International Journal of Scientific Research in Science, Engineering and Technology

Print ISSN - 2395-1990
Online ISSN : 2394-4099

OPEN ACCESS

Available Online at : www.ijsrset.com
doi : https://doi.org/10.32628/IJSRSET

Effect Flavonoids Rich Fraction of Mucuna Pruriens Leaf on Blood Glucose, Liver Function Indices and Lipid Profile of Alloxan-Induced Diabetes in RATS

Ahamefula Uchenna[1*], Ezeibe Anderson[2], Ajah Obinna[3], Nnaoma Ikenna[3]

[1]Department of Chemistry, Federal Polytechnic Nekede, Owerri, Nigeria.

[2]Department of Biology/Microbiology, Federal Polytechnic Nekede, Owerri, Nigeria

[3]Department of Pharmaceutical Technology, Federal Polytechnic Nekede, Owerri, Nigeria

Corresponding Email address : innaoma@fpno.edu.ng

ARTICLEINFO

Article History :

Accepted: 10 Sep 2023
Published: 29 Sep 2023

Publication Issue :
Volume 10, Issue 5
September-October-2023
Page Number :
159-165

ABSTRACT

Diabetes mellitus is spreading in an alarming way throughout the world and considered as a major cause of high economic loss which can in turn impede the development of nations. Moreover, uncontrolled diabetes lead to many chronic complications such as blindness, heart disease, renal failure, etc. This present study checked the effect of flavonoid fraction of *Mucuna pruriens* leaves on blood glucose level, liver function indices and lipid profile of alloxan induced diabetes in rats Diabetes was induced by intraperitoneal injection of Alloxan monohydrate (130 mg/kg b w) to rats. The rats were randomly divided into the following groups (n = 6). Group I (normal control) received distilled water, group II received 5 mg/kg b w glibenclamide, Group III (diabetic control) received distilled water, group IV, V and VI received 200, 400 and 600 mg/kg b w of the flavonoids fraction of Mucuna puriens (FFMU), respectively orally once daily for 14 days. The study showed that there was a significant reduction ($p<0.05$) in the blood glucose level. The serum levels of TC, TG, LDL-C, AST, ALT and ALP were significantly reduced in the alloxan-induced diabetic treated groups. There was remarkable increased in HDL-C of alloxan-induced diabetic treated groups. The result shows that the FFMU may be useful in the management of secondary complications associated with diabetes mellitus.

Keywords: Diabetes mellitus, flavonoids fraction, liver functions, lipid profile, *Mucuna pruriens*

I. INTRODUCTION

Diabetes is a growing threat to the public health in modern society (Xiang et al., 2007). It is a metabolic disorder of the pancrease in which blood sugar (glucoese) levels are abnormally high (hypoerglycaemia) because either the body does not produce enough insulin, the hormone produced by beta cells of the islet of langerhans that controls the amount of sugar in the blood (Rother 2007), or the insulin produced cannot be used by the body (Mayfield, 1998). Prevalence of diabetes was predicted to be 2.8 percent in the world in 2000 and 4.4 percent in 2030 for all age groups. As of 2010, 285 million persons globally, or 6.6% of the adult population, were estimated to have diabetes. It is anticipated that the number will rise to 438 million by 2030. (IDF, 2009). Currently, there are 382 million diabetics in the world. Another 316 million individuals with impaired glucose tolerance are at high risk of developing the illness, and by 2035, that number is anticipated to increase to 471 million, which is a concerning amount (IDF, 2013; (Mbanya et al., 2010). This statistic suggests that lifestyle may have a part in the prevalence of diabetes. In reality, it has been demonstrated that changing one's diet and lifestyle can lower the risk of developing diabetes (Hu, 2011). The prevalence of diabetic complications (Song, 2015) is evidence that additional research is necessary to understand the causes of the disease and to develop efficient therapies for the prevention and management of the disease, even though there are medications that target the control of plasma lipid and glucose (Song, 2015).

Herbal medicine has been used for many years. Unani and ayurvedic medications have lasted the test of time due to their toxicity, cultural acceptance, efficacy, and safety. Between 75 and 80 percent of the world's population, who are largely residents of developing countries, continue to get their primary medical care from plants. Additionally, the majority of contemporary drugs come from plants. Numerous currently available drugs were either directly or indirectly developed from phytochemicals.

Mucuna pruriens extracts have shown the presence of substances that exhibit a wide variety of pharmacological effects, including anti-diabetic, anti-inflammatory, neuroprotective and antioxidant, anti-epileptic, anti-neoplastic, anti-oxidants, anti-microbial, aphrodisiac, anti-neoplastic, anti-epileptic, anti-microbial, anti-venom anti-helminthic and analgesic (Olufunke, 2021). It has long been used in traditional Ayurvedic Indian medicine and low-income region of the world as an alternative treatment for Parkinson's disease. More commonly, Mucuna pruriens is used to promote muscle growth, increase strength and has been proven to raise levels of testosterone. It can help reduce menstrual discomfort in women. It can also help decrease psychological stress. Since the plant is a legume, it fixes nitrogen and fertilizes soils (Olufunke, 2021).

