International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 5, 2024

Original Article

THE EFFECT OF SUPPLEMENTATION OF CAPSULE WITH A COMBINATION OF MOMORDICA CHARANTIA FRUIT AND MORINGA OLEIFERA LEAVES EXTRACT ON THE BIOMARKER OF LIVER AND RENAL FUNCTION IN THE LIMITED HEALTHY VOLUNTEERS

FAHRUN NUR ROSYID[1*], HARYOTO[2], BETI KRISTINAWATI[1], AHMAD FADHLUR RAHMAN[3]

[1]Department of Medical Surgical Nursing, School of Nursing, Faculty of Health Science, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia. [2]Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia. [3]School of Nursing, Faculty of Health Science, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia

*Corresponding author: Fahrun Nur Rosyid; *Email: fnr100@ums.ac.id

Received: 06 May 2024, Revised and Accepted: 28 Aug 2024

ABSTRACT

Objective: This study examined the supplementation of the capsule with a combination of *Momordica Charantia Fruit* (MCF) and *Moringa Oleifera Leaves* (MOL) extract on the biomarkers of liver and renal function in limited healthy volunteers.

Methods: This study used a nonrandom test. 78 healthy volunteers were given 2 x 1000 g of the capsules of MCF and MOL extract. This intervention was conducted for 30 d. The levels of GOT/AST, GPT/ALT, alkaline phosphatase, gamma glutamyl transpeptidase, urea, and creatinine were measured at baseline and at the end of treatment. Data were analyzed using paired t-test and Wilcoxon sign test with a significant limit of $P \leq 0.05$.

Results: This study showed a significant decrease in GOT/AST ($P = 0.001$), GPT/ALT ($P = 0.001$), Alkaline phosphatase ($P = 0.007$), gamma glutamyl transpeptidase ($P = 0.002$), urea ($P = 0.009$) and creatinine ($P = 0.002$).

Conclusion: These findings indicated that supplementation of the capsule with a combination of MCF and MOL extract had a positive impact on liver and renal function biomarkers in limited healthy volunteers. This study contributes towards the extension of understanding the potential health benefits of supplementation of the capsule with the combination of MCF and MOL extract.

Keywords: Momordica charantia, Moringa oleifera, Liver function test, Renal function test

DOI: https://dx.doi.org/10.22159/ijap.2024.v16s5.52468 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Momordica charantia is a popular vegetable in many countries for possessing nutritional and medicinal benefits [1]. In addition, MCF is able to improve the life quality and protect against various diseases [2]. Belonging to *Cucurbitaceae* family, MCF is widely used as a medicinal food for the treatment of diabetes and hyperlipidemia in many countries in Asia, South America, East Africa and America [3-5]. In the last five decades, many active ingredients have been identified in the seeds, fruits and entire *Momordica charantia* plant, which can be classified into proteins, carbohydrates, lipids, triterpenes, saponins, alkaloids, steroids and flavonoids [6]. The main components of MCF that provide hypoglycemic effects include peptide insulin, alkaloids and charantin. Also, triterpenoids have hypoglycemic activity [7]. Various studies have reported that MCF has a number of beneficial effects on several chronic diseases such as cancer, cardiovascular disease, and metabolic disorders [1].

In most countries, MCF is well known as a food ingredient; however, it also has several side effects (e. g. hemolytic anemia, stomach ache, headache, abortion, and antifertility) [8]. The daily administration of MCF within 2 w does not cause hepatotoxicity or nephrotoxicity. In addition, the continuous administration of MCF in Diabetes Mellitus (DM) can result in a significant reduction in GOT/AST and GPT/ALT, markers of liver function, compared to diabetic controls. Additionally, there will be a significant reduction in calcium and potassium levels as an indication of the recovery of parts of renal function. The levels of other renal function parameters also decrease. These data indicated that MCF medical treatment overall does not cause further decline in renal function [9, 10]. Previous studies also showed that a repeated treatment with MCF did not cause systemic toxicity [10-13]. *Moringa oleifera*, as part of the *Moringaceae* family, is known as the "Miracle Tree", which is mostly found throughout the world [14] and has been used globally for centuries since it contains nutrients and improves health [15]. The content of *Moringa Oleifera Leaves* (MOL) includes saponins, tannins, flavonoids, glycosides, terpenoids, beta-carotene, vitamin C, vitamin E, and polyphenols [16], all of which show medicinal properties, and are protective on the kidneys, liver, neurons, antioxidant, anti-inflammatory, anti-carcinogenic, antimicrobial, and increases immunity [16-18].

