ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Anti-diabetic and nephrotoxicity effect of *Aegle marmelos* leaf on alloxan-induced diabetic rat

Mohd. Sufiyan Siddiqui[1], Gaurav Sharma[*1], Asha Sharma[2]

[1]School of Sciences, Suresh Gyan Vihar University, Mahal, Jagatpura, Jaipur-302017, Rajasthan, India
[2]Department of Zoology, Swargiya PNKS Government P.G College, Dausa, Rajasthan, India

Article History:

Received on: 08 Jan 2020
Revised on: 13 Feb 2020
Accepted on: 18 Feb 2020

Keywords:

Diabetes mellitus,
Aeglemarmelos,
Antidiabetic,
Alloxan induced diabetic rats

ABSTRACT

Aegle marmelos is widely found in india. The leaves, roots, fruits, bark, and seeds are extensively used in Ayurveda. *A. marmelos* is mainly used for the treatment of diabetes mellitus. Alloxan (150 mg/kg b.w) was used in the Wistar rats for making the diabetic model. The oral administration of leaf extract of *Aegle marmelos* (200 and 400 mg/ kg b.w) was given for four weeks. The effect of ethanolic leaf extract of *Aegle marmelos* leaf extract on serum blood glucose as well as kidney function test [urea, uric acid, albumin, protein, and creatinine] were measured in the alloxan-induced diabetic rats. In the acute toxicity study, the ethanolic leaf extract of *Aegle marmelos* leaf was non-toxic at 2000 mg/kg in rats. The level of albumin and protein had significantly increased along with the serum glucose and urea, uric acid levels when they were observed and reduced in diabetic rats treated with both doses of ethanol leaf extract of *Aegle marmelos* as compared to diabetic group. Histopathological studies were revealed toward normal. Ethanolic extract of *Aegle marmelos* leaf possesses the significant anti-diabetic and rejuvenating capability of kidney function tests.

*Corresponding Author

Name: Gaurav Sharma
Phone: + 91-9460032444
Email: jai_gaurav06@rediffmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2588

Production and Hosted by

IJRPS | www.ijrps.com

© 2020 | All rights reserved.

INTRODUCTION

Diabetes is a chronic metabolic disorder. The metabolic disorders lead to alteration in carbohydrate, protein, and fat. There is insufficient secretion of insulin or no release of insulin by the pancreas in the human body shown in diabetes (Care, 2009). It is one of the fastest-growing health problems in the twenty-first century that is spreading throughout the world (WHO, 2011).

Diabetes mellitus is an alarming non-infectious disease and once diagnosed, it becomes perinniel and has become a serious problem globally: the number of diabetic patients around the world are increasing continuously and the number of diabetic patients around the world is estimated at 71 million by 2030 (IDF, 2011). Most investigations have shown that the earliest detectable changes in the course of diabetic nephropathy in the human will be seen 10 years after diabetes mellitus initiation. However, morphometric studies showed that the signs can be diagnosed 18 months after diabetes beginning (Melmed *et al.*, 2011).

From ancient times, the medicinal plant has been used for curing diabetes across the world. The medicinal plant has a pivotal role to renovate the function of pancreatic tissues by the enhance insulin activity and attenuated the intestinal absorption of glucose. The survey of WHO has identified more than four hundred plants that can act as an antidote to diabetis. The recent search for diabetic drugs from medicinal plants are attractive

because these plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids and many more phytochemical constituents (Malviya *et al.*, 2010).

Due to their phytochemical constituent or antioxidant study of plant for hypoglycemic, antioxidant and hypolipidemic activities may give new pharmacological approaches in the treatment of diabetes mellitus (Dangi and Mishra, 2010). *Aegle marmelos* , commonly known as bael belonging to the Rutaceae family and used for the treatment of various diseases. Various parts of this plant such as leaves, fruit, and seed have hypoglycemic, hypolipidemic and blood pressure curing property (Lambole *et al.*, 2010).

A recent survey of WHO revealed that approximate 80% population is dependent on herbs. (Gangadhar *et al.*, 2012; Rahman *et al.*, 2011) . There are various biochemicals ingredient present in *A. marmelos* leaves such as alkaloids, glycosides, terpenoids, cardiac, saponins, tannins, flavonoids and steroids (Sivaraj *et al.*, 2011).

MATERIALS AND METHODS

Authentication and Collection

The leaf of *Aegle marmalose* was collected from a local nursery and identified by the Botanical Survey of India, Allahabad(BSI/CRC/PS-01/2015-2016).

