World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Effect of combined extract of leaves of *Medicago sativum* and seeds of *Garcinia kola* on biochemical indices of alloxan-induced diabetic rats.

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World Journal of Advanced Research and Reviews, 2025, 27(02), 1964-1971

Publication history: Received on 14 July 2024; revised on 28 May 2025; accepted on 31 May 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.2.2117

Abstract

Diabetes is a worldwide condition with complications, and herbal herbs such as *Garcinia kola* and *Medicago sativum* might provide alternative therapies. The aim of this study was to investigate the potential antidiabetic effect of the combined extract of *Garcinia kola* seeds and *Medicago sativum* leaves on biochemical indices of diabetic rats. The combined extract was prepared by maceration. A single dose of alloxan 150 mg/kg body weight, BW) intraperitoneally injected into the experimental groups caused them to become diabetic. Rats with fasting blood glucose levels ≥250 mg/dl were considered diabetic and were divided into six groups. Group 1 serves as a normal control (no induction of diabetes). Three groups (4, 5, and 6) of diabetic animals were orally administered daily with 500 mg/kg G. kola extract, 750 mg/kg M. sativum + 250 mg/kg G. kola, and 750 mg/kg G. kola + 250 mg/kg, respectively. One group of alloxan rats was treated as diabetic control (group 2), and another group was orally administered 0.5 mg/kg BW glipalamide daily (group 3). Blood glucose level, liver function indices, kidney function indices, lipid profile and antioxidant enzyme level of diabetic rats treated with *Garcinia kola* seeds and *Medicago sativum* leaves combined extract. The result reveals that blood glucose levels (BGL) at 0, 7, 14, and 21 days for +ve control, std control, and all extract-treated groups were significantly higher and different ($p<0.05$) compared to the normal control group. Nephropathy result shows urea, creatinine urea, uric acid and globulin level of the rats were also maintained by the extract to normal level when compared to the healthy rats. Observation from the study shows a significant increase ($p<0.05$) in the alanine amino transferase (ALT) and aspartate amino transferase (AST) level of untreated diabetic rats which confirms the fact that hepatic injury is associated also with diabetes. Extract treated group ALT and AST levels was significantly reduced in comparison to the normal control group. However, there was no significant difference in ALP levels among the rats in any groups ($p>0.05$). The findings from this study revealed that the combination of methanol extract of *Garcinia kola* seeds and *Medicago sativum* leaves elicits better ameliorative effects from the disruptions caused by the induction of alloxan.

Keywords: Diabetes; *Garcinia kola*; *Medicago sativum;* Rats

1. Introduction

Diabetes is linked to an increase in reactive oxygen species generation and a decrease in antioxidant defenses (Cheng et al, 2013). Pathogenetically significant in the development of diabetes problems is the diabetic-induced oxidative stress (Naito, 2004). It is a chronic illness with pandemic traits due to its widespread global distribution (WHO/IDF, 1999). Globally, the prevalence for all age groups was expected to reach 2.8% in 2000 and 4.4% in 2030. According to estimates, 285 million adults worldwide, or 6.6% of the adult population, had diabetes as of 2010. It is expected that by 2030, that figure will have increased to 438 million (IDF, 2009).

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Alfalfa, sometimes known as Medicago, is a perennial herb with flowers that belongs to the Fabaceae family of peas. One of the most well-known medical plants, alfalfa may reach a height of three feet and has vibrant green leaves and bluish-violet flowers (Bagavathiannan and Van Acker, 2009). Both people and animals can benefit from eating alfalfa. Alfalfa has health benefits for both people and animals. People can benefit from alfalfa sprouts, tender stems, and dried alfalfa leaves (available as a nutritional supplement in forms like tablets, powders, and tea). M. sativa has a long history of usage as an ayurveda and homeopathic treatment for problems of the central nervous system. According to reports, the plant contains antidiabetic, anti-inflammatory, and antioxidant properties (Kundan and Anupam, 2011). One such plant that has received extensive use for its therapeutic and dietary properties is *Garcinia kola*. Numerous ethnobotanical and pharmacological studies have highlighted all of this plant's parts, including the nut, leaf, stem, bark, and root, however, the nut is still the most popular one. *Garcinia kola* is the name of a species of flowering plant that is a member of the Clusiaceae or Guttiferae family of tropical plants (Tcheghebe et al., 2016).