Moringa oleifera is able to prevent DM complications [20] as it has antidiabetic effects [21], improving the healing of diabetic ulcers [22–24]. This plant also has antimicrobial, anti-inflammatory, anti-cancer and anti-diabetic activity [25, 26]. *Moringa oleifera* exhibits protective effects on the kidney against heavy metal toxicity through the regulation of renal function and prevention of oxidative damage and inflammatory reactions. It also prevents or treats various chronic diseases, such as tilmicosin-induced nephrotoxicity [27], toxicity to the rat heart [28], and reproductive toxicity [29]. *Moringa oleifera* has low toxicity and is safe to be consumed by human even at high doses [26-28].

To the best of our knowledge, the supplementation of capsule with a combination of *Momordica Charantia Fruit* (MCF) and MOL extract has prolonged safety. This study examined the supplementation of capsule with a combination of MCF and MOL extract on the biomarkers of liver and renal function in limited healthy volunteers. This study is to provide a scientific perspective on the safety of the supplementation of the capsule with a combination of MCF and MOL extract as an evaluation of new foods.

MATERIALS AND METHODS

Making a combination of MCF and MOL extract

Making MCF extract was carried out using 300 g of dry bitter melon fruit powder that has been macerated with 2250 ml of 70% ethanol filter fluid. It was then left for 5 y, stirred occasionally, and then filtered using flannel cloth (filtrate 1). The dregs were extracted using 70% ethanol as much as 750 ml and then filtered using flannel cloth (filtrate 2). The results of filtrate 1 and filtrate 2 were then mixed and added with 70% ethanol through the dregs to cover the volume deficiency. The filtrate was evaporated using a vacuum rotary evaporator at a temperature of 60 °C for approximately 5 h.

Making MOL extract. Fresh green Moringa leaves were selected and picked. The leaves were then aerated for 24 h at a room temperature. It was then followed by drying in a cabinet dryer at a temperature of 50 °C for 5-6 h. The dried sample was blended until being smooth simplicia). 400 g of simplicia powder was soaked in 96% ethanol (ratio 1:5) for 2 x 24 h and stirred occasionally. The maceration yields were filtered to obtain filtrate 1. Subsequently, remaceration was carried out for 2 x 24 h and then filtered to obtain filtrate 2. Filtrate 1 and filtrate 2 were then mixed and concentrated using a Rotary Evaporator at a temperature of 40-60 °C.

Subsequently, the bitter melon extract and Moringa leaves were mixed into a mortar and 70% ethanol was added, stirred until being homogeneous. Following this, 20% dextrin were added, mixed evenly and poured into a container lined with aluminum foil before going to the drying process at a temperature of 40 °C until being dry. It was continued by crushing and sifting the mixture by means of a sieve to make powder.

Design of study

The Study conducted a non-random trial to evaluate the safety of liver and renal function after obtaining 2x1000 g of capsules of a combination of MCF and MOL extract.

Participants

Seventy-eight healthy volunteers participated in this study. The participants in this trial were the healthy volunteers aged 19-53 y. They were recruited through local advertisements. Those with allergies impaired liver and kidney function were excluded from this study. This study protocol has received approval from the Health Research Ethics Committee Dr. Moewardi General Hospital/School of Medicine Sebelas Maret University of Surakarta, Indonesia by letter number 1.544/VIII/HREC/2023.