Preparation of plant extract

The *Aegle marmalose* leaves were first washed well and dried at room temperature. The dried leaves were powdered through the grinder and stored at 5°C until further use. The residue was extracted with 70% Ethanol by soxhlet. The ethanol was evaporated in a rotary evaporator at 40-50°C under reduced pressure and using this extract preliminary phytochemical screening for various plant constituents (Harborne, 1998). After estimation of the LD_{50} value, the dose was 200 and 400 mg/kg body weight (OECD, 2001).

Induction of experimental diabetes

The Wistar rats were injected with alloxan monohydrate (2, 4, 5, 6- tetraoxyprimidine) which was dissolved in normal saline at a dose of 150 mg/kg body weight intraperitoneally. Since alloxan is capable of destroying pancreatic β- cells and induces diabetes mellitus. After three days diabetes was confirmed by the fasting blood glucose level more than 250 mg/dl with the help of a glucometer was considered as a diabetic model and used in this study.

Selection of the dose of the test drug

After established acute toxicity study, the two different doses (200mg/kg, and 400mg/kg) of the *Aegle marmalose* leaf extract were selected (van Herck *et al.*, 1998)

Animals

Adult Wister Albino rats weighing around 120-150 g were used in this experiment. The animals were kept in polypropylene cages in an air-conditioned room. The animal experiments were conducted as per protocol approved by the institutional animal ethics committee (UIP/IAEC/APRIL-2015/08).

Experimental Design

For the study the rat were divided into the following groups,

1. Normal control fed with a normal diet.
2. Alloxan treated [150mg/kg i. p]
3. Alloxan treated + ethanolic extracts of Aegle marmelos (200mg/kg b. w.)
4. Alloxan treated + ethanolic extracts of Aegle marmelos (400 mg/kg b. w.)
5. Alloxan treated + standard drug Metformin standered drug (200mg/kg b. w.).

Biochemical determination

A blood sample was collected by orbital sinus puncture at the End of the experiment and allowed to clot for 1hr then centrifuged at 3000 rpm for 15 min at room temperature for serum (Trinder, 1969) Collected serum was kept at -20° C until analysis biochemical analysis (Kleiner *et al.*, 2005).

Histopathological studies

The liver tissue was dissected out and cut into small pieces then kept into a 10 % formaldehyde solution. The small portion of the liver was dehydrated in alcohol then embedding in paraffin wax. With the help of a microtome, 4-5μm thin section was collected and stained with hematoxylin-eosin (Sen *et al.*, 2010).

RESULTS AND DISCUSSION

Determination of glucose level

The serum level of glucose was significantly high after alloxan induction; the increased levels were down after administration of ethanolic leaf extract of *aegle marmalose* at the dose of 200 and 400 mg/kg bw. However, standard drug metformin at 200 mg.kg b.w was reduced blood glucose level as a comparison to alloxan-induced diabetic rats.

Estimation of Kidney Function Test of *Aegle Marmalose*

Kidney function parameters (Albumin, urea, uric acid, creatinine, and protein) were estimated on 28^{th} day of study after treatment with extracts. Results are tabulated in Table 1 and graphically represented in Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6.

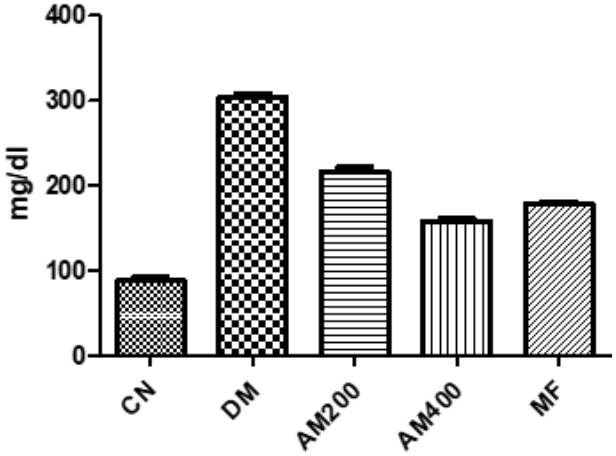

Figure 1: Effect of *Aegle marmalose* on Blood glucose level in alloxan-induced diabetic model. Values are statistically significant at p<0.05

The albumin level had significantly reduced (Table 1) after alloxan induction (1.200±0.1018g/dl) when compared to control (3.200±0.7305g/dl) , the reduced level was down after oral administration of the ethanolic leaf extract of AM at the dose of 200 & 400 mg /kg BW 2.610±0.1018; 2.010±0.1018/dl for four week. However, standard drug Metformin at the dose of 200 mg/kg BW was found to enhance (2.560±0.1018g/dl).

