Acta Scientific Women's Health (ISSN: 2582-3205)

Volume 3 Issue 2 February 2021

Research Article

Beta vulgaris Impact on Splenic Toxicity Diclofenac-induced in Swiss Albino Mice

Hussah M Alobaid[1]*, Tahani M Al-Hazani[2], Doaa M Elnagar[1,3] and Norah A Althobaiti[4]

[1]Zoology Department, College of Science, King Saud University, Riyhad, Saudi Arabia

[2]Biology Department, College of Science and Humanities, Prince Sattam bin Abdulaziz University, Alkharj, Saudi Arabia

[3]Zoology Department, Faculty of Women, Ain Shams University, Cairo, Egypt

[4]Biology Department, College of Science and Humanities, Shaqra University, Al Quwaiiyah, Saudi Arabia

***Corresponding Author:** Hussah M Alobaid, Zoology Department, College of Science, King Saud University, Riyhad, Saudi Arabia.

Received: December 22, 2020

Published: January 22, 2021

Abstract

The present work aimed to investigate the possible effect of beetroot juice to overcome the splenic toxicity diclofenac - induced. 40 male albino mice were divided, 1st group deal as control group, 2nd treated with 8ml/kg of freshly prepared beetroot juice, 3rd treated with oral daily dose 20 mg/kg of diclofenac and 4th pre-treated with beetroot juice to diclofenac administration. Diclofenac treatment resulted in significant increase of liver and kidney functions, marked pathological alterations as diffusion of white pulp, minimized lymph nodes areas and volumes, increased pathological score, neutrophils and oxidative stress. The pre-treatment of beetroot to diclofenac revealed significant increase in liver and kidney functions, noticeable improvement in the spleen tissue manifested by decreasing of pathological score, neutrophils and oxidative stress.

Keywords: Spleen; Liver; Diclofenac; Beetroot; Pathological Score

Introduction

The diclofenac (DCLF) considered antipyretic, analgesic and anti-inflammatory non-steroidal drug which is the most popular one [1]. It has a proven effect in treating symptoms of numerous clinical signs, like fever, lower back pain, cerebral pain, headache, and osteoarthritis [2]. Its action occurs through inhibition of prostaglandin synthesis, and it does that by inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Diclofenac can amass in the systemic circulation and inflamed tissues, where it can diffuse quickly into the intracellular spaces to exert its therapeutic effect [3].

Diclofenac treatment might be resulted in various deteriorations all over the body as hepatotoxicity, kidney failure and spleen disorders [4]. A study in 2014 conducted on female students in Saudi Arabi and investigated the use of NSAIDs found that the use of these drugs was higher in high school student than undergraduate students [5]. NSAIDs are available in Saudi Arabia as both OTC and medical prescription. A recent study declared that the use of NSAIDs is high in metropolitan areas of Saudi Arabia [6].

Fruits and vegetables are always recommended to be a source for nutrients rich with vitamins, minerals and antioxidants [7]. Beetroot are distributed in cool countries such as Europe, North

America and also in some parts of West Africa because it is grown better under the cool conditions. Beetroots enlarge near or just above the soil surface to form bulbous root [8]. In addition to betanin, beetroots contain principal bioactive components compounds such as phenolic compounds, fibers, minerals and antioxidant substances [9]. In the past, beetroot juice was used as a remedy to overcome sexual weakness problem due to its capability to enhance human sex hormones, also it increases the blood volume and flow of blood to brain. On the other hand, several studies postulated the anti-inflammatory, antioxidant and cardioprotective effect of beetroot [10-12].

So far, studies that deal with the toxic effect of diclofenac on the spleen was few, so that the present work was achieved to determine expansively the effect of diclofenac on the spleen, also to investigate if the beetroot juice could reverse the spleen injury due to diclofenac treatment.

Materials and Methods

Animals and Experimental design

Male albino mice (25 ± 5 g) were collected at 22 ± 2˚C with free access to food and clean water from the King Saud University animal house, subjected to 12 hours of light-dark period. After 1 week of adaptation phase, mice were randomly divided into four groups, the first group served as control, the second group administered 8 ml/kg of freshly prepared beet root juice daily oral dose, the third group administered 20 mg/kg of diclofenac daily oral dose, the fourth group administered a one-hour pre-administration daily oral combined dose of beet root juice to diclofenac. The length of the experiment was 30 days; one day after the last dose, all mice were sacrificed.