Biochemical assays

A colorimetric spectrophotometer was used to measure the levels of GOT/AST, GPT/ALT serum, alkaline phosphatase, and gamma glutamyl transpeptidase, urea, and creatinine. Fresh reagents were prepared and procedures were carried out using a standard operating procedure (SOP). Each sample was analyzed in duplicate.

Statistical analysis

Data from 78 healthy volunteers were depicted as mean±SD. The data were analyzed using Paired Samples Test and Wilcoxon Signed Ranks Test. Here, it was declared significant if $P<0.05$.

RESULTS AND DISCUSSION

In this study, initially 80 healthy volunteers participated. Then, 2 participants were excluded from the analysis since they did not take the posttest. On average, 78 participants were aged 23.63±9.11 y and have completed the study. In terms of sex, there were 33 men (42.3%), and 45 women (57.7%). As shown in table 1, the average body weight was 58.44±11.67 kg, BMI was 22.28±3.38 kg/m2), heart rate was 91.53±13.09 beats/minute, systolic blood pressure was 120.43±18.29 mmHg, and diastolic blood pressure was 82.60±8.44 mmHg. Biochemical test results as presented in table 2 showed significant changes in the markers of liver and kidney function. Regarding liver function biomarkers, this study showed a decrease in the levels of GOT/AST serum with an average difference of 5.28 U/l and was significant ($P \leq 0.001$), levels of GPT/ALT serum was in an average difference of 7.36 U/l and significant ($P \leq 0.001$), level of Alkaline phosphatase serum showed a mean difference of 12.12 U/l and significant ($P \leq 0.007$), level of Gamma glutamyl transpeptidase serum was in the mean difference of 6.89 U/l and significant ($P \leq 0.002$) at the end of the study. This decrease showed that the capsule of the combination of MCF and MOL extract was not toxic to the liver.

Renal function biomarkers showed a decrease in urea levels with a mean difference of 2.68 mg/dl and was significant ($P \leq 0.009$), creatinine with a mean difference of 0.12 mg/dl and significant ($P \leq 0.002$) at the end of the study. This decrease showed that the capsule of the combination of MCF and MOL extract was not toxic to the kidneys.

Table 1: Baseline of subject characteristics (mean±SD)

Variable	Total (%)	Mean±SD
Age (Year)		23.63±9.11
Sex		
Male	33 (42.3%)	
Female	45 (57.7%)	
Weight (kg)		58.44±11.67
BMI (kg/m^2)		22.28±3.38
Heart Rate (beats/minute)		91.53±13.09
Blood Pressure		
Systolic (mmHg)		120.43±18,29
Diastolic (mmHg)		82.60±8.44

Data are presented in the numerical form (proportion of n (%) or mean±deviation standards (SD)

Table 2: Data of Biochemical Test before and after intervention of combination of MCF and MOL for 30 d

Variable	Baseline mean±SD	Posttest mean±SD	P-value
GOT/AST (U/l)	24.83±8.52	19.55±9.68	0.001*
GPT/ALT (U/l)	23.18±16.20	15.82±13.74	0.001*
Alkaline phosphatase (U/l)	74.58±29.99	62.46±31.13	0.007*
Gamma-glutamyl transpeptidase (U/l)	26.11±17.04	19.22±13.56	0,002*
Ureum (mg/dl)	21.01±6.73	18.33±7.26	0.009**
Kreatinin (mg/dl)	0.84±0.25	0.72±0.29	0.002**

*Significant difference between means at $p \leq 0.05$ by using paired sample t-test. **Significant difference between means at $p \leq 0.05$ by using the Wilcoxon sign test.