2. Materials and Methods

2.1. Collection of plant material

Fresh leaves of *Medicago sativum* and *Garcinia kola* seeds were obtained from a compound in Obazu Mbieri Autonomous Community in Mbaitolu L.G.A. of Imo State of Nigeria. The plant samples were identified by a Botanist, Dr. Duru, C.N. of Environmental Biology, Federal University of Technology Owerri. Fresh leaves of *Medicago sativum* and Seeds of *Garcinia kola* were washed, air-dried for two (2) weeks, ground into powder using a pulverizer, and then stored in an air-tight container.

2.2. Animals

Albino rats (8-12 weeks old) with an average weight of 120.11 g were used for this study. These animals were purchased from a local breeder in Ihiagwa, Owerri-West L.G.A of Imo State. The animals were kept in well-aerated stainless steel wire cages in the animal house of the Department of Biochemistry. The rats were given standard feed for at least two weeks after purchase to acclimatize them to the laboratory environment before used for the assay.

2.3. Extraction of plant

Fresh leaves of *Medicago sativum* and *Garcinia kola* seeds were homogenized with the pulverizer and were extracted using the Maceration method involving soaking 250g each of plants powder in 5000ml of methanol for 3 days and was later filtered to discard the residue. The filtrate was further concentrated by evaporation to obtain crude extract and Rotary evaporator was used.

2.4. Induction of diabetes

Hyperglycemia was induced in albino rats by the single dose of alloxan (150 mg/kg, intraperitoneally) reconstituted in normal saline after overnight fasting. Rats with fasting blood glucose levels of 250 mg/dl were considered for the hyperglycemic condition (Misra and Aiman, 2012).

2.5. Experimental design

A total of Twenty-four (24) Wistar rats of either sex was divided into five (6) groups of four (4) rats each grouped as follows:

- **Group 1:** (Normal control) was administered distilled water.
- **Group 2**: (Positive control group) served as the positive control group after being induced.
- **Group 3**: (Standard control group) administered with 0.5 mg/kg of glibenclamide (standard drug substance)
- **Group 4**: (Test group) diabetic rats administered with 500 mg/kg *G. kola* +
- 500 mg/kg *M. sativum*
- **Group 5**: (Test group) diabetic rats administered with 750 mg/kg *M. sativum* +
- 250 mg/kg *G. kola*
- **Group 6:** (Test group) diabetic rats administered with 750mg/kg *G. kola* + 250 mg/kg *M. sativum*
- Fasting blood glucose levels was measured before the administration of extracts. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected by snipping of the rat tail. Blood glucose levels were measured by using the glucose oxidase peroxidase reactive strips and a glucometer (One touch glucometer).

2.6. Sacrifice of animals

At the end of 28 days, a transverse incision was made through the ventral wall of the abdomen of each rat under slight chloroform anaesthesia. Blood samples were also obtained from the descending abdominal aorta and was centrifuged at 2000rpm for ten minutes then stored in a plain bottle for biochemical assay estimation.

3. Liver Profile estimation

3.1. Assay of alanine aminotransferase (ALT) activity

Serum ALT activity was estimated by the method of Reitman and Frankel (1957)

Principle: This method is based on the production of pyruvate by the transamination activity of alanine amino transferase. Pyruvate reacts with 2, 4 dinitrophenylhydrazone (DNPH) to give a brown coloured hydrazone that is measured colorimetrically at 546 nm.

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \underset{\text{ALT}}{\longleftrightarrow} \text{L-glutamate} + \text{pyruvate}$$

3.2. Reagent composition

- R_1 is a reagent containing Phosphate buffer (100 mmol/L, pH7.4), L- Alanine (200 mmol/L) a-oxoglutarate (2 mmol/L).
- R_2 is a reagent containing 2, 4dinitrophenyl hydrazine (2 mmol/L).

Procedure: In two separate test tubes, a volume, (0.1 ml) of serum and 0.5ml of water were mixed with 0.5 ml of R_1 as test and blank, respectively. The reaction mixture was solutions were mixed and incubated, respectively for 30 minutes at 37˚C. Next, 0.5ml of R2 was added to both test-tubes (test and blank), incubated for 20 minutes at 25˚C, and followed by adding of 5ml of sodium hydroxide (NaOH) solution. The two solutions were mixed and the absorbance of test sample against reagent blank was read after 5 minutes at 546 nm.