Biochemical analysis

The collected serum samples were subjected to UV-VIS spectrophotometer measurements for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) levels as liver enzymes. Blood Urea Nitrogen (BUN) and creatinine as kidney function.

Histopathological analysis

Spleen samples were gathered and cut into small bits, then 10% neutral buffered formalin was fixed. Using ascending alcohol grades, samples were dehydrated and washed with xylene. Samples were integrated and sectioned at 5µ thickness and then Hematoxylin and Eosin (HE) stained. Sections were photographed using light microscopy and subjected to morphometric measurements (Motic-2000).

Pathological scoring system

A semiquantitative splenic scoring system was used for the following criteria: It was scored for the diffusion of white pulp in the red pulp (0=absent; 1=slight; 2=moderate; and 3=pronounced), congestion of red pulp, number of macrophages (1-2=slight; 2-4=moderate; ≤ 5=intense), number of granular cells (1-10=slight; 11-20=moderate; < 20=intense), hyaline degeneration and presence of pigments (0=absent and 1=present). Scoring of each tissue sample represented the mean score of ten different high microscopic power fields.

Morphometry

Images were submitted using the microscope program to calculate areas of lymph nodes at 100X magnification for 10 different fields per section. Lymph node volumes were determined according to the formula: a sphere's volume is V= 4/3 πr3. For 10 different fields per section, macrophages were counted at 400X magnification. Macrophage areas were determined, and volumes were estimated according to the spherical volume formula.

Immunohistochemistry

Spleen paraffin sections were deparaffinized and rehydrated. sections were immersed in citrate buffer (pH 6) and heated in microwave for 5 minutes. Sections were blocked with peroxidase solution for 10 minutes. Sections were incubated with the primary antibody over night at 4˚C anti-malondialdehyde (Anti-MDA ab 94671) and anti-neutrophils (Anti-NIMP-R14 ab2557). Sections were incubated with secondary antibody for 30 minutes followed by avidin-biotin complex for 30 minutes. Sections were incubated with DAB for 10 minutes and then with Mayer's hematoxylin counter stain. Sections were subjected to (IHC Tool Box – Image J system) for nuclei stained with H-DAB for neutrophils counting in 20 field x 200 mag per each group, then results analyzed statistically by SPSS (17).

Statistical analysis

Data was subjected to ANOVA test by (SPSS 17), values are considered significant at $p \leq 0.05$. Data expressed as mean ± SEM.

Results

Beta vulgaris (beetroot) regulated the liver and kidney functions tests that disturbed due to diclofenac treatment

As shown in table 1, treatment of mice with diclofenac resulted in significantly raising of liver enzymes (AST, ALT, ALP) activity levels and BUN levels compared to control group ($p \leq 0.05$). Whereas, pre- treatment with beetroot fresh juice to diclofenac 1 hour revealed significant decrease of liver enzymes activities and BUN level compared to the treated group with diclofenac only ($p \leq 0.05$), although, it still significantly higher than control group ($p \leq 0.05$). The creatinine levels showed no change among the groups.

Groups	AST	ALT	ALP	BUN	Creatinine
Control	80 ± 0.1	42 ± 0.1	135 ± 0.1	25 ± 0.1	0.3 ± 0.1
Beet root	82 ± 0.1	42 ± 0.2	138 ± 0.3	28 ± 0.2	0.3 ± 0.1
Diclofenac	212 ± 0.2^{*a}	88 ± 0.3^{*a}	200 ± 0.4^{*a}	48 ± 0.1^{*a}	0.3 ± 0.1
Diclofenac + beetroot	103 ± 0.1^{*a,b}	49 ± 0.4^{*a,b}	150 ± 0.4^{*a,b}	30 ± 0.2^{*a,b}	0.3 ± 0.1

Table 1: The effect of Beta Vulgaris (beetroot) on liver the liver and kidney functions tests.

Data=mean ± SEM.