DISCUSSION

The current study aims to examine whether the supplementation of the capsule with a combination of MCF and MOL extract has a positive or negative impact on liver and renal function biomarkers in healthy volunteers. Statistically, this study showed a significant and positive effect of the supplementation of the capsule with a combination of MCF and MOL extract on liver function biomarkers. The biomarkers of liver function, which included the level of GOT/AST, GPT/ALT, alkaline phosphatase, and gamma-glutamyl

transpeptidase, showed changes occurred within normal limits and clinically unimportant. The levels of GOT/AST serum decreased by – 5.28 U/l, GPT/ALT decreased by – 7.36 U/l, alkaline phosphatase decreased by – 12.12 U/l, and Gamma-glutamyl transpeptidase serum decreased by – 6.89 U/l. Similar findings were reported in a study on MCF by Deshmukh (2016) and Sagor *et al.*, (2015) [10, 29]. Similarly, Chung *et al.*, in 2022 concluded that MC is safe for long-term consumption. Another study related to MOL found that MOL can improve the levels of GOT/AST, GPT/ALT [30], and alkaline phosphatase (ALP) [34]. Asgari-Kafrani *et al.*, (2020) concluded that MOL has hepatoprotective and antioxidant activity [35].

Quercetin, gallic acid, and caffeic acid are a few of the antioxidants found in moringa that are crucial in preventing oxidative stress and lowering hepatic inflammation [27]. In animal liver tests, these antioxidants have been demonstrated to have hepatoprotective benefits by averting early liver injury and restoring antioxidant levels [16]. Additionally, it is well known that the antioxidant components in moringa scavenge free radicals, reducing fat buildup in the liver and maintaining a balanced lipid profile [36]. Higher doses of moringa extract have greater hepatoprotective effects, indicating that this antioxidant activity is dose-dependent. Overall, Moringa oleifera's hepatoprotective and antioxidant activity in non-alcoholic fatty liver disease (NAFLD) is associated with its capacity to eliminate oxidative stress, scavenge free radicals, avoid lipid buildup in the liver, and enhance liver function markers, all of which ultimately guard against steatosis and liver damage [37, 38].

The findings of this study showed that the supplementation of capsule with a combination of MCF and MOL extract had a significant positive impact on kidney function biomarkers. Urea significantly decreased by – 2.68 mg/dl and creatinine levels significantly decreased from 0.84±0.25 to 0.72±0.29 mg/dl. These findings are in line with the results of a research published by Deshmukh [33]. Likewise, Offor *et al.*, in 2018 reported that MCF extract at low and high doses significantly prevented the development of nephrotoxicity by reducing the markers of kidney injury such as BUN and creatinine [39, 40]. Studies related to MOL found that MOL was able to protect against kidney injury [27, 41]. Likewise, Hussein *et al.*, (2024) reported that MOL extract could protect against nephrotoxicity. MCF and MOL have both been studied separately for their potential health benefits, including their impact on kidney function [39, 40]. Research conducted by Bortolotti *et al.*, (2019) has shown that bitter melon extract can have a protective effect on the kidneys by protecting against oxidative damage and inflammation [1]. In addition, research by Oguntibeju *et al.*, (2020) also shows Moringa leaves have been associated with anti-inflammatory, antioxidant, and kidney protection properties [44].

Although this study contributes to the development of knowledge that the supplementation of capsule with a combination of MCF and MOL extract has a positive impact on liver and renal function biomarkers in healthy volunteers, there are still a number of several limitations that must be concerned. The small size of sample of the study and study design may limit the generalizability of the results. Also, the short time period of this study may result in a less comprehensive understanding of the long-term implications of supplementation of the capsule with the combination of MCF and MOL extract on health. Future studies should have better study designs, larger and more diverse populations, as well as longer time duration to validate and deepen these findings.

CONCLUSION

This study evaluated the supplementation of capsule with a combination of MCF and MOL extract on liver and renal function biomarkers in healthy volunteers. Such supplementation was found to be capable of reducing AST, ALT, alkaline phosphatase, gamma-glutamyl transpeptidase, urea and creatinine. Then, it can be concluded that such supplementation was not hepatotoxic and nephrotoxic. The capsule with the combination of MCF and MOL extract is safe to be consumed.

