

Original Research Article

Assessment of hepatic profile changes in rat diabetes induced with streptozotocin and nicotinamide

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Abstract

Purpose: To determine the changes in hepatic profile during the evaluation of herbal mixtures of *Moringa oleifera* and *Raphanus sativus* in streptozotocin-nicotinamide (STZ- NA)-induced diabetic rat model.

Methods: Forty-eight (48) albino rats were randomly divided into eight groups of eight rats each. Diabetes was induced in all the groups except Group I (normal control) using nicotinamide (110 mg/kg) and streptozotocin (55 mg/kg). Groups I and II (untreated control) received only distilled water (2 mL/kg) while Group III received 100 mg/kg of metformin. Groups IV to VIII were treated with a dose of 200 mg/kg of the herbal mixtures (HEMA-C) at different ratios of *M. oleifera* and *R. sativus*. Blood samples were analyzed for biochemical markers while liver histopathology was assayed after 28 days of treatment.

Results: Acute toxicity study showed that the herbal mixtures did not exhibit mortality or adverse effect in rats up to a dose of 2000 mg/kg ($LD_{50} > 2000$ mg/kg) with a safe dose of 200 mg/kg (1/10th LD_{50}). Induction of diabetes significantly increased serum aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatases (ALP) and bilirubin levels in untreated control compared to normal control ($p < 0.001$). Administration of the herbal mixtures, especially HEMB, significantly ($p < 0.001$) restored these liver marker levels to levels comparable to metformin. Furthermore, histological examination showed that HEMB significantly restored the STZ-NA-induced liver damage in rats.

Conclusion: The herbal mixture possesses hepatoprotective effect and ameliorates the adverse diabetic conditions caused by STZ-NA-induced diabetes in rats. The herbal mixture also demonstrated better and extended therapeutic potential than the single herbs. Further pharmacological and biochemical investigations will elucidate the mechanisms of action and clarify the potentials of the herbal mixture as a therapeutic tool as an anti-diabetic therapy.

Keywords: Streptozotocin, Nicotinamide, Diabetes, *Moringa oleifera*, *Raphanus sativus*, Liver

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INTRODUCTION

Diabetes mellitus (DM) is a complex and progressive metabolic disorder characterized by increased blood glucose levels and disturbance

in insulin secretion or insulin resistance [1]. The global prevalence of diabetes is rising every year and it is estimated to reach 10.2 % (578 million) by 2030. The prevalence of diabetes is higher in urban areas (10.8 %) in comparison to rural

areas (7.2 %) [2]. An increase in weight is a major factor responsible for the increased prevalence of DM. Dyslipidemia also increases the risk of stroke, coronary heart disease, myocardial infarction and peripheral vascular disease in many folds, which are contributory to diabetes mellitus [3]. Also, abnormality in lipoprotein metabolism is commonly observed in diabetic patients and hyperlipidemia represents a major risk factor for atherosclerosis and cardiovascular complications development [4,5]. This disorder also leads to several complications in the heart, kidneys, eyes, brain and feet [6,7]. Previous studies have also shown that DM creates complications in the liver and causes liver diseases, such as fibrosis, cirrhosis, hepatic steatosis and nonalcoholic steatohepatitis [8,9]. Dysfunction of the hepatocytes in diabetic animals due to a decrease in oxidative phosphorylation increases oxidative stress and ultrastructural abnormalities in diabetic patients [10]. Increases in the level of glycosylated hemoglobin (HbA1c), decreased glycogen levels in the liver and hydroxyl radical-induced apoptosis of hepatocytes in diabetic rats were also observed in the condition of hyperglycemia.

Ayurvedic herbal preparations, comprising complex mixtures of plant materials, are widely practiced throughout the Indian subcontinent and are increasingly utilized worldwide as home remedies across various healthcare settings. Often touted as "natural" and "safe" alternatives to conventional medicines, herbal products, including dietary supplements containing herbs, are promoted to the public. However, the study of herb-drug interactions, an expanding area of research in modern medicine, faces challenges due to the lack of specific information resulting from ongoing investigations.