*a=significant difference against control group

*b=significant difference against diclofenac group

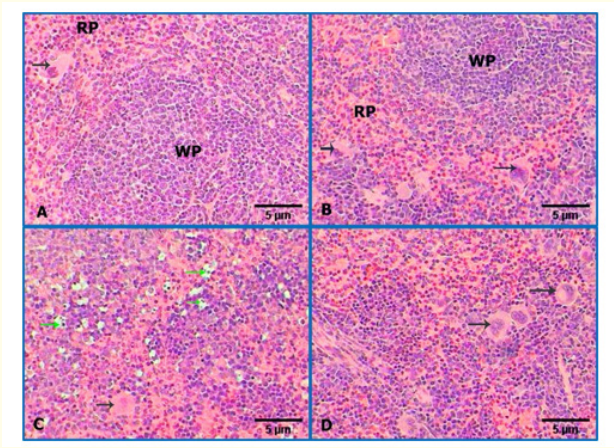

Figure 1: The effect of beetroot on splenic pathological changes diclofenac – induced.

Photomicrographs of spleen tissue (1A) control spleen showing white pulp (WP), red pulp (RP), macrophage (arrow), (1B) spleen of mice treated with beetroot juice displaying abundant white pulp (WP), red pulp (RP), activation for macrophages (arrows), (1C) spleen of mice treated with diclofenac revealing diffused white pulp, macrophage (black arrow), necrotic foci (green arrows), (1D) spleen of mice pre-treated with beetroot juice to diclofenac showing improved section, numerous giant macrophages (arrows) (HE-400X).

Beta vulgaris (beetroot) ameliorated pathological alterations diclofenac-induced

The control splenic tissue showed normal structure that white pulp was well-defined than red pulp, a few number of macrophages and granular cells (Figure 1A), also the spleen of mice treated with beetroot juice revealed no pathological alterations with some increase of macrophages (Figure 1B). Whereas, the spleen of mice treated with diclofenac displayed marked pathological signs as diffusion of white pulp in the red pulp with many necrotic foci filled with inflammatory granular cells (Figure 1C). While, the pre-treated mice with beetroot juice to diclofenac treatment showed less diffusion of white pulp in the red pulp besides to activation of macrophages (Figure 1D).

Beta vulgaris (beetroot) reduced the pathological scoring system raised due to diclofenac treatment

The pathological scoring system of normal control spleen resembled by 2, the score of mice treated with beetroot juice scored 3 due to the activation of macrophages. Whereas, diclofenac treatment highly raised the pathological score to reach 8 because of pronounced diffusion of white pulp in red pulp, heavy incidence of granulocytes and congestion of red pulp but mice pre-treated with beetroot juice to diclofenac treatment resulted in decrease of pathological score that raised by diclofenac treatment to reach 6 because of reduction of diffusion and granulocytes but intense presence of macrophages (Table 2).

Score Criteria	Control	Beetroot	Diclofenac	Beetroot and diclofenac
The diffusion of white pulp in the red pulp	0	0	3	1
Number of macrophages	1	2	1	3
Number of granular cells	1	1	3	2
Congestion of red pulp	0	0	1	0
Hyaline degeneration	0	0	0	0
Presence of pigments	0	0	0	0
Total	2	3	8	6

Table 2: The effect of Beta Vulgaris (beetroot) on the splenic pathological scoring system raised by diclofenac.

The effect of *Beta vulgaris* (beetroot) treatment to adjust the morphometric measurements that desolated due to diclofenac treatment

As shown in table 3, diclofenac treatment significantly decreases the areas and volumes of lymph nodes in the spleen tissue compared to control and the group treated with beetroot juice ($p \leq 0.05$). Whereas, the pre-treatment with beetroot juice to diclofenac treatment displayed significant increase of lymph nodes areas and volumes compared to the treated group with diclofenac only, but it still significantly less than control group. Table 4 revealed that both treatment with beetroot juice and diclofenac only caused insignificant increase of macrophages count ($p \geq 0.05$) compared to control group. While the pre-treated group with beetroot juice to diclofenac treatment resulted in significant increase of macrophages count ($p \leq 0.05$) compared to control group. Additionally, it was found that treatment with beetroot juice only or as pre-treatment to diclofenac significantly ($p \leq 0.05$) increased the areas and volumes of macrophages compared to control and group treated with diclofenac, so macrophages looked larger.