ACKNOWLEDGEMENT

Supporting sources: This study was funded by DRTPM DIRJENDIKTIRISTEK KEMENDIKBUDRISTEK Republik Indonesia, based upon the Decree Number 182/E5/PG.02.00. PL/2023. We also thank all participants in this study.

AUTHORS CONTRIBUTIONS

Concept and design (Fahrun Nur Rosyid, Haryoto), data collection and ethical clearance (Fahrun Nur Rosyid, Beti Kristinawati), analysis and interpretation of data (Fahrun Nur Rosyid, Beti Kristinawati, Haryoto), manuscript draft and translation (Fahrun Nur Rosyid, Ahmad Fadhlur Rahman).

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Bortolotti M, Mercatelli D, Polito L. Momordica charantia a nutraceutical approach for inflammatory-related diseases. Front Pharmacol. 2019;10:486. doi: 10.3389/fphar.2019.00486, PMID 31139079.
2. Saeed F, Afzaal M, Niaz B, Arshad MU, Tufail T, Hussain MB. Bitter melon (Momordica charantia): a natural healthy vegetable. Int J Food Prop. 2018;21(1):1270-90. doi: 10.1080/10942912.2018.1446023.
3. Oyelere SF, Ajayi OH, Ayoade TE, Santana Pereira GB, Dayo Owoyemi BC, Ilesanmi AO. A detailed review on the phytochemical profiles and anti-diabetic mechanisms of momordicacharantia. Heliyon. 2022;8(4):e09253. doi: 10.1016/j.heliyon.2022.e09253, PMID 35434401.
4. Zhang F, Zhang X, YU J, Tan Y, Guo P, WU C. The gut microbiota confers the lipid-lowering effect of bitter melon (Momordica charantia L.) in high fat diet (HFD)-Induced hyperlipidemic mice. Biomed Pharmacother. 2020 May;131:110667. doi: 10.1016/j.biopha.2020.110667, PMID 32861068.
5. Yan JK, WU LX, Qiao ZR, Cai WD, MA H. Effect of different drying methods on the product quality and bioactive polysaccharides of bitter gourd (Momordica charantia L.) slices. Food Chem. 2019;271:588-96. doi: 10.1016/j.foodchem.2018.08.012, PMID 30236720.
6. Jia S, Shen M, Zhang F, Xie J. Recent advances in Momordica charantia: functional components and biological activities. Int J Mol Sci. 2017 Nov 28;18(12):2555. doi: 10.3390/ijms18122555, PMID 29182587.
7. Han JH, Tuan NQ, Park MH, Quan KT, Oh J, Heo KS. Cucurbitane triterpenoids from the fruits of momordica charantia improve insulin sensitivity and glucose homeostasis in streptozotocin induced diabetic mice. Mol Nutr Food Res. 2018;62(7):e1700769. doi: 10.1002/mnfr.201700769, PMID 29405623.
8. Basch E, Gabardi S, Ulbricht C. Bitter melon (Momordica charantia): a review of efficacy and safety. Am J Health Syst Pharm. 2003 Feb;60(4):356-9. doi: 10.1093/ajhp/60.4.356, PMID 12625217.
9. Mejia LA, Korgaonkar CK, Schweizer M, Chengelis C, Novilla M, Ziemer E. A 13-week dietary toxicity study in rats of a napin rich canola protein isolate. Regul Toxicol Pharmacol. 2009;55(3):394-402. doi: 10.1016/j.yrtph.2009.09.008, PMID 19766157.
10. Pamungkas AR, Indrayudha P. Aktivitas sitotoksik ekstrak etanol fraksi etanol-air etil asetat serta n-heksana buah pare (momordica charantia) pada sel mcf-7 secara *in vitro*. Pharmacon. 2019;16(2):73-82. doi: 10.23917/pharmacon.v16i2.9049.
11. Sagor AT, Chowdhury MR, Tabassum N, Hossain H, Rahman MM, Alam MA. Supplementation of fresh ucche (Momordica charantia L. var. muricata Willd) prevented oxidative stress fibrosis and hepatic damage in CCl4-treated rats. BMC Complement Altern Med. 2015;15:115. doi: 10.1186/s12906-015-0636-1, PMID 25884170.
12. Choudhary SK, Chhabra G, Sharma D, Vashishta A, Ohri S, Dixit A. Comprehensive evaluation of the anti-hyperglycemic activity of fractionated *Momordica charantia* seed extract in alloxan-induced diabetic rats. Evid Based Complement Alternat Med. 2012;2012:293650. doi: 10.1155/2012/293650, PMID 23320026.