Calculation: The alanine phosphate activity was calculated as follows:

$$\text{Activity of ALT (IU/L)} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 100$$

3.3. Assay of serum aspartate aminotransferase (AST) activity

3.3.1. Assay of aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was evaluated according to the method of Reitman and Frankel (1957).

Principle: Oxaloacetate reacts with AST and is decarboxylated spontaneously to pyruvate. The pyruvate is measured by hydrazone formation after pyruvate reacts with 2, 4 dinitrophenylhydrazine (DNPH) to give a brown coloured hydrazone which can be measured at 546nm.

$$\alpha\text{-oxoglutamate} + \text{L-Aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{oxaloacetate.}$$

3.4. Reagent composition

- R_1 is a reagent containing Phosphate buffer (100 mmol/L, pH7.4), L Aspartate (100 mmol/L), a-oxoglutarate (2 mmol/L)
- R_2 is a reagent containing 2, 4-dinitrophenyl hydrazine (2 mmol/L).

Procedure: A volume,0.1ml of serum and 0.1ml blank in different test-tubes were mixed with 0.5 ml of R_1. The solution was incubated for 30 minutes at 37˚C. Next 0.5 ml of R_2 was added to both test-tubes and allowed to stand for 20 minutes at 25˚C. Then 5 ml of sodium hydroxide (NaOH) was added, the solution was mixed. The absorbance of sample was read against the reagent blank at 546nm after 5 minutes.

Calculation: The aspartate aminotranfarase activity was calculated as follows:

$$\text{Activity of AST (IU/l)} \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 1746$$

3.5. Assay of serum alkaline phosphatase (ALP) activity

The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss (1976).

Principle: The principle is that, in the presence of magnesium and zinc ions, p-nitrophenol phosphate is hydrolyzed by phosphatase to form phosphate and p-nitrophenol. The p-nitrophenol released is proportional to the alkaline phosphatase (ALP) activity and can be measured photometrically at 405 nm.

$$\text{P-nitrophenolphosphate} + H_20 \xrightarrow{\text{Alkaline Phosphatase}} \text{Phosphate} + \text{P nitrophenol}$$

Procedure: To 0.1ml of serum in a test tube labeled 'test', 0.5ml of reagent (p-nitrophenol phosphate) was added, mixed and the initial absorbance read immediately while timer was started simultaneously. It was read again after I -, 2- and 3minutes intervals. p-nitrophenol concentration was estimated by reading off its absorbance at the time intervals spectrophotochemically at 405nm.s

3.5.1. Calculation

The mean absorbance per minute was used in the calculation:

$$\text{Activity of ALP (UI/L)} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 2760$$

3.6. Kidney Function Test

Urea

Urea concentration was determined using the method of Bartels and Bohmer (1972) as described in Randox Kit.

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically at 230nm.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} \quad + \text{Phenol} \xrightarrow{} \text{Indophenol (blue compound)}$$

Reagents (R)

- R1: EDTA (116mmol/L), Sodium Nitroprusside (6 mmol/L) and (1g/L)
- R2: Phenol (120mmol/L)
- R3: Sodium hypochlorite (27mmol/L) and Sodium (0.14N)

Procedure

Ten microliters (10µl) of distilled water (blank), standard calibrator (urea) and sample were added to three test tubes and labeled appropriately. This was followed by the addition of 100 µl of reagent1 to each of the test tubes. They were subsequently mixed and incubated at 37^0C for 10 minutes.

The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the blank was read at 546nm

3.6.1. Calculation

$$\text{Urea Conc.} = \frac{\text{Abs of Sample x Standard conc (mmol/L or mg/dl)}}{\text{Abs of Standard}}$$

1 mg of urea corresponds to 0.467mg of urea nitrogen.

Creatinine

The serum creatinine was determined using the method of Bartels and Bohmer (1972) as outlined in Randox kit.

Principle

Creatinine in an alkaline solution reacts with picric acid to form a coloured complex. The amount of the coloured complex formed is directly proportional to the creatinine concentration.

