase in PLT count in Group III (773.3 ± 169.89x10^3/µL), and a significant decrease ($P < 0.001$) in WBC when compared to the values of group IV. Figure 2 shows an increase in hematocrit and a decrease in MCHC values as well as hemoglobin and RBC values in Group II. The rise in neutrophils and the decrease in lymphocyte count is a good marker for overdose toxicity as highlighted in Figure 3. Therefore, the extract has a higher protective effect against paracetamol (single overdose) than silymarin plus.
Acetaminophen toxicity is iatrogenic due to hematological effects. Paracetamol overdose causes liver damage based on the dose and this damage causes alterations in the red blood cell count and packed cell volume.[25,26] Paracetamol has the potential to inhibit glycoprotein unleashed from the kidneys,[25] leading to a reduction in erythrocytes production, Hb concentration, and Hct value that could result in anemia. Furthermore, the decrease in haematological parameters caused by paracetamol could also be attributed to the hyper-activity of bone marrow in the damaged integrity of red blood cells that were simply destroyed within the circulation.[27] The results obtained in the current study showed that treatment with paracetamol induces changes in some hematological parameters in rats. Overdose of paracetamol resulted in hematotoxicity and an increase in the number of RBCs. The number of WBC decreased, which led to changes within the synthesis and performance of blood cells, redoubled decomposition, and hematolysis. Paracetamol affects biological processes and is linked to decreased erythrocyte production in circulation.[28] In agreement with the findings of Gomma,[29] acetaminophen (paracetamol) elicited a significant decrease in the Hgb concentration and WBC count during the entire experiment compared with the control group. This may be due to inflammatory changes that were induced in the liver by acetaminophen in the treated rats. The results showed that paracetamol conjointly caused a rise in the total leukocyte count (TLC) whereas it induced a decrease in the total erythrocyte count (TEC), hemoprotein (Hb), and packed cell volume (PCV).[30] These findings are in agreement with the results obtained in the previous studies.[31,32] The World Health Organization attributed the reduction in the total erythrocyte count to a lack of broken internal organ parenchyma to supply erythropoietinogen.[33]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Serum Malondialdehyde (MDA) (nmol/g) levels in the control and treated groups

N = 10	Control G1	Acetaminophen G2	Acetaminophen+ sugar cane peel extract G3	Acetaminophen+ Silymarin plus G4
Range	3.46-5.20	13.35-17.58	3.98-9.36	7.02-17.52
Mean	4.51	14.86	6.97	11.43
SD	0.596	2.88	1.76	3.48
F			35.67	
P of LSD vs G1		0.000 ***	0.03 *	0.000 ***
P of LSD vs G2			0.000 ***	0.003 **
P of LSD vs G3				0.000 ***

***: P < 0.001; **: P < 0.01; *: P < 0.05; P > 0.05: Non-significant (NS); G1-4: Groups 1-4.

Table 2: Serum glutathione (GSH) (mmol/g) activity in the control and treated groups

N = 10	Control G1	Acetaminophen G2	Acetaminophen+ sugar cane peel extract G3	Acetaminophen+ Silymarin plus G4
Range	1.40-1.87	0.63-0.79	0.851.23	0.94-1.14
Mean	1.69	0.72	1.10	1.03
SD	0.16	0.05	0.13	0.07
F			139.12	
P of LSD vs G1		0.000 ***	0.000 ***	0.000 ***
P of LSD vs G2			0.000 ***	0.000 *
P of LSD vs G3				0.133 NS

***: P<0.001; **: P<0.01; *: P<0.05; P>0.05: Non-significant (NS); G1-4: Groups 1-4.

Table 3: Serum superoxide dismutase (SOD) (U/g) activity in the control and treated groups

N = 10	Control G1	Acetaminophen G2	Acetaminophen + sugar cane peel extract G3	Acetaminophen + Silymarin plus G4
Range	3.36-4.77	1.12-2.22	2.0-4.10	2.09-2.56
Mean	4.04	1.61	3.097	2.29
SD	0.49	0.42	0.72	0.142
F		45.81		
P of LSD vs G1		0.000 ***	0.000 ***	0.000 ***
P of LSD vs G2			0.000 ***	0.004 **
P of LSD vs G3				0.001 **

***: P<0.001; **: P<0.01; *: P<0.05; P>0.05: Non-significant (NS); G1-4: Groups 1-4.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Serum catalase (CAT) (U/g) activity in the control and treated groups