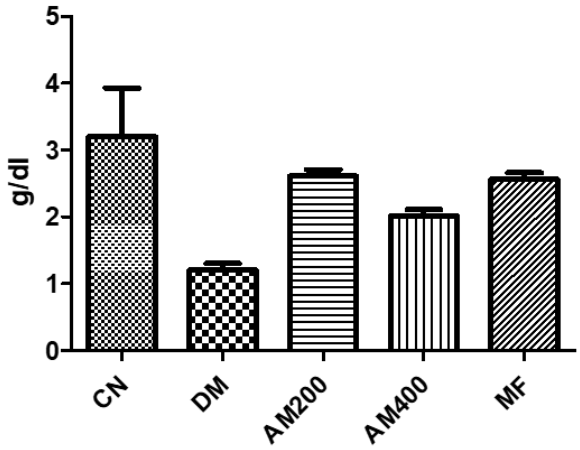

Figure 2: Effect of *Aegle marmalose* on Albumin level in alloxan induced diabetic model. Values are statistically significant at p<0.05

The urea level was significantly high (Table 1) after alloxan induction (62.62±1.806 mg/dl) when compared to control group (33.53±1.289 mg/dl) the increased level was down 5532±1.691 mg/dl; 43.29±1.194 mg/dl after administration of A.M. for four week, however treatment of standard drug Metformin at the dose of 200 mg/kg BW low (42.22±1.691 mg/dl) compared with alloxan induced diabetic rat. The obtained results showed that alcoholic extract at the dose of 400 mg/ kg BW was more significant than 200 mg/kg BW

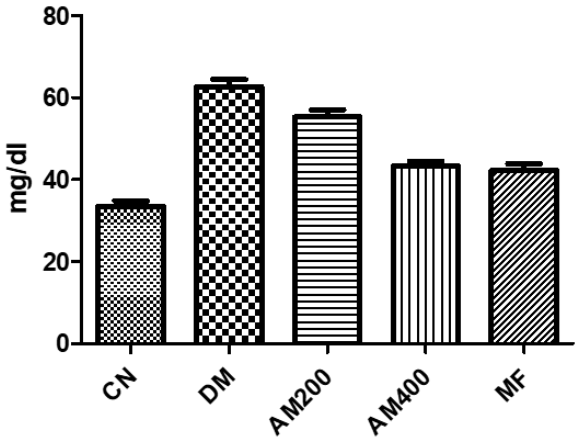

Figure 3: Effect of *Aegle marmalose* on urea level in alloxan induced diabetic model. Values are statistically significant at p<0.05

The level of uric acid was significantly high (Table 1) after alloxan induction (15.25±1.289 mg/dl) when compared to control group (6.500±1.289 mg/dl) that increased level was down 12.21±1.194 mg/dl; 11.25±1.194 mg/dl after administration of A.M. for four weeks. However treatment of standard drug Metformin at the dose of 200 mg/kg BW low (14.65±1.691 mg/dl) compared with alloxan-induced diabetic rat. The obtained results showed that alcoholic extract at the dose of 400 mg/ kg BW was more significant than 200 mg/kg BW.

The creatinine level was significantly high (Table 1) after alloxan induction (1.100±0.1018 mg/dl) when compared to control group (0.67200±0.1167 mg/dl) that increased level was down 0.9800±0.1018 mg/dl; 0.9500± 0.1326 mg/dl after administration of A.M. for four weeks, however treatment of standard drug Metformin at the dose of 200 mg/kg BW low (0.8200± 0.1018mg/dl) compared with alloxan induced diabetic rat. The obtained results were showed (Figure 4) that alcoholic extract at the dose of 400 mg/ kg BW was more significant than 200 mg/kg BW

The protein level had significantly reduced (Table 1)

© International Journal of Research in Pharmaceutical Sciences

Table 1: Effect of Aegle Marmalose leaf extracts on the serum albumin, creatinine, protein, urea and uric acid level of normal, diabetic induced and drug treated rats.