Moringa oleifera (Moringaceae family), also known as the oil tree, drumstick tree, horseradish tree and miracle tree, holds significant nutritional and medicinal values attributed to its leaves, which boast a plethora of beneficial properties including anti-diabetic, antioxidant, anti-hypertensive, anti-hyperlipidemic, anti-atherosclerotic, anti-ulcer, anti-inflammatory, anti-bacterial and anti-arsenic toxicity effects. These effects are facilitated by the presence of crucial chemical constituents such as flavonoids, phenols, tannins, alkaloids, steroids, chlorogenic acid, and amines. Similarly, radish (*Raphanus sativus*), an edible root vegetable of the Brassicaceae family, is prized for its medicinal benefits, particularly in treating hypertension, cardiometabolic disorders, and as an antimicrobial and antioxidant agent.[11] The present investigation aims to observe the

changes in the hepatic profile during the evaluation of Streptozotocin-Nicotinamide-induced diabetes in rats.

EXPERIMENTAL

Collection, authentication and preparation of hydroalcoholic extracts of plant material

The leaves of *Moringa oleifera* and roots of *Raphanus sativus* plants were sourced from the Government Nursery, Moinabad, in Hyderabad in February 2019. It was authenticated by scientists at the Botanical Survey of India (BSI) and also deposited at the BSI with voucher no. BSI/DRC/2019-20/Tech/609. The plant materials were sundried and subsequently ground into powdered form. Powdered leaves 70 g of *M. oleifera* and roots (70 g) of *R. sativus* were taken separately and initially defatted with petroleum ether, after which the solvent was allowed to evaporate completely. Thereafter, each residue was dissolved in hydroalcoholic solvent of 45 mL water and 135 mL ethanol (water: ethanol solution of 30:70 v/v) and extracted with a Soxhlet extractor. The resulting hydroalcohol extract was filtered and concentrated in a rotary evaporator [12,13]. The crude extracts were preserved at low temperatures for further investigation.

Preparation of herbal mixtures

Following the extraction process, three distinct mixtures (designated A, B and C), containing the herbal extracts, were formulated as follows: Herbal mixture A (HEMA) was composed of 50 % *M. oleifera* and 50 % *R. sativus* while herbal mixture B (HEMB) comprised 70 % *M. oleifera* and 30 % *R. sativus*. Lastly, Herbal extracts mixture C (HEMC) had 30 % *M. oleifera* and 70 % *R. sativus* [14].

Design

Forty-eight (48) healthy albino Wistar rats of either sex, weighing between 180-220 g, were sourced from the Animal Facility, Jeeva Life Sciences, Hyderabad, India. The animals were kept for two weeks on a normal diet and water *ad libitum* before the start of the experiment. The rats were handled according to the approved methods of the institutional committee. Ethical clearance for the animal study was obtained from the Institutional Animal Ethics Committee (IAEC) of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Jeeva Life Sciences, Hyderabad, Telangana, India, (approval no. CPCSEA/IAEC/JLS/011/11/19/

018). The study protocol followed international guidelines for animal studies.

Oral acute toxicity of herbal mixtures

The OECD guideline 420 was used to conduct the acute toxicity investigation. The herbal mixtures (HEMA, HEMB and HEMC) were each orally administered at doses of 5, 50, 300 and 2000 mg/kg body weight to observe the acute toxicity, with each dose administered to 3 rats, while control animals received distilled water. Rats' behavioural changes were tracked for 14 days to determine the mean lethal dose (LD₅₀) value [15,16].

Anti-diabetic study

Rats were randomly assigned to the following groups after becoming acclimated; each group comprised six animals. All administrations of herbal mixtures and the standard drug (metformin) were done orally, once daily for 28 days. The groups and their treatment are shown in Table 1.

Induction of diabetes and administration of herbal mixtures

Diabetes was induced in overnight fasted rats of Group II to VIII (40 rats in total) by injecting a single dose of Nicotinamide (110 mg/kg b.wt.) followed by a single dose of Streptozotocin (55 mg/kg b.wt.) in citrate buffer, with a pH of 4.5 [17]. Then, rats were fed with a 5 % glucose solution for the next 24 hours to prevent fatal hypoglycemia and after 72 hours, they were

examined for diabetes by determining the blood glucose levels of rats using a glucometer. The rats were further stabilized for 7 days [18]. All animals were sacrificed by cervical decapitation on the 29th day of study after an overnight fast. Blood samples were collected by cardiac puncture, serum separated and used to evaluate biochemical parameters.

