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RESEARCH ARTICLE

Renal Protective Effects of *Passiflora edulis* in a Rat Model of Fructose-induced Diabetes

Kinjal P. Patel* and Rajesh A. Maheshwari

Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara - 391760, Gujarat, India; kinjalpatel54@gmail.com

Abstract

Background: The global prevalence of diabetes mellitus is escalating rapidly, with fructose consumption emerging as a significant risk factor for metabolic disorders. Extract of *Passiflora edulis* (EPE) (passion fruit) has gained attention for its potential therapeutic benefits in managing diabetes. **Aim:** This study aims to investigate the efficacy of EPE in mitigating fructose-induced diabetic complications. **Methods:** Acute oral toxicity tests revealed the safety of EPE up to 5000 mg/kg. Subsequent pharmacological studies demonstrated that EPE treatment significantly reduced glycated haemoglobin levels and kidney index in fructose-fed rats. **Results:** EPE administration effectively ameliorated renal dysfunction by decreasing serum creatinine, uric acid, Blood Urea Nitrogen (BUN) and urea levels. Additionally, EPE treatment attenuated pro-inflammatory mediators, C Reactive Protein (CRP), Tumour Necrosis- Alpha (TNF-α), and Interleukin (IL-6) levels in fructose-fed rats. Histopathological analysis further supported the protective effects of EPE, showing improvements in kidney morphology. These findings suggest that EPE has the potential to mitigate fructose-induced renal damage and may offer therapeutic benefits in diabetes management. **Conclusion:** Further research is warranted to explore the clinical applications of EPE in diabetic patients.

Keywords: Diabetes Mellitus, Fructose, Glycated Haemoglobin, *Passiflora edulis* Extract, Pro-inflammatory Mediators

1. Introduction

The global prevalence of diabetes mellitus has reached alarming levels, with an estimated 537 million (10.5%) people projected to be living with the condition by 2025. If no adequate measures are taken to tackle the issue, it is anticipated that by 2030, the estimated number of people living with diabetes will reach 643 million comprising 11.3% of the global inhabitants. Should current trends persist, this figure is projected to surge to an astonishing 783 million, accounting for 12.2% of the population by 2045[1]. Among the various factors contributing to this epidemic, excessive fructose consumption has come out as a significant risk parameter for the onset of metabolic syndrome[2]. Fructose, abundantly present in processed foods and sugary beverages, has detrimental effects on glucose metabolism, insulin sensitivity, and lipid profiles, predisposing individuals to insulin resistance, obesity and ultimately, type 2 diabetes[3,4]. In the quest for effective strategies to combat fructose-induced diabetes, natural remedies have garnered increasing attention for their potential therapeutic benefits. Among these, *P. edulis*, commonly known as passion fruit, has emerged as a promising candidate due to its rich phytochemical composition and traditional use in folk medicine for various health ailments[5]. *Passiflora edulis* is renowned for its antioxidant, blood pressure-lowering, anti-cancer, blood sugar-lowering, cholesterol-lowering and other properties, making it a subject of interest for researchers exploring alternative therapies for diabetes management[5]. This study aims to unveil the therapeutic potential of the extract of *Passiflora edulis* (EPE) in mitigating fructose-induced diabetes. By reviewing existing literature and elucidating the underlying mechanisms of action, the study seeks to

*Author for correspondence

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provide insights into the efficacy and mechanisms of EPE as a natural intervention for diabetes prevention and management.

2. Materials and Methods

2.1 Drugs and Chemicals

Fructose was purchased from a local market. Standard biochemical kits and analytical-grade chemicals and reagents were used in the study.

2.2 Experimental Animals

The Zydus Research Centre in Ahmedabad provided the female Wistar rats that were used in this study. The rodents weighed between 200 and 250 g. Every rat was housed in an ideal environment, which featured a light/dark cycle with a 12-hour duration, temperature regulated at 24 ± 1 °C, and humidity ranging from 35% to 60%. They were given unrestricted access to a pellet diet and water throughout the research period. Before commencing the experiment, a 48-hour adaptation period was allowed for the rats. The Institutional Animal Ethics Committee (IAEC) gave its approval for the study.