N = 10	Control G1	Acetaminophen G2	Acetaminophen+ sugar cane peel extract G3	Acetaminophen+ Silymarin plus G4
Range	425.0-481.0	278.0-290.0	278-494	384-393
Mean	454.3	284.4	428.8	387.80
SD	22.60	4.35	68.26	3.23
F			43.06	
P of LSD vs G1		0.000 ***	0.123 NS	0.000 ***
P of LSD vs G2			0.000 ***	0.000 ***
P of LSD vs G3				0.015 *

***: P<0.001; **: P<0.01; *: P<0.05; P>0.05: Non-significant (NS); G1-4: Groups 1-4.

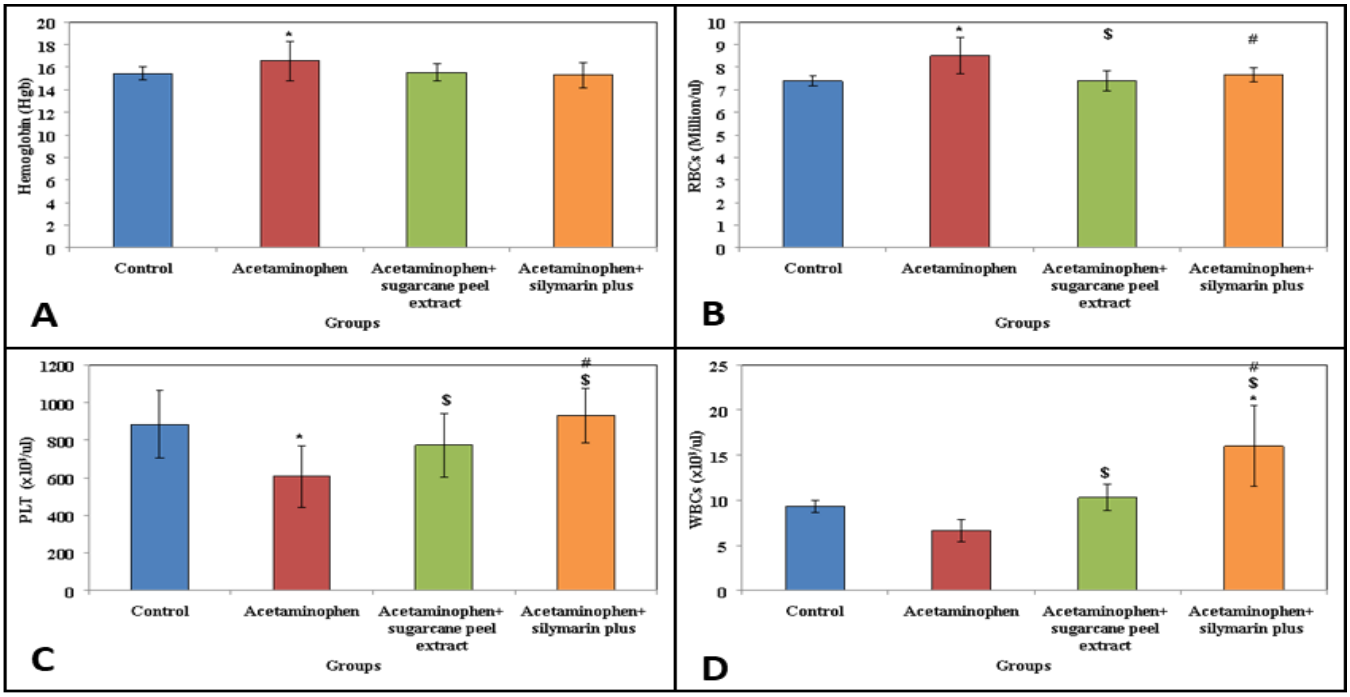

Figure 1: Impact of *Saccharum officinarum* peel extract on vital hematological indices in adult male Sprague Dawley rats. *: Significant *VS* control; $: Significant *VS* acetaminophen; #: Significant *VS Saccharum officinarum* extract.