Determination of biochemical parameters

The concentration of aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatases (ALP) and total bilirubin in the serum were determined according to standard procedures.

Histopathological examination

Tissue samples of harvested rat liver were placed in 10 % neutral buffered formalin (NBF). They were subsequently embedded in paraffin wax to form a homogenous mass and further processed for staining. After mounting, tissues were stained with eosin and hematoxylin and viewed under the microscope [20].

Statistical analysis

The results are expressed as mean \pm standard error of the mean (SEM). Data were analyzed statistically using one-way analysis of variance (ANOVA). Furthermore, Duncan's multiple range (DMR) test was applied in the case of significant differences among the groups at a 5 % level of significance [21].

Table 1: Groups and their treatment

Group	Treatment
I	Non-diabetic (ND) rats given distilled water and used as a normal control.
II	STZ-NA diabetic rats given distilled water and used as disease control
III	STZ-NA diabetic rats given metformin (100 mg/kg b.wt. per day up to 28 days) orally and served as standard.
IV	STZ-NA diabetic rats given hydroalcohol concentrate of HEMA (200 mg/kg b. wt.) administered orally.
V	STZ-NA diabetic rats given hydroalcohol concentrate of HEMB (200 mg/kg b. wt.) administered orally.
VI	STZ-NA diabetic rats given hydroalcohol concentrate of HEMC (200 mg/kg b. wt.) administered orally.
VII	STZ-NA diabetic rats given hydroalcohol extract of <i>M. oleifera</i> (200 mg/kg b. wt.) administered orally.

RESULTS

Acute toxicity of herbal mixtures

The non-toxic nature of herbal mixtures was demonstrated by acute toxicity experiment, wherein treated rats exhibited normal behaviour and no significant changes in neurological or behavioural responses up to 2000 mg/kg b.wt. Also, there was no toxicity or mortality up to 2000 mg/kg b.wt. (Table 2). The results of acute toxicity showed an LD₅₀ above 2000 mg/kg. Therefore, the therapeutic dose was determined to be 1/10th of 2000 mg/kg (200 mg/kg b. wt.) of the concentrates, which was used for further investigation.

Table 2: Acute toxicity of herbal mixtures

Observation	1 st Day	7 th Day	14 th Day
Gross activity	+	+	+
Respiration	-	-	-
Writhing	-	-	-
Tremor	-	-	-
Convulsions	-	-	-
Hind limb Paralysis	-	-	-
Sense of touch and sound	+	+	+
Salivation	+	+	+
Urination	+	+	+
Diarrhoea	-	-	-
Mortality	-	-	-

Hepatoprotective potentials of the herbal mixtures

The hepatoprotective effect of herbal mixtures on serum biochemical parameters of diabetes-

induced hepatotoxicity in rats is shown in Table 3. It was observed that serum ALT, AST and ALP levels were significantly increased in disease control in comparison to other groups ($p < 0.001$). Metformin significantly decreased the mean serum ALT, AST and ALP levels during the experiment but administration of the herbal mixture B (HEMB) significantly ($p < 0.001$) restored these liver markers levels. In addition, the mean serum bilirubin levels increased significantly in the disease control. However, administration of the herbal mixture B (HEMB) significantly restored the bilirubin levels to the same concentration as the standard drug (metformin).

Effect of herbal mixture on liver histopathology

Figure 1 displays photomicrographs produced from the examination of the liver's histopathology. The results from the histopathological examination of the liver corroborate the biochemical enzyme assays. The control group's liver section displayed normal sinusoids, a typical look of the hepatic portal region as well as a typical appearance of the hepatic cell in the parenchyma. On the other hand, the liver of the disease control group exhibited congestion of hepatic cells.