2.3 Acute Toxicity Study

Acute oral toxicity testing was carried out on albino Wistar rats weighing 200 to 250g by OECD 425 guidelines. Three female rats were given different dosages of EPE orally (100, 200, 500, 1000, 2000 and 5000 mg/kg). Their symptoms were observed every day for 14 days, with intervals of 0, 30, 60, 120, 180 and 240 minutes in between.

2.4 Experimental Design

Five groups were formed randomly in which six animals were assigned to each group. In this experimental setup, various groups of rats were subjected to different treatments to evaluate the effects of EPE on fructose-induced conditions. Group I functioned as the normal control, which was given distilled water orally. Group II consisted of rats consuming a fructose solution in their drinking water, establishing a fructose control condition. Groups III, IV and V comprised fructose control rats treated with increasing doses of EPE (250, 500 and 1000 mg/kg correspondingly) administered orally. These groups aimed to assess the potential dose-dependent effects of the EPE on the parameters under investigation. Diabetes was induced by administering fructose (20%) to all groups except for group I[6]. During these 8 weeks, the animals in each group received their respective therapies daily. Glass capillaries were used to take blood samples from the retro-orbital plexus of anaesthetised rats once the experiments were finished. These samples were collected with or without disodium ethylenediaminetetraacetate for the estimation of biochemical parameters. Following a 15-minute clotting period, centrifugation of blood was done at 5000rpm for around 20 minutes to separate the serum. The storage condition was maintained at -20°C until any other biochemical investigation could be conducted. Glycated Haemoglobin (HbA1C) levels were measured using whole blood samples. This kidney index was used to assess kidney enlargement or hypertrophy. Serum samples were utilised to estimate levels of creatinine, urea, BUN and uric acid, CRP, TNF-α and IL-6 employing a standard diagnostic kit.

2.5 Histopathology

Following euthanasia, kidneys from each experimental group were swiftly extracted and rinsed with saline before fixation in 10% formalin buffered with phosphate. Subsequently, the samples were embedded in paraffin, divided into sections with a thickness of 5µm and stained with hematoxylin and eosin. To examine these slices, a light microscope (Olympus B × 10, Tokyo, Japan) was used and identify any histopathological alterations and photomicrographs were captured using an Olympus DP12 camera (Japan). Importantly, the individual conducting the histopathological assessment remained unaware of the specific treatment group assigned to each animal, ensuring unbiased evaluation.

2.6 Statistical Analysis

The outcomes were presented as mean ± SEM (n=6). One-way ANOVA helped to find a statistical difference among various groups. Then Bonferroni multiple comparisons post hoc test was done using Prism and GraphPad software and interpreted statistically as a p-value of less than 0.05, statistical relevance.

3. Results

3.1 Acute Oral Toxicity

Administering EPE at doses up to 5000 mg/kg exhibited no toxicity-related symptoms, nor was there any mortality observed over 14 days. Consequently, pharmacological studies were conducted using EPE at dose levels of 1/20th (250 mg/kg), 1/10th (500 mg/kg) and 1/5th (1000 mg/kg) of the maximum dose.

3.2 Impact of EPE on Glycated Haemoglobin and Kidney Index

Fructose-fed control rats exhibited a marked increase in HbA1C levels and kidney index compared to rats of normal control group. Treatment with EPE at doses of 500 mg/kg and 1000 mg/kg considerably lowered HbA1C levels relative to the disease control group. Moreover, rats administered with EPE exhibited a considerable reduction in kidney index across all dosage levels (Figure 1).