Reagent

R1a: Picric acid 35 (mmol/L)

R1b: Sodium hydroxide (0.32 mol/L)

3.6.2. Procedure

Two milliliters (2ml) of the working reagent were mixed with 1ml of standard (creatinine) and incubated for 30 seconds. The same was done for the blood sample. The absorbance A1 of the sample and standard were taken at 492 nm. Exactly 2 minutes later, the absorbances A2 of the sample and standard were taken again. The serum creatinine was calculated thus:

$$\text{Serum Creatinine Conc.} = \frac{\Delta\text{Abs of Sample x standard conc.(mg/dl)}}{\Delta\text{Abs Standard}}$$

- A1= absorbance 1
- A2= absorbance 2
- ΔA =A2-A1= change in absorbance (ΔA sample or ΔA standard)
- This could either be in mg/dl or µmol/L

3.6.3. Serum uric acid

Serum uric acid level was determined using uricase method as described by Trivedi *et al.* (1978). Thoroughly 1.00 ml of serum (3) and 3.00 ml of the dilute acetic acid were mixed in stoppered centrifuge tubes and Placed in a boiling water bath for 5 mm, later cooled under tap water, and centrifuge for 5 mm. A water blank and the uric acid working standard were treated similarly. Then 1.00 ml of the supernatant fluid was transferred into 15 mm X 125 mm test tubes. Two (2.00) ml of uricase reagent was added, then the test tubes was stoppered and the time record, then mixed gently by inversion. The reaction proceeded for 30 mm at room temperature. 2.0 ml of 0.10 molar hydrochloric acid, was added followed by 3.00 ml of the chromogen reagent. Then the reaction proceeded for 5 mm. The solution was gently extracted once with 5.00 ml of n-butyl acetate and the absorbance of the butyl acetate against the blank was measured at 492 nm.

Calculations:

$$\text{Uric acid, mg/liter} = \frac{\text{Abs of Sample x Standard conc (mg/L)}}{\text{Abs of Standard}}$$

3.7. Globulin levels

The concentration of rabbit's serum globulin was calculated by using the Bradford method and Simorangkir (Simorangkir *et al.,* 2020). Rabbit serum globulin levels in this study were tested using the Bradford method. In this method, rabbit serum globulin levels are determined based on their absorbance using a UV-vis spectrophotometer at a wavelength of 595 nm. Measurement of rabbit serum globulin was carried out by repeating 3 times for each treatment group.

4. Results

4.1. Blood glucose level of rats treated with combined extract of *G. kola* seeds and *M. sativum* leaves

Table 1 BGL of diabetic rats treated with combined extract of *G. kola* seed and *M. sativum* leaves

Blood glucose level				
Groups	**0 Day**	**7 Days**	**14 Days**	**21 Days**
Normal control	99.15 ± 0.13d	102.20 ± 0.12a	104.25 ± 0.96a	101.50 ± 1.29a
Positive control	102.25 ± 0.13f	471.50 ± 1.29e	580.75 ± 0.96e	401.50 ± 1.29f
Standard control	101.15 ± 0.13e	351.50 ± 1.29d	170.75 ± 0.96b	141.50 ± 1.29b
500GC:500MS	96.25 ± 0.13a	401.50 ± 1.29f	501.00 ± 1.41f	350.75 ± 0.96e
750MS:250GC	97.30 ± 0.18b	323.00 ± 1.83c	271.75 ± 1.70c	220.75 ± 0.96c
750GC:250MS	98. 30 ± 0.18c	250.75 ± 0.96b	300.75 ± 0.96d	281.50 ± 1.29d

Key: GC= *Garcinia kola*; MS= *Medicago sativum*

Table 2 Liver function indices of rats treated with Combined extract of G.kola seeds and M.sativum leaves.

	Liver Function indices (U/I)		
Groups	**ALT**	**AST**	**ALP**
Normal Control	28.75 ± 0.96	47.50 ± 1.29	136.50 ± 1.29
Positive Control	41.50 ± 1.29	56.50 ± 1.29	142.75 ± 1.70
Std Control	30.75 ± 0.96	50.00 ± 0.82	140.75 ± 0.96
500 GC:500 MS	31.50 ± 1.29	52.75 ± 0.96	141.50 ± 1.29
750 MS:250 GC	27.50 ± 1.29	45.50 ± 1.29	130.50 ± 1.29
750 GC:250MS"	26.50 ± 1.29	51.50 ± 1.29	142.50 ± 1.29

Key: GC= *Garcinia kola*; MS= *Medicago sativum*

Kidney function indices of rats treated with Combined extract of *G.kola* seeds and *M.sativum* leaves.