Groups	Albumin g/dl	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Protein g/dl
Control(CN)	3.200±0.7305	33.53±1.289	6.500±1.289	0.67200±0.1167	7.475 ±1.335
Diabetic control(DM)	1.200±0.1018	62.62±1.806	15.25±1.289	1.100±0.1018	4.287 ±1.322
Aegle marmalose (AM) (200mg/kg BW)	2.610±0.1018	55.32±1.691	12.21±1.194	0.9800±0.1018	5.753 ±1.003
Aegle marmalose (AM) (400mg/kg BW)	2.010±0.1018	43.29±1.194	11.25±1.194	0.9500± 0.1326	7.278 ±1.716
Standard drug (Metformin)	2.560±0.1018	42.22±1.691	14.65±1.691	0.8200± 0.1018	7.27 ± 1.726

The results were expressed as mean ± SD; n=6 animals in each group; * P<0.05

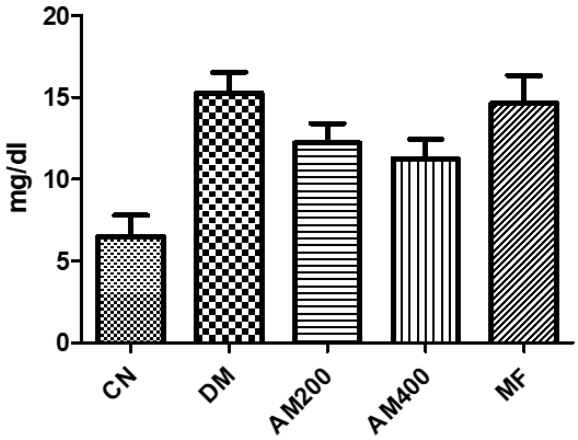

Figure 4: Effect of *Aegle marmalose* on uric acid level in alloxan induced diabetic model. Values are statistically significant at p<0.05

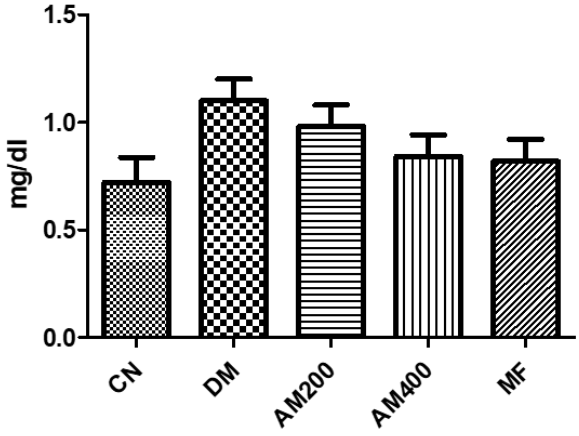

Figure 5: Effect of *Aegle marmalose* on creatinine level in alloxan induced diabetic model. Values are statistically significant at p<0.05

after alloxan induction (4.287 ±1.322 g/dl) when compared to control (7.475 ±1.335 g/dl), that reduced level was increased after oral administration of the ethanolic leaf extract of A.M. at the dose of 200 & 400 mg /kg BW 5.753 ±1.003; 7.278 ±1.716g/dl for four weeks. However, standard drug metformin at the dose of 200 mg/kg BW was found to enhance (7.27 ± 1.726 g/d) the activity.

The interest of humans in the medicinal plant is not only providing a source of vital nutrients largely but also helps to attenuate different types of diseases. The phytochemical constituent is presented in the medicinal plant such as alkaloids, glycosides, essential and fatty oils, resins, gums, mucilage, tannins (Jorns *et al.*, 1997).

The present study was investigated to assess the anti-diabetic and nephrotoxicity activities of medicinal plant i.e. *Aegle marmalose leaf*. In this study, alloxan (150 mg/kg b. w) was used for making the diabetic model. Alloxan acts as a diabetogenic agent due to the destruction of beta-cells of the islet of Langerhans (LeDoux *et al.*, 1986). Increased activity of reactive oxygen species causes the increase in cytosolic calcium concentration which leads to the destruction of beta-cells destruction (Szkudelski, 2001; Dewanjee *et al.*, 2008), due to this effect secretion of insulin alter as well as enhances blood glucose levels (Latha *et al.*, 2004; Naquvi *et al.*, 2011).