3.3 Impact of EPE on Renal Function Tests

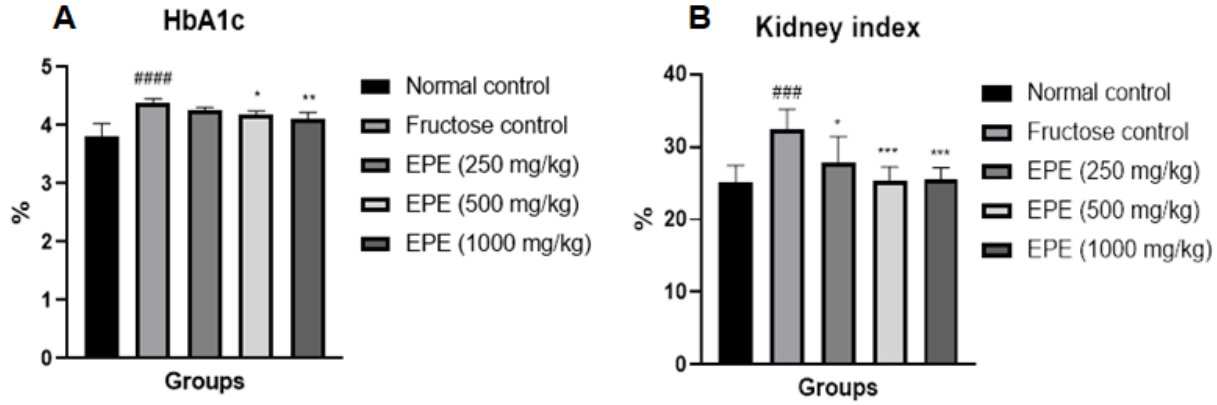
Fructose control rats displayed a significant elevation in serum creatinine, BUN, urea and uric acid levels compared to rats of the normal control group. Administration of EPE at doses of 250, 500 and 1000 mg/kg caused a substantial lessening in serum creatinine ($P<0.001$; $P<0.0001$; $P<0.0001$), urea ($P<0.05$; $P<0.001$; $P<0.001$), BUN ($P<0.001$; $P<0.0001$) and uric acid ($P<0.05$; $P<0.0001$; $P<0.0001$) levels relative to the fructose control rats. Additionally, the treatment with 250 mg/kg EPE did not significantly alter BUN levels when compared to rats that were only given fructose (Figure 2).

3.4 Impact of EPE on TNF-α, IL-6 and CRP

Fructose control animals showed drastic changes in the levels of TNF-α, IL-6 and CRP compared to normal control rats. Treatment with EPE having doses of 250, 500 and 1000 mg/kg resulted in a notable decline in TNF-α ($P<0.05$; $P<0.01$; $P<0.001$), IL-6 ($P<0.01$; $P<0.0001$), and CRP ($P<0.01$; $P<0.0001$) levels relative to the fructose control group. Furthermore, administering 250 mg/kg of EPE did not significantly affect IL-6 and CRP levels compared to untreated fructose control rats (Figure 3).