The present study indicated that paracetamol administration and treatment caused an increase in RBC count and Hgb values. This observation is linked to paracetamol overdoses that caused liver harm and alterations within the red corpuscle count and PCV.[26] Moreover, a decrease resulted in hematological indices. The hyperactivity of bone marrow caused by paracetamol is also attributed to the damaged integrity of RBCs and simple destruction in the circulation.[28] However, paracetamol did not cause significant changes in total corpuscular volume and protoplasm counts,[34] co-administration of SOPE protected against paracetamol-induced hepatotoxicity. This can be attributed to the antioxidative and anti-inflammatory effects of SOPE. The oxidative stress imbalance between assembly and reactive oxygen species (ROS) scavenging is correlated with impaired inhibitor defense mechanisms. Traditional cellular metabolism of endogenous ROS products may be scavenged by inhibitor enzymes like CAT, SOD, GSH, and antioxidant (GPX).[35] Nonetheless, the production of ROS overwhelms the inhibitor system and results in oxidative stress that damages lipids, proteins, and nucleic acids, and even causes necrosis[36,37] The levels of ROS increased because of a disease range. Diseases such as chronic inflammation promote the discharge of assorted pro-inflammatory factors.[35,38] The protective effects of sugarcane extract are similar to the ameliorative effects of *Saussurea lappa* root extract. This reason provided a significant safeguard against toxicant-induced depletion of marker enzymes and oxidative stress to the extent of affecting the quality of natural antioxidants (α-tocopherol). Because of the high quantity of flavonoids, phenolic acid, steroids, and chlorogenic acid, this impact could be linked to reduced membrane damage and fluidity.[39] As the largest peripheral lymphatic organ, the spleen contains around one-fourth of the body's lymphocytes and plays a vital role in initiating immune responses.[40,41] The current results are consistent with another investigation that found that nonsteroidal anti-inflammatory drugs (NSAIDs) and panadol have adverse effects on immune-related organs such as bone marrow, spleen, and lymph nodes.[42]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

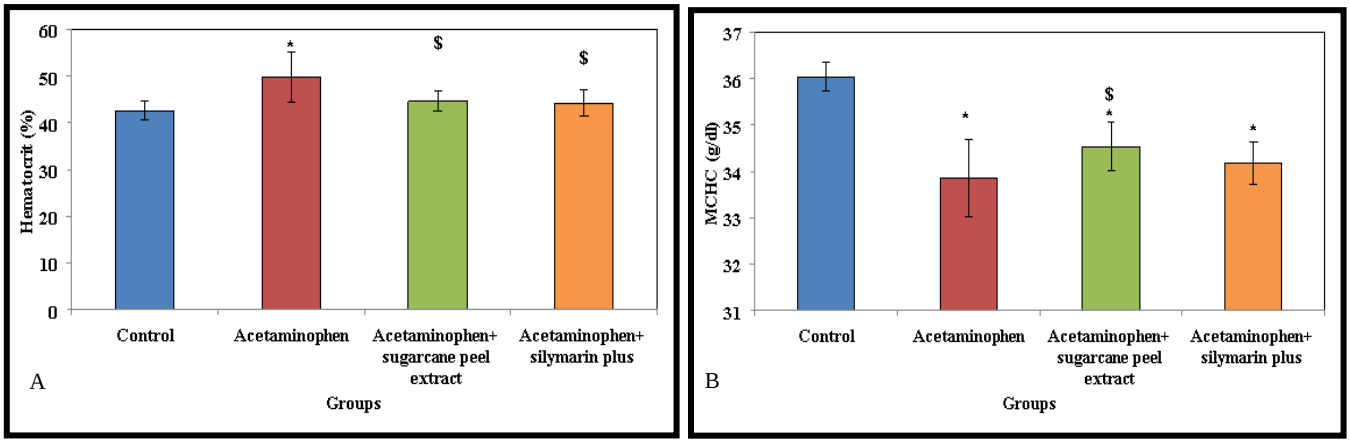

Figure 2: Effects of *Saccharum officinarum* peel extract on (A) Hematocrit (%); and (B) corpuscular hemoglobin concentration (MCHC) in adult male Sprague Dawley rats.
*: Significant *VS* control; $: Significant *VS* acetaminophen; #: Significant *VS Saccharum officinarum* extract.