Kidney Profile 40.0

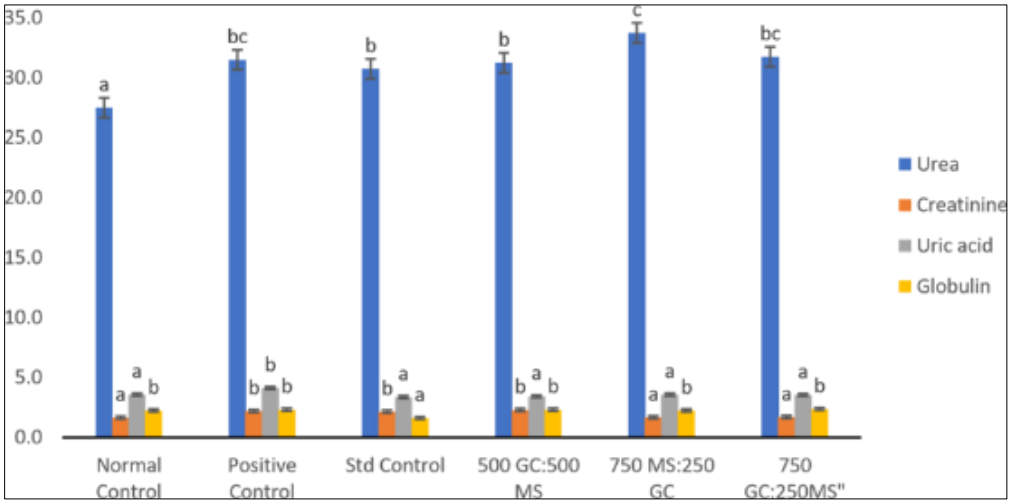

Figure 1 The effect of extract on kidney function indices of diabetic rats

5. Discussion

Diabetes is a chronic disease affecting millions of people worldwide. The WHO expert committee has aptly suggested that research should be aimed at investigating the traditional methods of treatment for refractory diseases like diabetes (Adiga et al., 2010). Alloxan, a beta-cytotoxic substance that causes diabetes in a variety of animal species by damaging the insulin-secreting mwere given alloxan appear to have hyperglycemia, according to the literature. Even though the animals developed permanent diabetes, treatment with smaller dosages of alloxan (100 mg/kg b.wt.) resulted in partial loss of pancreatic -cells (Kalaiyarasi, 2017). As a result, these animals have surviving beta-cells and are capable of regeneration. Sulfonylurea is widely known for its ability to treat moderate alloxan-induced diabetes by directly stimulating the islets of Langerhans' -cells to generate more insulin (Kalaiyarasi, 2017).

When glucose-loaded normal rats were given a mixed methanol extract of M. sativum and G. kola, reversal of hyperglycemia started on the first day, an effect that increased and peaked on the 21st day.it was found in this study that mixed methanol extracts significantly regulated blood glucose levels within acceptable limits in both normal and alloxan-induced diabetic rats. This effect was however less effective than that of standard drug. It is generally known that glibenclamide produce hypoglycemia by causing the pancreatic beta cells to release more insulin (Adiga et al., 2010). The combined extract's similar effect to the standard drug's hypoglycemic effect suggests that they might have a similar mode of action. Naturally occurring compounds, the phytochemicals are thought to be largely responsible for the protective health benefits of plant-based foods and beverages (González-Castejón and Rodriguez-Casado, 2011).

Liver enzymes such as AST (aspartate aminotransferase), ALT (alanine aminotransferase), and ALP (alkaline phosphatase) are valuable markers for assessing liver function and integrity. However, it's important to note that elevated levels of these enzymes can indicate a variety of liver conditions, including but not limited to acute hepatotoxicity, mild hepatocellular injury, viral hepatitis, fatty liver disease, and drug-induced liver injury. While disruption of the liver cell membrane can lead to the release of cytosolic enzymes into the bloodstream, the specific pattern of enzyme elevation along with patient history and additional tests are crucial for accurately diagnosing specific liver conditions. Not all elevated liver enzymes solely indicate membrane disruption, as some enzymes, like ALT, primarily reside in the cytosol and their elevation often signifies direct injury to liver cells. In this study the significant ($p<0.005$) elevations increase in the ALT and AST activities of the untreated diabetic rats strongly suggested that hepatic injury is associated also with diabetes. The mean ALT and AST activities of the extract treated groups were significantly ($p<0.005$) reduced in comparison with the normal control group. However, ALP activities of all the rats were not significantly ($p<0.005$) changed across all groups.