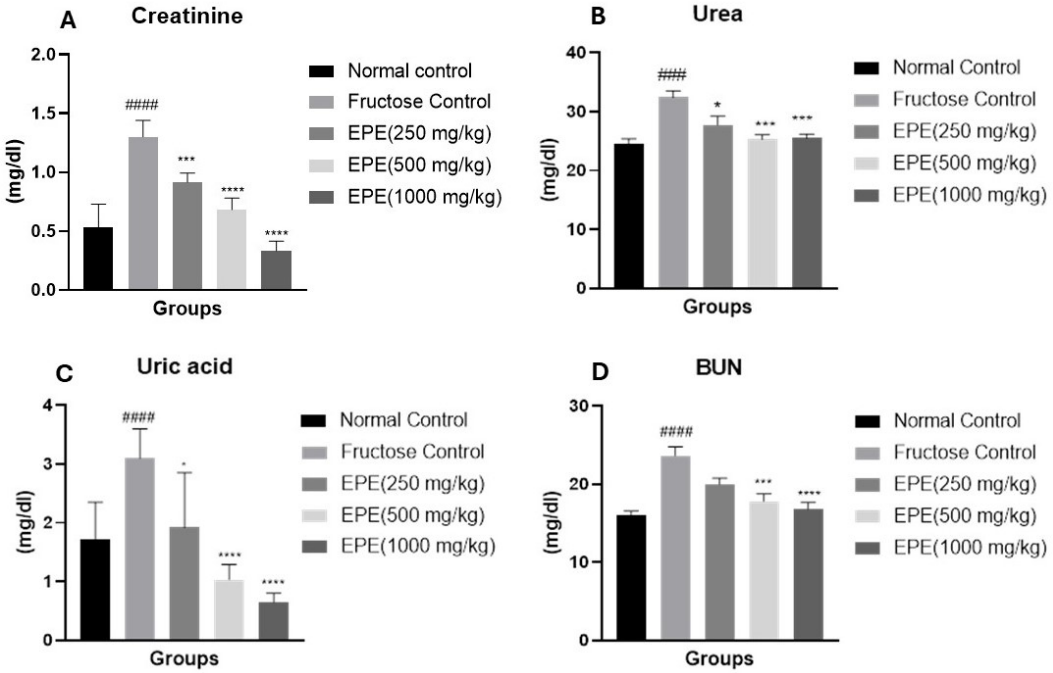
3.5 Histopathological Study

The glomeruli and tubules of the renal tissue that were examined histopathologically in the normal control group were found to have a normal appearance. In contrast, kidney tissue from fructose control rats exhibited dilation of Bowman's capsular space, extensive necrosis of the proximal convoluted tubules and tubular granular degeneration. Treatment with EPE resulted in mild to moderate dilation of Bowman's capsular space and reduced severity of necrosis in the



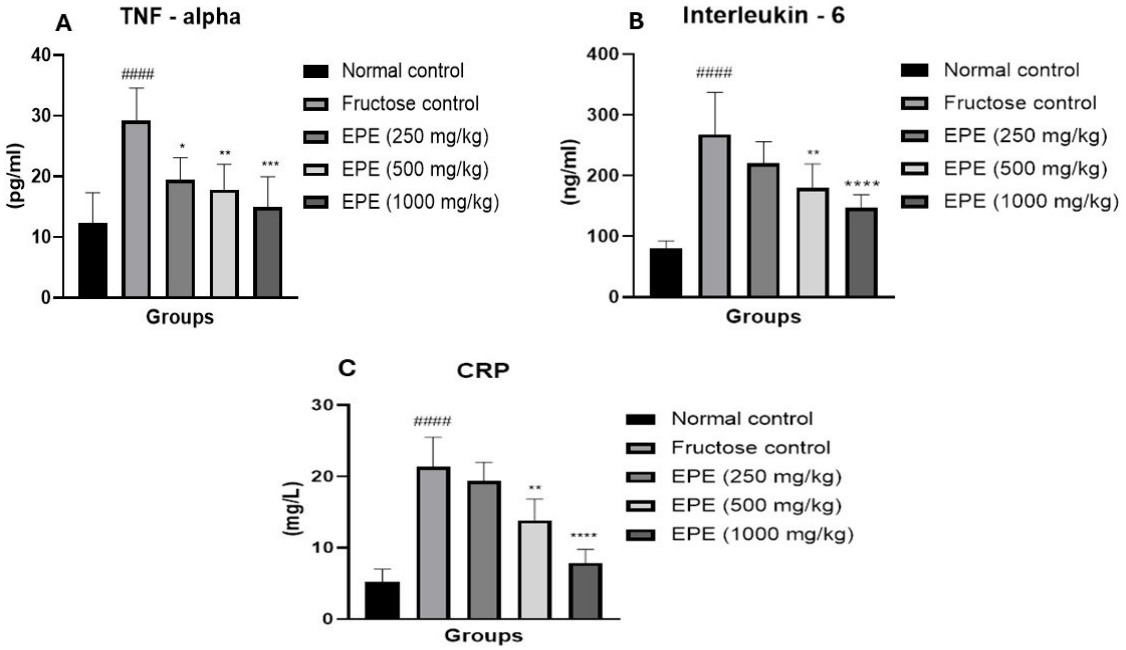
Mean ± SEM is used to express all values (n=6).
$^{###}P<0.001$, $^{####}P<0.0001$, compared to normal control; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to fructose control.

Figure 1. Effect of EPE on, **(A).** HbA1c; **(B).** Kidney index levels against fructose-induced diabetes.



The Mean ± SEM is used to express all values (n=6).
$^{###}$P<0.001, $^{####}$P<0.0001, compared to normal control; *P<0.05, ***P<0.001, ****P<0.0001compared to fructose control.

Figure 2. Effect of EPE on **(A).** Creatinine; **(B).** Urea; **(C).** Uric acid; **(D).** BUN levels against fructose-induced diabetes.



The Mean ± SEM is used to express all values (n=6).
####P<0.0001 compared to normal control; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to fructose control..

Figure 3. Effect of EPE on **(A).** TNF-α; **(B).** Interleukin-6; **(C).** CRP levels against fructose-induced diabetes.