Treatment with the standard drug, Metformin, revealed almost normal arrangements of hepatic cells and hepatic triad. However, photomicrograph of liver section of herbal mixture A (HEMA)-treated group showed a poor ameliorative effect characterized by congestion in hepatic cells and fusion of portal triad. Furthermore, rats treated with herbal mixture B (HEMB) revealed a normal appearance of

Table 3: Hepatoprotective effect of herbal mixtures on serum biochemical parameters of diabetes-induced hepatotoxicity in rats

Group (mg/kg)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)
Normal control	43.43±0.34	45.74±0.31	114.11±0.41	0.447±0.01
Disease control	105.93±0.26###	128.28±0.29###	199.01±0.15###	1.75±0.01###
Standard	42.80±0.22***	40.97±0.54***	136.23±0.32***	0.565±0.01***
HEMC (200)	51.59±0.25**	47.22±0.34**	158.29±0.59**	1.09±0.105**
HEMB (200)	42.99±0.12***	40.95±0.105***	139.45±0.35***	0.55±0.01***
HEMA (200)	60.865±0.19**	57.46±0.34**	166.06±0.23**	0.74±0.01**
HEMO (200)	65.79±0.44**	60.40±0.513**	164.10±0.62**	1.22±0.17**
HERS (200)	67.01±0.62**	62.94±0.87**	166.078±1.18**	1.29±0.15**

HEMA (Herbal mixture A); HEMB (Herbal mixture B); HEMC (Herbal mixture C); HEMO (Herbal extract of *M. oleifera*); HERS (Herbal extract of *R. sativus*); ALT (Alanine aminotransferase); AST (Aspartate aminotransferase); ALP (Alkaline phosphatase). Values are presented as mean ± SEM (n = six rats per group). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ^{ns} $p > 0.05$; ### values are extremely significant at $p < 0.001$ when compared to normal control; *** values were extremely significant at $p < 0.001$ when compared to standard group; ** values were highly significant at $p < 0.01$ when compared to standard group