13. Mardani S, Nasri H, Hajian S, Ahmadi A, Kazemi R, Rafieian Kopaei M. Impact of momordica charantia extract on kidney function and structure in mice. J Nephropathol. 2014;3(1):35-40. doi: 10.12860/jnp.2014.08, PMID 24644542.
14. Bhattacharya A, Tiwari P, Sahu PK, Kumar S. A review of the phytochemical and pharmacological characteristics of Moringa oleifera. J Pharm Bioallied Sci. 2018;10(4):181-91. doi: 10.4103/JPBS.JPBS_126_18, PMID 30568375.
15. Vergara Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in moringa oleifera leaves protect against chronic disease. Antioxidants (Basel Switzerland). 2017 Nov 16;6(4):91. doi: 10.3390/antiox6040091, PMID 29144438.
16. Helal FI, El-Badawi AY, El-Wardany I, Ali NG, Aboelazab OM. Effect of dietary moringa (Moringa oleifera) and rosemary (Rosmarinus officinalis) leaves or their mixture on productive performance carcass characteristics and antioxidant enzymes of rabbits reared under heat stress conditions. Agric Eng Int CIGR J. 2017;2017:184-92.
17. El Shanawany EE, Fouad EA, Keshta HG, Hassan SE, Hegazi AG, Abdel Rahman EH. Immunomodulatory effects of Moringa oleifera leaves aqueous extract in sheep naturally co-infected with fasciola gigantica and clostridium novyi. J Parasit Dis. 2019;43(4):583-91. doi: 10.1007/s12639-019-01130-6, PMID 31749528.
18. Abd Elhakim YM, El Bohi KM, Hassan SK, El Sayed S, Abd Elmotal SM. Palliative effects of moringa olifera ethanolic extract on hemato immunologic impacts of melamine in rats. Food Chem Toxicol. 2018;114:1-10. doi: 10.1016/j.fct.2018.02.020, PMID 29438774.
19. Obembe OO, Raji Y. Effects of aqueous extract of Moringa oleifera seed on cadmium-induced reproductive toxicity in male wistar rats. Afr Health Sci. 2018 Sep;18(3):653-63. doi: 10.4314/ahs.v18i3.23, PMID 30602998.
20. Mthiyane FT, Dludla PV, Ziqubu K, Mthembu SX, Muvhulawa N, Hlengwa N. A review on the antidiabetic properties of *Moringa oleifera* extracts: focusing on oxidative stress and inflammation as main therapeutic targets. Front Pharmacol. 2022 Jul 11;13:940572. doi: 10.3389/fphar.2022.940572, PMID 35899107.
21. Al-Ghanayem AA, Alhussaini MS, Asad M, Joseph B. Effect of Moringa oleifera leaf extract on excision wound infections in rats: antioxidant antimicrobial and gene expression analysis. Molecules. 2022 Jul;27:14. doi: 10.3390/molecules27144481, PMID 35889362.
22. Mohammad Shafie N, Raja Shahriman Shah RN, Krishnan P, Abdul Haleem N, Tan TY. Scoping review: evaluation of *Moringa oleifera* (Lam.) for potential wound healing in *in vivo* studies-N. I, Krishnan, Abdul Haleem, TYC Tan. Molecules. 2022;27. doi: 10.3390/molecules27175541, PMID 36080308.
23. Syahruni R, Umar AH, Asnar NH. Karakterisasi tiga tumbuhan obat antihiperkolesterolemia dengan pendekatan berbasis profil anatomi, histokimia dan fitokimia pharmacon. J Farm Indones. 2022;19(2):176-86. doi: 10.23917/pharmacon.