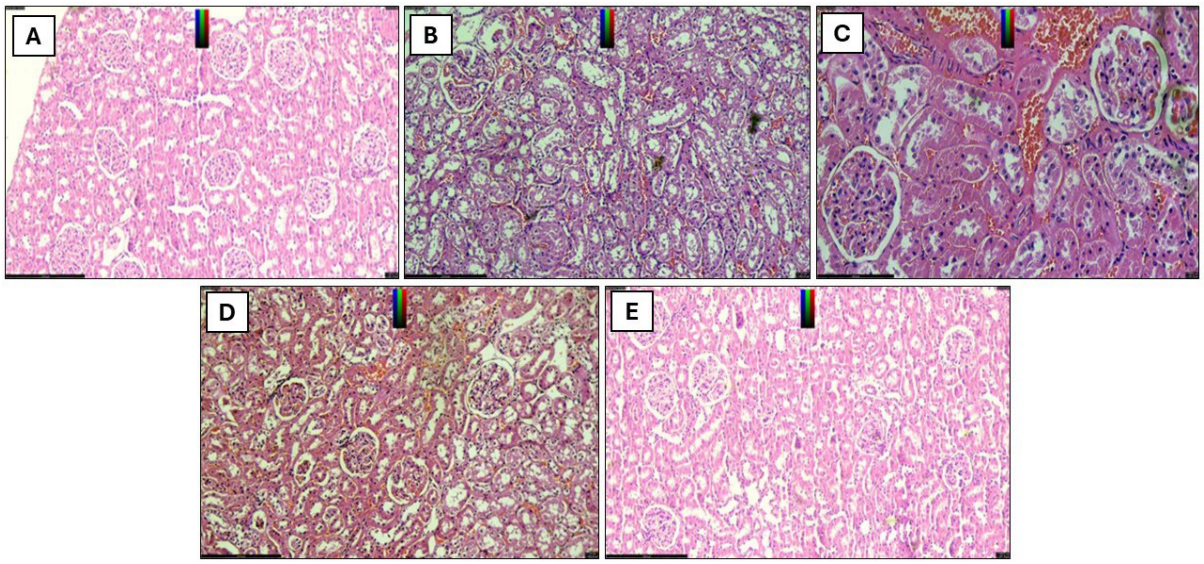


Figure 4. Rat kidney tissues under light microscopy. Groups represented as control group, fructose control group, EPE 250 mg/kg, EPE 500 mg/kg and EPE 1000 mg/kg with alphabet A, B, C, D and E respectively.

proximal convoluted tubules and tubular granular degeneration (Figure 4).

4. Discussion

The main cause of end-stage renal disease and a major influence on world morbidity and death is diabetic nephropathy[7]. Stopping diabetic nephropathy from getting worse still presents a major hurdle for biomedical research. Diabetic nephropathy can be detected by raised levels of HbA1C, serum creatinine, uric acid, BUN and urea[8,9]. Fructose-induced diabetic renal damage is characterised by elevated renal function test markers such as creatinine, uric acid and urea, along with increased levels of HbA1C. Research indicates that high fructose intake can lead to metabolic changes that exacerbate kidney function decline in diabetic conditions. Elevated levels of these markers are indicative of renal impairment and are commonly used to assess the extent of diabetic nephropathy[6,10-13]. In our investigation, rats subjected to a fructose-rich diet exhibited a notable rise in HbA1C, serum creatinine, uric acid, BUN and urea levels, consistent with the findings reported in the aforementioned study. Nevertheless, administering EPE having 250, 500 and 1000 mg/kg doses exhibited a marked lessen in the aforementioned biomarkers, indicating that EPE may possess a protective effect against fructose-induced diabetic renal damage. In a prior investigation, rats consuming fructose-infused water exhibited a notable increase in pro-inflammatory mediators, contributing to renal damage in diabetic rats[14]. In our current research, rats in the fructose control group demonstrated a considerable rise in the aforementioned parameters, together with, CRP, IL-6 and TNF-α which are implicated in causing renal damage in diabetic rats, in line with the results documented in the previously mentioned investigation. Administering EPE resulted in a noticeable drop in CRP, TNF-α and IL-6 levels in comparison with rats of the fructose untreated group. Additionally, the histopathological examination reinforces the findings of the biochemical parameters in our study.