During this experiment, the serum glucose level was significantly high when compared with the normal

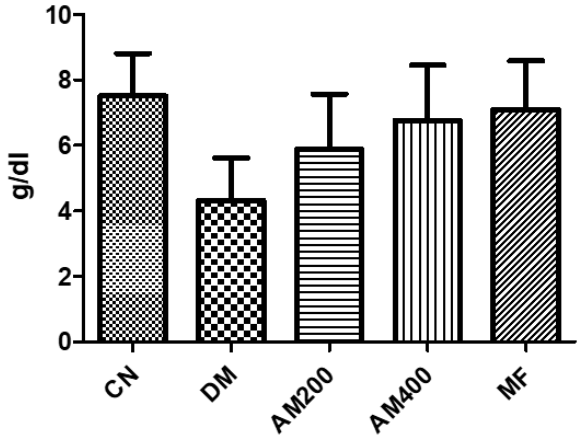

Figure 6: Effect of *Aegle marmalose* on protein level in alloxan-induced diabetic model. Values are statistically significant at p<0.05

group. On the other hand, oral administration with extract at the dose of 200 and 400 mg/kg body weight on the alloxan-induced diabetic groups significantly reduced the elevated blood glucose level.

In this experiment. kidney function test (urea, creatinine, uric acid) values were increased and albumin, protein had decreased in the diabetic groups. The formation of urea from ammonia in the liver and that is an end product of protein catabolism, urine constitute about half of the total urinary solids. Urea is formed in a cyclic pathway that is known as the urea cycle. In this cycle, amino groups donated by ammonia and L- aspartate are converted to urea (Sugumar *et al.*, 2016).

By active and passive carrier modulated process uric acid is mostly reabsorbed in the proximal tubules. Glomerulus is freely filtered urea but about 40% of the urea reabsorbed by tubules, urea reabsorption rate varies inversely with the tubular flow. Uric acid is a hetro-cyclic compound of hydrogen, oxygen, nitrogen, and carbon. By the metabolic breakdown of purine nucleotides, uric acid is formed. The high concentration of uric acid is seen in diabetes. In the blood, creatinine is a chemically waste product that passes through the kidneys to be filtered and eliminated in urine. When the kidneys do not function properly, the Creatinine level in the blood increases. It cannot be reabsorbed or secreated.. The urinary concentration of creatinine is about 70 times more than of plasma.

In this study, the level of urea, uric acid, and creatinine level had increased when comparison with normal groups after induction of alloxan and these results are similar to the previous study. Decreased level of protein and albumin had significantly increased after treatment with the extract. This result was similar to other authors (Dabla, 2010) and that confirms that this extract has a potent role of vital kidney tissues thereby reducing the causation of diabetes in the experimental animals. (Sur, 2016; Lombardo *et al.*, 2016).

CONCLUSION

In conclusion, Leaf extract of *Aegle marmalose* has a potential ability to attenuate blood glucose level as well as kidney function test in alloxan-induced diabetes mellitus through its phytochemical constitutents.

ACKNOWLEDGEMENT

The authors are humbly thankful to Mr. Sunil Sharma (chancellor) and Dr. Sudhansu Sharma (chief mentor) of Suresh Gyan Vihar university , Jaipur India who gave valuable suggestions and support for this work. Authors also thank Dr. Alok Mukerjee who provided animal housing facilities to perform the animal studies

Conflict of Interest

None.

Funding Support

None.

REFERENCES

Care, D. 2009. Diagnosis and Classification of Diabetes Mellitus. *American Diabetes Association*, 32(Supplement_1):62–67.

Dabla, P. K. 2010. Renal function in diabetic nephropathy. *World Journal of Diabetes*, 1(2):48–56.

Dangi, K. S., Mishra, S. N. 2010. Antihyperglycemic, antioxidant and hypolipidemic effect of Capparis aphylla stem extract in streptozotocin induced diabetic rats. *Biology and Medicine*, 2(4):35–44.

Dewanjee, S., Bose, S., Sahu, R., Mandal, S. 2008. Antidiabetic effect of matured fruits of Diospyros peregrina in alloxan-induced diabetic rats. *International Journal of Green Pharmacy*, 2(2):95–99.

Gangadhar, M., Shraddha, K., Ganesh, M. 2012. Antimicrobial screening of garlic (Allium sativum) extracts and their effect on glucoamylase activity in-vitro. *Journal of Applied Pharmaceutical Science*, 2(1):106–108.

Harborne, J. 1998. Phytochemical Methods: A guide to modern techniques of plant analysis. *In Journal of Chemical Information and Modeling.*, page 302.