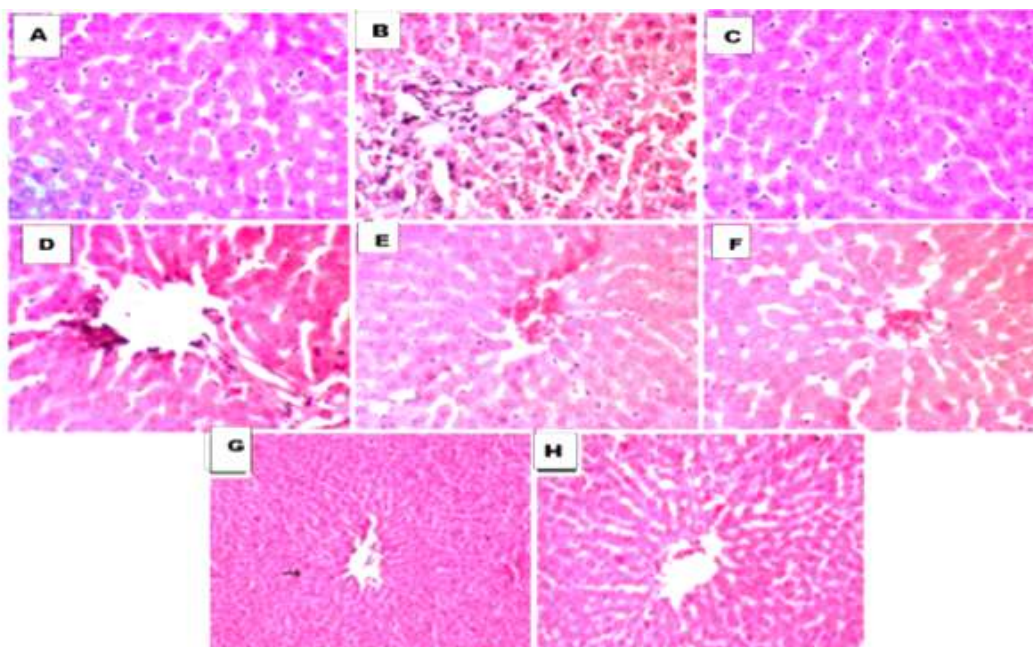


Figure 1: A-H: Photomicrographs of liver histopathology of control, Disease control, Metformin, Herbal mixture A, B, C, *M. oleifera* and *R. sativus* treated Wistar rats (H&E staining 40X). (A) Control rat's liver with normal sinusoids, hepatic portal region, and a typical appearance of the hepatic cell in the parenchyma. (B) The photomicrograph of the liver of the disease control group shows congestion of hepatic cells (C) Metformin-treated animals revealed almost normal arrangements of hepatic cells and hepatic triad. (D) Photomicrograph of liver section of herbal mixture A (HEMA)-treated group revealed the poor ameliorative effect as indicated due to congestion in hepatic cells and fusion of portal triad. (E) Photomicrograph of herbal mixture B (HEMB) treated group revealed a normal appearance of hepatocytes with mild congestion in the hepatic area. (F) Photomicrograph of liver section of herbal mixture C (HEMC)-treated group revealed mild degree congestion in hepatocytes showing partial ameliorative effect. (G) Photomicrograph of liver section of *M. oleifera* treated group revealed mild degree congestion in hepatocytes showing partial ameliorative effect. (H) Photomicrograph of the liver section of the *R. sativus* treated group revealed a poor ameliorative effect as indicated due to congestion in hepatic cells

hepatocytes with mild congestion in the hepatic area. On the other hand, liver sections of diabetic rats treated with HEMC was characterized with mild degree congestion in hepatocytes indicating a partial ameliorative effect. Interestingly, treatment with 200 mg/kg b.wt. of *M. oleifera* (HEMO) and *R. sativus* (HERS) revealed a partial or poor ameliorative effect due largely to congestion in the hepatic cells.

DISCUSSION

The liver is an organ that plays an important role in the maintenance of systemic glucose homeostasis and gluconeogenesis. Increased hepatic gluconeogenesis is a sign of liver complication, which causes chronic hyperglycemia, mostly observed in diabetes. This study investigated the changes in the hepatic profile during the evaluation of streptozotocin- and nicotinamide-induced diabetes in rats [22]. An acute toxicity test was performed according to the OECD guidelines. Based on acute toxicity tests, doses were selected to assess the anti-diabetic activity of the herbal mixture in STZ-NA-induced diabetic rats.

In this study, STZ-NA induced diabetic rats had significantly higher serum levels of the liver function markers ALT, AST and ALP than control group. This could be because the enzymes in diabetic conditions leaked into the bloodstream following hepatocellular injury, disrupting the liver markers levels. The herbal mixtures produced significant reversible effects and restored the liver biomarkers. This could be due to phyto-constituents such as flavonoids, terpenoids and alkaloids in the mixture which produce antioxidant and hepatoprotective effects in treated animals [23]. Furthermore, liver histopathology was done in all groups of rats and untreated STZ-NA-induced diabetic rats had congestion of hepatocytes in the liver. Compared to other herbal mixture treatment groups, the results showed that herbal mixture B (HEMB) considerably improved hepatocytes with normal appearance and mild to moderate congestion.

CONCLUSION

The result shows that herbal mixture B (HEMB) possesses a better hepatoprotective effect and ameliorates adverse diabetic conditions imposed

by streptozotocin-nicotinamide-induced diabetes in rats. Compared to single plant, herbal mixtures have better and extended therapeutic potential. Further pharmacological and biochemical investigations will aid the elucidation of the mechanisms of action and clarify the therapeutic potentials of the herbal mixture.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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REFERENCES