5. Conclusion

In conclusion, EPE treatment effectively mitigated elevated glycated haemoglobin levels and kidney index induced by fructose feeding. Additionally, EPE demonstrated significant improvements in renal function tests, including serum creatinine, uric acid, BUN and urea levels, compared to fructose control rats. Moreover, EPE administration resulted in a notable decline in pro-inflammatory indicators IL-6, CRP and TNF-α levels. Histopathological examination confirmed the protective effects of EPE, showing

amelioration of renal damage features observed in fructose control rats. These findings collectively suggest the potential therapeutic efficacy of EPE in mitigating fructose-induced renal damage and warrant further investigation into its clinical application.

6. References

1. International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: International Diabetes Federation. 2021.
2. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, *et al*. Potential role of sugar (fructose) in the epidemic of hypertension, obesity metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr. 2007; 86(4):899-906. https://doi.org/10.1093/ajcn/86.4.899 PMid:17921363
3. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev. 2010; 90(1):23-46. https://doi.org/10.1152/physrev.00019.2009 PMid:20086073
4. Johnson RJ, Perez-Pozo SE, Sautin YY, Manitius J, Sanchez-Lozada LG, Feig DI, *et al*. Hypothesis: Could excessive fructose intake and uric acid cause type 2 diabetes? Endocr Rev. 2009; 30(1):96-116. https://doi.org/10.1210/er.2008-0033 PMid:19151107 PMCid: PMC2647706.
5. He X, Luan F, Yang Y, Wang Z, Zhao Z, Fang J, *et al*. *Passiflora edulis*: An insight into current researches on phytochemistry and pharmacology. Front Pharmacol. 2020; 11:617. https://doi.org/10.3389/fphar.2020.00617 PMid:32508631 PMCid: PMC7251050.
6. Mamikutty N, Thent ZC, Sapri SR, Sahruddin NN, Mohd Yusof MR, Haji Suhaimi F. The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. BioMed Res Int. 2014; 2014:263897. https://doi.org/10.1155/2014/263897 PMid:25045660 PMCid: PMC4086416.
7. Ntemka A, Iliadis F, Papanikolaou NA, Grekas D. Network-centric analysis of genetic predisposition in diabetic nephropathy. Hippokratia. 2011; 15(3):232-7.
8. Idonije BO, Festus O, Oluba OM. Plasma glucose, creatinine and urea levels in type 2 diabetic patient attending a Nigerian Teaching Hospital. Res J Med Sci. 2011; 5:1-3 https://doi.org/10.3923/rjmsci.2011.1.3
9. Maheshwari RA, Balaraman R, Sen AK, Seth AK. Effect of coenzyme Q10 alone and its combination with metformin on streptozotocin-nicotinamide-induced diabetic nephropathy in rats. Indian J Pharmacol. 2014; 46(6):627-32. https://doi.org/10.4103/0253-7613.144924 PMid:25538335 PMCid: PMC4264079.
10. Gersch MS, Mu W, Cirillo P, Reungjui S, Zhang L, Roncal C, *et al*. Fructose, but not dextrose, accelerates the progression of chronic kidney disease. Am J Physiol Renal Physiol. 2007; 293(4):F1256-61. https://doi.org/10.1152/ajprenal.00181.2007 PMid:17670904.
11. Jia G, Aroor AR, Whaley-Connell AT, Sowers JR. Fructose and uric acid: Is there a role in endothelial function? Curr Hypertens Rep. 2014; 16:1-7. https://doi.org/10.1007/s11906-014-0434-z PMid:24760443 PMCid: PMC4084511.
12. Patel R, Shah P, Deshpande S, Shah G, Gohil P. Fructose diet and low dose streptozotocin treatment induces the development of diabetic nephropathy in rats. Orient Pharm Exp Med. 2015; 15:305-12. https://doi.org/10.1007/s13596-015-0193-7
13. Toyoda K, Suzuki Y, Muta K, Masuyama T, Kakimoto K, Kobayashi A, *et al*. High fructose diet feeding accelerates diabetic nephropathy in Spontaneously Diabetic Torii (SDT) rats. J Toxicol Sci. 2018; 43(1):45-58. https://doi.org/10.2131/jts.43.45 PMid:29415951.
14. Mogorosi MS. The influence of Oleanolic acid on high fructose-induced oxidative stress and inflammation in Sprague Dawley Rats (Doctoral dissertation, North-West University (South Africa).