IDF 2011. IDF Diabetes Atlas - 5th Edition. *International Diabetes Federation*.

Jorns, A., Munday, R., Tiedge, M., Lenzen, S. 1997. Comparative toxicity of alloxan, N-alkylalloxans and ninhydrin to isolated pancreatic islets in vitro. *Journal of Endocrinology*, 155(2):283–293.

Kleiner, D. E., Brunt, E. M., Natta, M. V., Behling, C., Contos, M. J., Cummings, O. W., Ferrell, L. D., Liu, Y.-C., Torbenson, M. S., Unalp-Arida, A., Yeh, M., McCullough, A. J., and, A. J. S. 2005. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*, 41(6):1313–1321.

Lambole, V. B., Murti, K., Kumar, U., Sandipkumar, P., Gajera, V. 2010. Phytopharmacological properties of Aegle marmelos as a potential medicinal tree: An overview. *International Journal of Pharmaceutical Sciences Review and Research*, 5(2):67–72.

Latha, M., Pari, L., Sitasawad, S., Bhonde, R. 2004. Insulin-secretagogue activity and cytoprotective role of the traditional antidiabetic plant Scoparia dulcis (Sweet Broomweed). *Life Sciences*, 75(16):2003–2014.

LeDoux, S. P., Woodley, S. E., Patton, N. J., Wilson, G. L. 1986. Mechanisms of Nitrosourea-Induced -Cell Damage: Alterations in DNA. *Diabetes*, 35(8):866–872.

Lombardo, G., Pighi, J., Corrocher, G., Mascellaro, A., Lehrberg, J., Marincola, M., Nocini, P. F. 2016. Aesthetic Surgical Approach for Bone Dehiscence Treatment by Means of Single Implant and Interdental Tissue Regeneration: A Case Report with Five Years of Follow-Up. *Case Reports in Dentistry*, 2016:1–10.

Malviya, N., Jain, S., Malviya, S. 2010. Antidiabetic potential of medicinal plants. *Acta Poloniae Pharmaceutica*, 67(2):113–118.

Melmed, S., Polonsky, K., Larsen, P. R., Kronenberg, H. 2011. Williams Textbook of Endocrinology, 12th Edition. . pages 937–2061.

Naquvi, K., Ali, M., Ahamad, J. 2011. Antidiabetic activity of aqueous extract of coriandrum sativum L. fruits in streptozotocin induced rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(1):239–241.

OECD 2001. OECD Guideline for Testing of Chemicals (423). *Acute oral toxicity*, pages 1–14.

Rahman, M. S., Salehin, M. F., Ja, M. A. H. M., Parvin, A., Alam, M. K. 2011. Antibacterial Activity of Argemone mexicana L. against Water Borne Microbes. *Research Journal of Medicinal Plant*, 5(5):621–626.

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. S. R., De, B. 2010. Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research*, 3(1):91–100.

Sivaraj, R., Balakrishnan, A., Thenmozhi, M., Venckatesh, R. 2011. Preliminary phytochemical analysis of aegle marmelos, ruta graveolens, opuntia dellini, euphorbia royleana and euphorbia antiquorum. *International Journal of Pharmaceutical Science and Research*, 2:132–136.

Sugumar, M., Doss, D. A., Maddisetty, P. P. 2016. Hepato-renal protective effects of hydroethanolic extract of Senna alata on enzymatic and nonenzymatic antioxidant systems in streptozotocin induced diabetic rats. *Integrative Medicine Research*, 5(4):276–283.

Sur, A. 2016. Evaluation of Serum Creatinine and Cockcroft-Gault Estimated GFR as an Early Biomarker of Renal Impairment in Patients with Type 2 Diabetes Mellitus. *Journal of Clinical & Experimental Nephrology*, 01(04):1–21.

Szkudelski, T. 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, 50(6):537–546.

Trinder, P. 1969. Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. *Annals of Clinical Biochemistry*, 6(1):24–27.

van Herck, H., Baumans, V., Brandt, C. J. W. M., Hesp, A. P. M., Sturkenboom, J. H., van Lith, H. A., van Tintelen, G., Beynen, A. C. 1998. Orbital sinus blood sampling in rats as performed by different animal technicians: the influence of technique and expertise. *Laboratory Animals*, 32(4):377–386.

WHO 2011. World Health Statistics. 1-170. *World Health Organization.*, pages 1–170.