24. Samodra G, Alfathani NF, Octaviani P. Uji aktivitas antioksidan ekstrak etanol kombinasi daun kersen (Muntingia calabura L.) dan daun kelor (*Moringa oleifera* L) dengan Metode DPPH (2,2-Diphenyl-1-Picrylhydrayl). Pharmacon. 2023;20(1):19-26. doi: 10.23917/pharmacon.v20i1.22293.
25. Iswandana R, Lestari DA, Cholimi S. Combination of HPMC and PEG 400 as a taste masking agent of film-coated tablets containing momordica charantia linn. extract. Int J App Pharm. 2018;10(3):8-12. doi: 10.22159/ijap.2018v10i3.24025.
26. Melebary SJ, Elnaggar MH. Impact of *Moringa oleifera* leaf extract in reducing the effect of lead acetate toxicity in mice. Saudi J Biol Sci. 2023;30(1):103507. doi: 10.1016/j.sjbs.2022.103507, PMID 36458096.
27. Abou Zeid SM, Ahmed AI, Awad A, Mohammed WA, Metwally MM, Almeer R. Moringa oleifera ethanolic extract attenuates tilmicosin induced renal damage in male rats via suppression of oxidative stress inflammatory injury and intermediate filament proteins mRNA expression. Biomed Pharmacother. 2021 Jan;133:110997. doi: 10.1016/j.biopha.2020.110997, PMID 33197759.
28. Khalil SR, El Bohi KM, Khater S, Abd El-Fattah AH, Mahmoud FA, Farag MR. Moringa oleifera leaves ethanolic extract influences DNA damage signaling pathways to protect liver tissue from cobalt-triggered apoptosis in rats. Ecotoxicol Environ Saf. 2020;200:110716. doi: 10.1016/j.ecoenv.2020.110716, PMID 32450433.
29. Mansour MF, Arisha AH, Al-Gamal M, Yahia R, Elsayed AA, Saad S. *Moringa oleifera* leaves extract ameliorates melamine-induced testicular toxicity in rats. Adv Anim Vet Sci. 2020;8(1s):47-54. doi: 10.17582/journal.aavs/2020/8.s1.47.54.
30. Soliman MM, Aldhahrani A, Alkhedaide A, Nassan MA, Althobaiti F, Mohamed WA. The ameliorative impacts of *Moringa oleifera* leaf extract against oxidative stress and methotrexate-induced hepato renal dysfunction. Biomed Pharmacother. 2020 May;128:110259. doi: 10.1016/j.biopha.2020.110259, PMID 32485567.
31. Qamar H, Rehman S, Chauhan DK. Current status and future perspective for research on medicinal plants with anticancerous activity and minimum cytotoxic value. Curr Drug Targets. 2019;20(12):1227-43. doi: 10.2174/1389450120666190429120314, PMID 31486747.
32. Ouedraogo M, Lamien Sanou A, Ramde N, Ouedraogo AS, Ouedraogo M, Zongo SP. Protective effect of *Moringa oleifera* leaves against gentamicin-induced nephrotoxicity in rabbits. Exp Toxicol Pathol. 2013;65(3):335-9. doi: 10.1016/j.etp.2011.11.006, PMID 22197459.
33. Deshmukh NS. Safety assessment of McB-E60 (extract of a Momordica sp): subchronic toxicity study in rats. Toxicol Rep. 2016;3:481-9. doi: 10.1016/j.toxrep.2016.05.006, PMID 28959571.