1. Chen M, Zheng H, Xu M, Zhao L, Zhang Q, Song J, Zhao Z, Lu S, Weng Q, Wu X, et al. Changes in hepatic metabolic profile during the evolution of STZ-induced diabetic rats via ¹H NMR-based metabonomic investigation. *Biosci Rep* 2019; 39 (4): BSR20181379.
2. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J Clin Invest* 1973; 52 (7):1544-1568.
3. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018; 138:271-281.
4. Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. *Hepatology* 2014; 59(2):713-723.
5. McKimmie RL, Daniel KR, Carr JJ, Bowden DW, Freedman BI, Register TC, Hsu FC, Lohman KK, Weinberg RB, Wagenknecht LE. Hepatic steatosis and subclinical cardiovascular disease in a cohort enriched for type 2 diabetes: the diabetes heart study. *Am J Gastroenterol* 2008; 103(12): 3029-3035.
6. Solomon SD, Uno H, Lewis EF, Eckardt KU, Lin J, Burdman EA, de Zeeuw D, Ivanovich P, Levey AS, Parfrey P, et al. Erythropoietic response and outcomes in kidney disease and type 2 diabetes. *N Engl J Med* 2010; 363(12): 1146-1155.
7. Chiang DJ, Pritchard MT, Nagy LE. Obesity, diabetes mellitus, and liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2011; 300(5): G697-G702.
8. Dey A, Swaminathan K. Hyperglycemia-induced mitochondrial alterations in liver. *Life Sci* 2010; 87(7-8): 197-214.
9. Parveen K, Khan MR, Mujeeb M, Siddiqui WA. Protective effects of Pycnogenol on hyperglycemia-induced oxidative damage in the liver of type 2 diabetic rats. *Chem Biol Interact* 2010; 186(2): 219-227.
10. Uma P, Venkatachalam VV, Manichandrika P. Preparation and evaluation of polyherbal Formulation (PHF) Extracts and its phytochemical investigation. *IJBPAS* 2020; 9(7): 1525-1531.
11. Arulselvan P, Senthilkumar GP, Sathish Kumar D, Subramanian S. Anti-diabetic effect of *Murraya koenigii* leaves on streptozotocin-induced diabetic rats. *Pharmazie* 2006; 61(10): 874-877.
12. Dhanalakshmi S, Aleema SU, Lakshmi M, Lokesh K, Sangeetha G. Anti-Diabetic Activity of Herbal Mixture. *J Pharm Res* 2017; 11(4): 278-280.
13. Lanjhiyana S, Garabadu D, Ahirwar D, Bigoniya P, Rana AC, KC Patra, Kumar SL, Karuppai M. Hypoglycemic activity studies on root extracts of *Murraya koenigii* root in Alloxan-induced diabetic rats. *J Nat Prod Plant Resour* 2011; 1(2): 91-104.

14. Ng SH, Mohd Zain MS, Zakaria F, Wan Ishak WR, Wan Ahmad WA. Hypoglycemic and Antidiabetic Effect of *Pleurotus sajor-caju* Aqueous Extract in Normal and Streptozotocin-Induced Diabetic Rats. *Biomed Res Int* 2015; 214918.
15. Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. *Exp Biol Med (Maywood)* 2012; 237 (5): 481-90.
16. Bancroft JD, Gamble M. *Theory and practice of histological techniques*. Philadelphia, Churchill Livingstone 2007; p. 131-133.
17. Steel RGD, Torri JH, Dicky DA. *Principles and procedures of statistics: a biometrical approach (3rd Ed.)*. McGraw Hill Book Co. Inc., New York. 2007
18. Arun Giridhari V, Malathi D, Geetha K. Anti-diabetic property of drumstick (*Moringa oleifera*) leaf tablets. *Int J Health Nutr* 2011; 2: 1–5
19. Khidr BM, El-Sokkary GH, Saleh SM. Study on morphological changes induced by aspartame on liver of normal and diabetic male albino rats. *J Histol Histopathol* 2017; 4: 1, <https://doi.org/10.7243/2055-091X-4-1>
20. Kumakura K, Kato R, Kobayashi T, Sekiguchi A, Kimura N, Takahashi H, Takahashi A, Matsuoka H. Nutritional content and health benefits of sun-dried and salt-aged radish (takuan-zuke). *Food Chem* 2017; 231: 33–41.
21. Ouyang J, Sun F, Feng W, Sun Y, Qiu X, Xiong L, Liu Y, Chen Y. Quercetin is an effective inhibitor of quorum sensing, biofilm formation and virulence factors in *Pseudomonas aeruginosa*. *J Appl Microbiol* 2016; 120: 966-974.
22. Abed SA, El-Shazely MO, Ahmed KA, Abdelmawla EM, Ibrahim AK. Pathological, immunohistochemical and biochemical studies on the therapeutic effect of *Raphanus sativus* Oil on streptozotocin-induced diabetic rats, *Egyptian J Comp Pathol Clin Pathol* 2015; 28: 1-17.
23. Pocasap P, Weerapreeyakul N, Barusrux S. Cancer preventive effect of Thai rat-tailed radish (*Raphanus sativus* L. var. *Caudatus Alef*). *J Funct Foods* 2013; 5: 1372-1381.