The immunohistochemical analysis against malondialdehyde (Anti-MDA) and neutrophils (Anti-NIMP-R14)

Spleen of control mice and treated with beetroot juice stained against MDA and Anti-NIMP-R14 exhibited slight immune response represented by a few number of stained cells (Figure 2A,3A, 2B, 3B) and low optical density of images. Whereas, spleen tissue of mice treated with diclofenac displayed intense immune response against MDA and Anti-NIMP-R14, significant number of cells stained versus MDA and Anti-NIMP-R14 (Figure 2C,3C) and significantly high optical density ($p \leq 0.05$) compared to control group. Moreover, spleen of pre-treated mice with beetroot juice to diclofenac showed moderate immune response against MDA and Anti-NIMP-R14 with less stained number of cells (Figure 2D,3D) and significant lowered optical density ($p \leq 0.05$) compared to the group treated with diclofenac only (Table 5,6).

Photomicrographs of spleen tissue stained immunohistochemically against NIMP (3A) control spleen revealing a few number of

Groups	Lymph nodes areas $x10^4$ µm^2	Lymph nodes volumes $x10^4$ µm^3
Control	154 ± 0.5	180 ± 0.5
Beet root	152 ± 0.1	176 ± 0.1
Diclofenac	51 ± 0.4^{*a}	34 ± 0.4^{*a}
Diclofenac + beetroot	112 ± 0.4^{*a,b}	111 ± 0.4^{*a,b}

Table 3: The effect of Beta Vulgaris (beetroot) treatment on lymph nodes areas and volumes decreased by diclofenac treatment.

Data=mean ± SEM

*a=significant difference against control group

*b=significant difference against diclofenac group.

Groups	Macrophages count	Macrophages areas $x10^3$ µm^2	Macrophages volumes $x10^3$ µm^3
Control	1 ± 0.2	2.6 ± 0.4	0.39 ± 0.4
Beet root	3 ± 0.3	5.2 ± 0.6^{*a,b}	1.1 ± 0.6^{*a,b}
Diclofenac	2 ± 0.0	3.4 ± 0.4	0.58 ± 0.4
Diclofenac + beetroot	5 ± 0.3^{*a,b}	7 ± 0.5^{*a,b}	1.7 ± 0.5^{*a,b}

Table 4: The effect of Beta Vulgaris (beetroot) treatment on the count , areas volumes of macrophages

Data=mean ± SEM.

*a=significant difference against control group

*b=significant difference against diclofenac group.

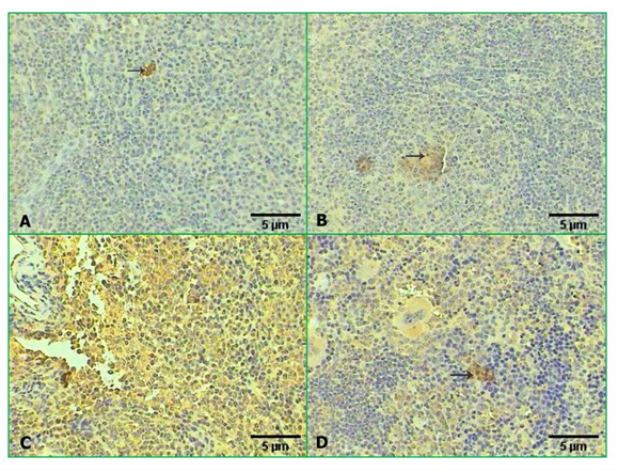

Figure 2: The immune response against MDA.

Photomicrographs of spleen tissue stained immunohistochemically against MDA (2A) control spleen revealing faint immune response (arrow), (2B) spleen of mice treated with beetroot juice displaying weak immune response (arrow), (2C) spleen of mice treated with diclofenac revealing intense immune response (arrow), (2D) spleen of mice pre-treated with beetroot juice to diclofenac showing moderate immune response. (ABC-400X).