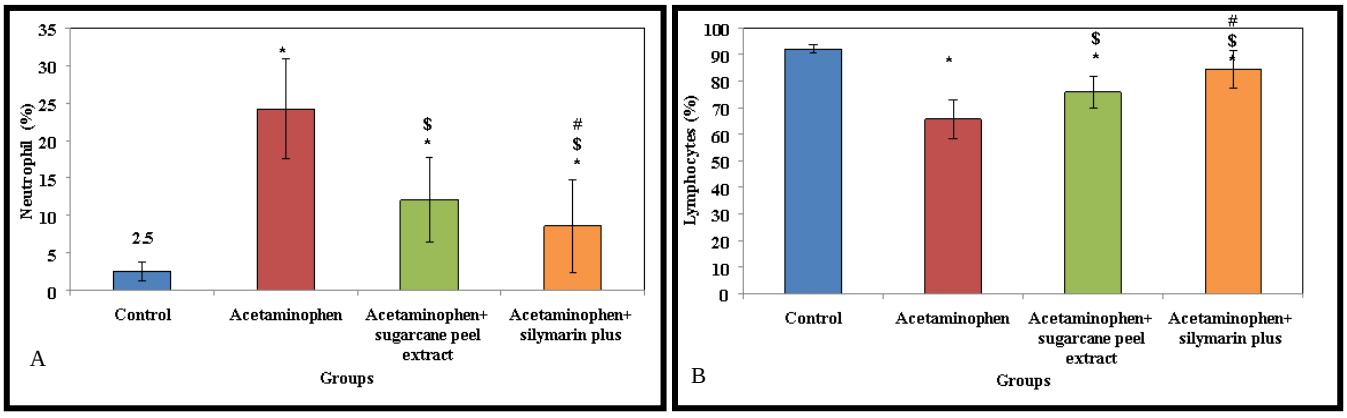

Figure 3: Impacts of *Saccharum officinarum* peel extract on (A) Neutrophil; and (B) Lymphocytes (%) in adult male Sprague Dawley rats.
*: Significant *VS* control; $: Significant *VS* acetaminophen; #: Significant *VS Saccharum officinarum* extract.

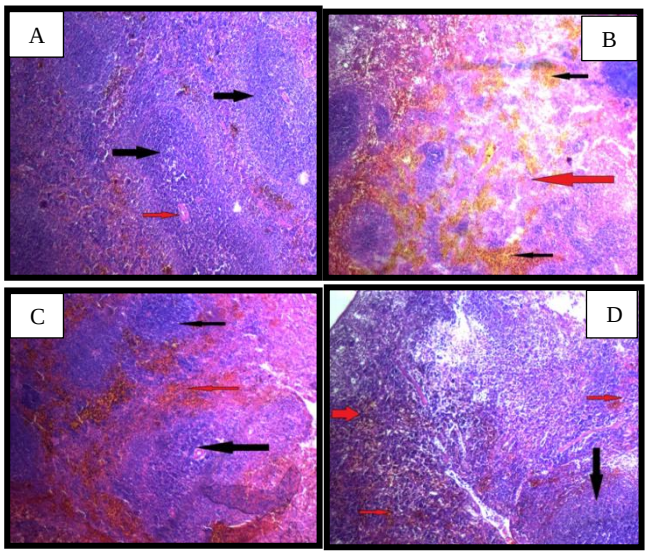

Figure 4: Light microscopic images of rat spleen of the control and treated groups. (H&Ex200).

A: Group I (control group) showing preserved architecture with lymphoid follicles (black arrow) and central arteriole (red arrow); B: showing Lymphoid follicle destruction, extensive hemorrhage (black arrows), and necrosis (red arrows); C: showing some preservation of follicles (black arrows), fewer areas of hemorrhage (red arrows); D: shows some preservation of follicles (black arrows) and minimal areas of hemorrhage (red arrows).

Moreover, the toxic metabolites of panadol® overdose damages hepatocytes, thus triggering an innate response to the immune system. Excessive activity of cells and leukocytes may be due to tissue damage caused by drug overdose.[43]

The results obtained for the histological examination of spleen tissues in all the groups (Figure 4) revealed that light microscopic images of rat spleen of Group I (control group) showed preserved architecture with lymphoid follicles (black arrow) and central arteriole (red arrow) as depicted in Figure 4A. Lymphoid follicle destruction, extensive hemorrhage (black arrows), and necrosis (red arrows) were revealed in Figure 4B. In Figure 4C, some preservation of follicles (black arrows), fewer areas of hemorrhage (red arrows), and a light microscopic image of rat spleen from Group IV were observed. Some preservation of follicles (black arrows) and minimal areas of hemorrhage (red arrows) were represented in Figure 4D.

Conclusion

The findings of this study revealed that the possible mechanism of the protective activity of *Saccharum officinarum* peel extract is due to its antioxidant and anti-inflammatory effects. The antioxidant activity of SOPE was confirmed by biochemical, antioxidant, and histopathological studies which were compared with other drug (silymarin plus). Therefore, treatment with *Saccharum officinarum* peel extract is an effective and possible cure for all hematotoxicity threats.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors thank Dr. Mai M. Abdelwahab of the Department of Pathology, Faculty of Medicine, Zagazig University, Sharkia, Egypt for her great help in the histopathological examinations. Also, the staff of the Scientific and Medical Research Center (ZSMRC), Zagazig University, Faculty of Medicine, Sharkia, Egypt are equally appreciated.

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