34. Akorede GJ, Ambali SF, Olatunji AO, Aremu A, Basiru A, Ahmed AO. Mitigative potentials of methanol leaf extract of *Moringa oleifera* on chronic carbamazepine induced haemo-biochemical and thyrotoxicity in male wistar rats. Pharmacological Research Modern Chinese Medicine. 2022 Mar;2:100055. doi: 10.1016/j.prmcm.2022.100055.
35. Asgari Kafrani A, Fazilati M, Nazem H. Hepatoprotective and antioxidant activity of aerial parts of *Moringa oleifera* in prevention of non-alcoholic fatty liver disease in wistar rats. S Afr J Bot. 2020 Mar;129:82-90. doi: 10.1016/j.sajb.2019.01.014.
36. Usman A, Kawu MU, Shittu, Saleh, Bilbonga. Comparative effects of *Moringa oleifera* and ascorbic acid on biochemical changes induced by subchronic lead toxicity in male wistar rats. Pharmacol Res Mod Chin Med. 2022 Jun;4(9):100140. doi: 10.1016/j.prmcm.2022.100140.
37. Akyuz E, Turkoglu S, Sozgen Baskan K, Tutem E, Apak MR. Comparison of antioxidant capacities and antioxidant components of commercial bitter melon (Momordica charantia L.) products. Turk J Chem. 2020;44(6):1663-73. doi: 10.3906/kim-2007-67, PMID 33488261.
38. WU S, Huang C, Chen YR, Huang HC, Huang WC, Lai YH. Momordica charantia leaf extract reduces hepatic lipid accumulation and diet induced dyslipidemia in zebrafish through lipogenesis and beta-oxidation. J Funct Foods. 2021;87:104857. doi: 10.1016/j.jff.2021.104857.
39. Offor U, Naidu EC, Ogedengbe OO, Jegede AI, Peter AI, Azu OO. Nephrotoxicity and highly active antiretroviral therapy: mitigating action of Momordica charantia. Toxicol Rep. 2018;5:1153-60. doi: 10.1016/j.toxrep.2018.09.003, PMID 30627515.
40. Wardana MS, Jufri M, Mun'im A. Physicochemical properties and nutrition of *Moringa oleifera* lam leaf extract: a preliminary study on preparation phytosomes as herbal supplement for children. Int J App Pharm. 2022;14(1):281-7. doi: 10.22159/ijap.2022v14i1.43477.
41. Muhaimin M, Chaerunisaa AY, Rostinawati T, Amalia E, Hazrina A, Nurhasanah S. A review on nanoparticles of *Moringa oleifera* extract: preparation characterization and activity. Int J App Pharm. 2023;15(4):43-51. doi: 10.22159/ijap.2023v15i4.47709.
42. Cortez Navarrete M, Martinez Abundis E, Perez Rubio KG, Gonzalez Ortiz M, Mendez Del Villar M. Momordica charantia administration improves insulin secretion in type 2 diabetes mellitus. J Med Food. 2018;21(7):672-7. doi: 10.1089/jmf.2017.0114, PMID 29431598.
43. Nakata H, Nakayama SM, Kataba A, Toyomaki H, Doya R, Beyene Yohannes Y. Does *Moringa oleifera* affect element accumulation patterns and lead toxicity in Sprague dawley rats. J Funct Foods. 2022 Sep;97:105242. doi: 10.1016/j.jff.2022.105242.
44. Oguntibeju OO, Aboua GY, Omodanisi EI. Effects of *Moringa oleifera* on oxidative stress apoptotic and inflammatory biomarkers in streptozotocininduced diabetic animal model. S Afr J Bot. 2020;129:354-65. doi: 10.1016/j.sajb.2019.08.039.