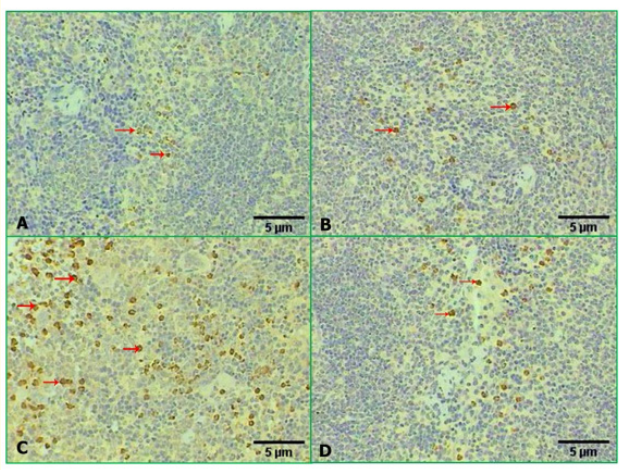

Figure 3: The immune response against NIMP.

Photomicrographs of spleen tissue stained immunohistochemically against NIMP (3A) control spleen revealing a few number of neutrophils (arrows), (3B) spleen of mice treated with beetroot juice displaying also a few number of neutrophils (arrows), (3C) spleen of mice treated with diclofenac revealing great number of neutrophils (arrows), (3D) spleen of mice pre-treated with beetroot juice to diclofenac showing less number of neutrophils (arrows). (ABC-400X).

neutrophils (arrows), (3B) spleen of mice treated with beetroot juice displaying also a few number of neutrophils (arrows), (3C) spleen of mice treated with diclofenac revealing great number of neutrophils (arrows), (3D) spleen of mice pre-treated with beetroot juice to diclofenac showing less number of neutrophils (arrows). (ABC-400X).

Groups	cells stained against MDA	Optical density
Control	1 ± 0.03	0.12 ± 0.02
Beet root	2 ± 0.05	0.14 ± 0.02
Diclofenac	28 ± 0.04*a	0.33 ± 0.01*a
Diclofenac + beetroot	6 ± 0.02*b	0.21 ± 0.03*a,b

Table 5: Counting of cells stained against Anti-MDA and images optical density.

Data=mean ± SEM

*a=significant difference against control group

*b=significant difference against diclofenac group.

Groups	Cells stained against Anti-NIMP	Optical density
Control	7 ± 0.06	0.14 ± 0.04
Beet root	6 ± 0.03	0.15 ± 0.08
Diclofenac	30 ± 0.02*a	0.30 ± 0.02*a
Diclofenac + beetroot	18 ± 0.04*a,b	0.20 ± 0.05*a,b

Table 6: Counting of cells stained against Anti-NIMP and images optical density.

Data=mean ± SEM

*a=significant difference against control group

*b=significant difference against diclofenac group.

Discussion

Diclofenac is considered a one of the most important triggers for hepatorenal damage which in turn commitment to disturbance

in liver and kidney functions. Thus, liver and kidney functions tests were used commonly as biomarkers reflecting its damage. While hepatocytes of liver exposed to damage its secretions of enzymes were flown in the circulatory blood lead to elevation of liver enzymes activities [13-15]. Several studies declared that administration of diclofenac especially long term treatment resulted highly significant increase of liver enzymes (ALT, AST and ALP) [15-17]. The present findings coincided with the previous studies that treatment with diclofenac for four weeks resulted in significant elevation of liver enzymes activities which attributed to liver cells damage.

Blood urea nitrogen (BUN) are the filtrated products eliminated by kidneys and their levels increasing considered a biomarker for kidney injury. Previous investigations displayed that administration of diclofenac caused increasing of BUN and creatinine levels in plasma [17,18]. The present results illustrated that diclofenac treatment raised the levels of BUN but not the creatinine as an evidence of some kidney injury which agreed with [19].

Many studies indicated the impacted effect of beetroot against several toxicants that distributed the liver and kidney functions as the beetroot could reduce the liver enzymes activities (ALT, AST and ALP) that elevated due to CCl4 treatment [8,20], paracetamol [21] and also silver nanoparticles [22]. The present results added that beetroot juice could significantly decrease the liver enzymes activities which increased by diclofenac treatment. Additionally, other studies illustrated previously the role of beetroot to protect the kidneys by amelioration of BUN, uric acid and creatinine that raised by gentamicin [23,24], chlorpyrifos [25]. On the other hand, the present study displayed the enhancement effect of beetroot juice to reduce the BUN levels diclofenac raised.