Studies have shown that rats with alloxan-induced diabetic nephropathy have decreased antioxidant defense function and increased oxidative stress (Olatunji et al., 2018). Findings from this study indicated that urea, creatinine urea, uric acid and globulin level of the rats were all maintained within acceptable limits by the combined extracts and significantly ($p<0.005$) tended to restore them to pre-induction levels when compared with those of healthy rats. High ROS production in diabetes leads to oxidative stress.

6. Conclusion

The findings from this study revealed that the combination of methanol extract of *Garcinia kola* seeds and *Medicago sativum* leaves had significant ($p<0.05$) ameliorative effects on the complication caused by the alloxan-induced diabetes in rats studied. The antioxidative ability of this combined extract showed that it could prevent diabetes related complication if used in the treatment and management of diabetes without any adverse drug reactions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

This present research followed and obeyed the guidelines and standard ethics of animal handling throughout the duration of this work.

References

[1] Adiga, S., Bairy, K. L., Meharban, A., & Punita, I. S. R. (2010). Hypoglycemic effect of aqueous extract of Trichosanthes dioica in normal and diabetic rats. International Journal of Diabetes in Developing Countries, 30(1), 38–42.

[2] Bagavathiannan, M.V. and Van Acker, R.C. (2009). The Biology and Ecology of Feral Alfalfa (Medicago sativa L.) and Its Implications for Novel Trait Confinement in North America.Crit. Rev. Plant Sci. 28(1):69-87

[3] Cheng, H. Y., Chuang, D. Y., Shyu, H. F., & Yang, Y. L. (2013). Antioxidant and anti-proliferative activities of extracts from Lycium barbarum and its active component, lycium barbarum polysaccharide. Food and Chemical Toxicology, 55, 436-442.

[4] Battell, M.L., Delgatty, H.L. and McNeill, J.H. (1998). Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. Molecular and Cellular Biochemistry.179:27-34.

[5] González-Castejón, M., & Rodriguez-Casado, A. (2011). Phytochemicals and health benefits of fruit and vegetables. Current Nutrition Reports, 1(1), 13–18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3650511/

[6] IDF (2009). International Diabetes Federation. Diabetes Atlas, 5th edition. https://diab

[7] Kalaiyarasi, C. (2017). Antidiabetic activity of berberis aristata leaves in streptozotocin induced diabetic model.

[8] Kundan, S.B., and Anupam, S. (2011). Evaluation of Antioxidant and Cerebroprotective Effect of Medicago sativa Linn.against Ischemia and Reperfusion Insult. Evid Based Complement Alternat. Med. 1-9.

[9] Misra, M., & Aiman, U. (2012). Alloxan: an unpredictable drug for diabetes induction?. Indian Journal of Pharmacology, 44(4), 538–539. https://doi.org/10.4103/0253-7613.99348

[10] Naito, Y. (2004). Prevention of diabetic nephropathy by treatment with astaxanthin in diabetic mice. Bio Factors.20,49-59

[11] Nostro, A., Germano, M.P., D'Angelo, V., Marino, A. and Cannatelli, M.A. (2000). Extraction methods and bio-authography for evaluation of medicina plant antimicrobial activity. Letters in Appl. Microbiol. 30,379-385

[12] Olatunji, O.J., Chen, H., and Zhou, Y. (2018).Lycium chinense leaves extract ameliorates diabetic nephropathy by suppressing hyperglycemia mediated renal oxidative stress and inflammation. Biomed Pharmacother; 102: 1145- 1151.

[13] REITMAN, S., & FRANKEL, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28(1), 56–63. https://do

[14] Simorangkir, M., Nainggolan, B., Doloksaribu, J. F., & Silaban, S. (2020). Effect of sarang banua (Clerodendrumfragransvent Willd) leaves extract on serum globulin levels of rabbit (oryctolagus cuniculus). Journal of Physics: Conference Series, 1485(1), 012016. https://doi.org/10.1088/17426596/1485/1/012016

[15] Tcheghebe, O.T., Signe, M., Seukep, A.J. and Tatong, F.N. (2016). Review on traditional uses, phytochemical and pharmacological profiles of *Garcinia kola* Heckel. Merit Res. J. Med. & Med. Sci. 4(11): 480-489.

[16] WHO/IDF (1999). Report of consultation: Definition, diagnosis and classification of diabetes mellitus and its complications, Geneva, World Health Organization, 1999.