Few studies mentioned that diclofenac treatment resulted in significant increase of spleen weight and splenocyte count [26,27]. The current results showed that diclofenac treatment induced marked pathological changes in the spleen as a wide diffusion of white pulp in the red pulp, necrotic foci, raising of the splenic pathological score, besides to significant decrease of lymph nodes areas and volumes, increase the number of neutrophils and high incidence of MDA.

There was a study displayed that beetroot administration could be reduce the pathological signs of spleen due to anemia [28], another study pointed out that reduced DNA damage of splenocytes attributed to gamma rays irradiation [29]. The present work demonstrated the effect of beetroot juice on the spleen injured by diclofenac to clarify that beetroot could improve the spleen by reducing the diffusion of white pulp, increasing lymph nodes areas and volumes, decreasing the count of neutrophils and detraction of MDA incidence but also it was markable the great increasing in the count and size of macrophages. The noticeable improvement might be attributed to the bioactive agents in the beetroot which could play an important role in the immunity system.

Conclusion

Diclofenac is a non-steroid drug triggers marked changes in the spleen tissue besides to oxidative stress. Whereas, the pre-treatment with beetroot before diclofenac could reduce the splenic toxicity and oxidative stress.

Acknowledgement

The authors acknowledge financial support from the Researchers Supporting Project number (RSP-2020/174). King Saud University, Riyadh, Saudi Arabia.

Conflict of Interest

The authors have no conflicts of interest to disclose contributions. All authors contribute to the search.

Bibliography

1. Kołodziejska J and Kołodziejczyk M. "Diclofenac in the treatment of pain in patients with rheumatic diseases". *Reumatologia* 56.3 (2018): 174-183.

2. Zhang X., *et al*. "Non-steroidal anti-inflammatory drug induced acute kidney injury in the community dwelling general population and people with chronic kidney disease: systematic review and meta-analysis". *BMC Nephrology* 18.1 (2017): 256-276.

3. Brune K and Furst D E. "Combining enzyme specificity and tissue selectivity of cyclooxygenase inhibitors: towards better tolerability?" *Rheumatology* 46.6 (2007): 911-919.

4. Roshi D., *et al*. "Users' Knowledge About Adverse Effects of Non-steroidal Anti-inflammatory Drugs in Tirana, Albania". *Materia Socio-Medica* 29.2 (2017): 138-142.

5. Karami N., *et al*. "Knowledge and attitude of analgesics use among Saudi population: A cross-sectional study". *International Journal of Medical Science and Public Health* 7.2 (2018): 137-142.

6. Qahl A M., *et al*. "Knowledge and attitude of analgesics usage among the Saudi population". *International Journal of Medicine in Developing Countries* 4.2 (2020): 318-324.

7. Sarfaraz S and Najam R. "Evaluation of anti-inflammatory effect of natural dietary supplement Beta vulgaris (Beet root) in animal models of inflammation". *Rawal Medical Journal* 42.3 (2017): 385-389.

8. Olumese F E and Oboh H A. "Hepatoprotective Effect of Beetroot Juice on Liver Injury in Male Sprague - Dawley Rats". *Annals of Tropical Pathology* 9.1 (2018): 83-88.

9. Baião D D S., *et al*. "Beetroot, a Remarkable Vegetable: Its Nitrate and Phytochemical Contents Can be Adjusted in Novel Formulations to Benefit Health and Support Cardiovascular Disease Therapies". *Antioxidants (Basel, Switzerland)* 9.10 (2020): 1-31.

10. Adhikari A., *et al*. "Evaluation of anti-inflammatory effect of beetroot extract in animal models". *International Journal of Basic and Clinical Pharmacology* 6.12 (2017): 2853-2858.

11. El-Beltagi H., *et al*. "Evaluation of some chemical constituents, antioxidant, antibacterial and anticancer activities of beta Vulgaris L. Root". *Fresenius Environmental Bulletin* 27.9 (2018): 6369-6378.

12. Mirmiran P., *et al*. "Functional properties of beetroot (Beta vulgaris) in management of cardio-metabolic diseases". *Nutrition and Metabolism* 17.3 (2020): 1-15.

13. Iqbal I., *et al*. "Hepatotoxicity of Cassia fi stula extracts in experimental chicks and assessment of clinical parameters". *Kuwait Journal of Science* 43.3 (2016): 135-141.

14. Adeyemi W J and Olayaki L A. "Diclofenac - induced hepatotoxicity: Low dose of omega-3 fatty acids have more protective effects". *Toxicology Reports* 5.1 (2018): 90-95.

15. Ogbe R J. "Comparative study on the effects of Alchornea cordifolia and Cassia spectabilis leaf extracts on diclofenac-induced liver and kidney injuries in rats". *Comparative Clinical Pathology* 29 (2020): 927-935.

16. Esmaeilzadeh M., *et al*. "Gallic acid mitigates diclofenac-induced liver toxicity by modulating oxidative stress and suppressing IL-1 β gene expression in male rats". *Pharmaceutical Biology* 58.1 (2020): 590-596.

17. Mostafa R E., *et al*. "Heliyon Protective effect of royal jelly against diclofenac-induced hepato-renal damage and gastrointestinal ulcerations in rats". *Heliyon* 6 (2020): 1-9.

18. Fattori V., *et al*. "Vinpocetine reduces diclofenac-induced acute kidney injury through inhibition of oxidative stress, apoptosis, cytokine production, and NF- B activation in mice". *Pharmacological Research* 120 (2017): 10-22.

19. El-ashmawy I M. "Hepato-Renal and Hematological Effects of Diclofenac Sodium in Rats". *Global Journal of Pharmacology* 7.2 (2013): 123-132.

20. Rose M., *et al*. "Effect of antioxidants and hepatoprotective activities of methanol extract of beet root (beta vulgaris l.) against carbon tetrachloride induced hepatotoxicity in rat models". *International Journal of Pharmaceutical Sciences and Research* 5.6 (2014): 2536-2545.

21. Al-qtaitat A. "The possible protective effect of fresh beetroot juice against". *International Journal of Pharmaceutical Sciences and Research* 11.2 (2020): 844-852.

22. Albrahim T and Alonazi M A. "Role of Beetroot (Beta vulgaris) Juice on Chronic Nanotoxicity of Silver Nanoparticle-Induced Hepatotoxicity in Male Rats". *International Journal of Nanomedicine* 15 (2020): 3471-3482.

23. Gamal A A., *et al*. "Beetroot (Beta vulgaris L) Extract Ameliorates Gentamicin-Induced Nephrotoxicity Associated Oxidative Stress, Inflammation, and Apoptosis in Rodent Model. Mediators of Inflammation (2014): 1-12.

24. Iahtisham-Ul-Haq., *et al*. "Nephroprotective effects of red beetroot-based beverages against gentamicin-induced renal stress". *Journal of Food Biochemistry* 43.7 (2019): e12873.

25. Albasher G., *et al*. "Nephroprotective Role of Beta vulgaris L. Root Extract against Chlorpyrifos-Induced Renal Injury in

Rats". *Evidence-Based Complementary and Alternative Medicine* (2017): 1-9.

26. Gomaa S. "Immunomodulatory and hematological effects induced by diclofenac, ibuprofen or paracetamol toxicity in Swiss albino mice". *European Journal of Biological Research* 7.4 (2017): 348-359.

27. Gomaa S. "Adverse effects induced by diclofenac, ibuprofen, and paracetamol toxicity on immunological and biochemical parameters in Swiss albino mice". *The Journal of Basic and Applied Zoology* 79.5 (2018): 1-9.

28. Elaby S and Ali J. "The anti-anemic effect of dried beet green in phenylhydrazine treated rats". *Archives of Pharmaceutical Sciences Ain Shams University* 2.2 (2018): 54-69.

29. Cho J., *et al*. "Beetroot (Beta vulgaris) rescues mice from c -ray irradiation by accelerating hematopoiesis and curtailing immunosuppression". *Pharmaceutical Biology* 55.1 (2017): 306-316.