Because of the unprecedented availability of chemical variety, natural products from medicinal plants, whether as pure compounds or as standardized extracts, provide limitless prospects for new therapeutic leads. Numerous studies have shown that these natural antioxidants have a wide range of pharmacological effects, including neuroprotective, anti-diabetic, anti-cancer, and anti-inflammatory effects. These effects may be related to the capacity of antioxidant compounds to scavenge free radicals in diabetic patients, thereby preventing disease. Therefore, it is imperative to assess the protective impact of the combined *Mucuna puriens* extract in diabetic rats.

II. Materials and Method

Plant Collection

Fresh leaves of *M. puriens* were collected from Nekede Owerri, Imo State Nigeria. The plants were identified and authenticated by a Plant Taxonomist at the Michael Okpara University of Agriculture Umudike. The leaves were washed with distilled water and air-dried for seven days. The dried leaves were pulverized

into a fine powder using Pulverize machine (5126 TP) and preserved in cellophane bags until when used.

Preparation of Plant extract and Flavonoid Extraction

One thousand five hundred gram (1500g) of powdered leaves was macerated in 2.5L of 80% methanol at room temperature for 72h. It was continuously mixed and then filtered using filter paper (Whatman size No.1). The filtrate was dried in a water bath at 45ºC and the concentrate was kept in airtight bottle at 4°C until used. The extract obtained was subsequently extracted in petroleum ether, diethyl ether, and ethyl acetate following the method of Subramanian and Nagarajan (1969) as used by Ajah *et al*. (2022). Petroleum ether fraction was discarded due to its being rich in fatty substances. Ether fraction was used for free flavonoids whereas ethyl acetate fraction for bound flavonoids. The ethyl acetate fraction of the sample was hydrolyzed further with 7% Sulphuric acid for 24 hours and was then re-extracted with ethyl acetate. The fraction obtained was repeatedly washed with distilled water to neutrality, dried, and weighed. Then use as flavonoid fraction of M. puriens (FFMU).

Experimental Animals

Thirty six Adult Albino rats were obtained from the Animal House of Nnamdi Azikiwe University Awka. The animals were house in cages and Standard laboratory protocols for animal studies were maintained. Care of experimental animals was taken as per the guidelines given by NRC (2011) and approval for animal studies was obtained from the Ethical Committee of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike with the Ethical Clearance Number - MOUAU/VPP/EC/20/021. The animals were acclimatized for two weeks and maintained at the optimum temperature and relative humidity with 12 h light/dark cycle. The animals were allowed feed and water *ad libitum*.

Acute Toxicity Test: Determination of LD_{50}

The acute toxicity test FFMU was done according to the method described by Lorke (1983). A total of 22 mice will be used for acute toxicity test.

Induction of diabetes

Hyperglycemia was induced in albino rats by the single dose of alloxan (130 mg/kg, intraperitoneally) reconstituted in normal saline after overnight fasting. Rats with fasting blood glucose levels of 200 mg/dl and above were considered for the hyperglycemic condition (Ahmad *et al*., 2014)

III. Experimental Design

The rats were divided into six groups of six (6) rats each as follows:

Group I: was administered water and feed only which served as Normal control.

Group II was alloxan induced diabetic rats treated with glibenclamide (5mg/kg b.w) for 14 days

Group III: was diabetes untreated rats

Group IV: was diabetic rats administered flavonoid fraction of *Mucuna puriens* leaf (200mg/kg b.w) for 14 days

Group V: was diabetic rats administered flavonoid fraction of *Mucuna puriens* leaf (400mg/kg b.w) for 14 days

Group VI: was diabetic rats administered flavonoid fraction of *Mucuna puriens* leaf (600mg/kg b.w) for 14 days

Fasting blood glucose levels were checked on 0th, 7th, 14th day of the treatment period from snipping of the rat tail. Blood glucose levels were measured by using the glucose oxidase peroxidase reactive strips and a glucometer (One touch glucometer).

At the end of the study, Rats were sacrificed using cervical dislocation and blood were collected by cardiac puncture. Collected blood was centrifuged at 1300 rpm for 15 min to obtain serum.

Liver Function parameters

Alanine amino transferase (ALT) activity, aspartate aminotransferase (AST) activity and alkaline phosphatase (ALP) activity will be assayed using the method of Reitman and Frankel (1957) and Kochmar and Moss (1976).

Lipid Profile

Total cholesterol will be estimated by modifified Roeschlau *et al* (1974) method. High-density lipoprotein cholesterol, level will be determined based on the method of Trinder (1969).

Triglycerides will be estimated by enzymatic GPO-PAP method, as described by Annoni *et al.* (1982).

LDL and VLDL will be determined by calculation.

IV. Statistical Analysis

Statistical analysis was carried out using SPSS version 23 for Windows (IBM Statistics for Social Sciences). One-way analysis of variance (ANOVA) followed by Duncan's posthoc test for multiple comparisons was performed to determine differences between treatment groups. A p-value less than 0.05 was considered statistically significant. Results were expressed as mean ± standard error of the mean (SEM).

V. RESULTS AND DISCUSSION

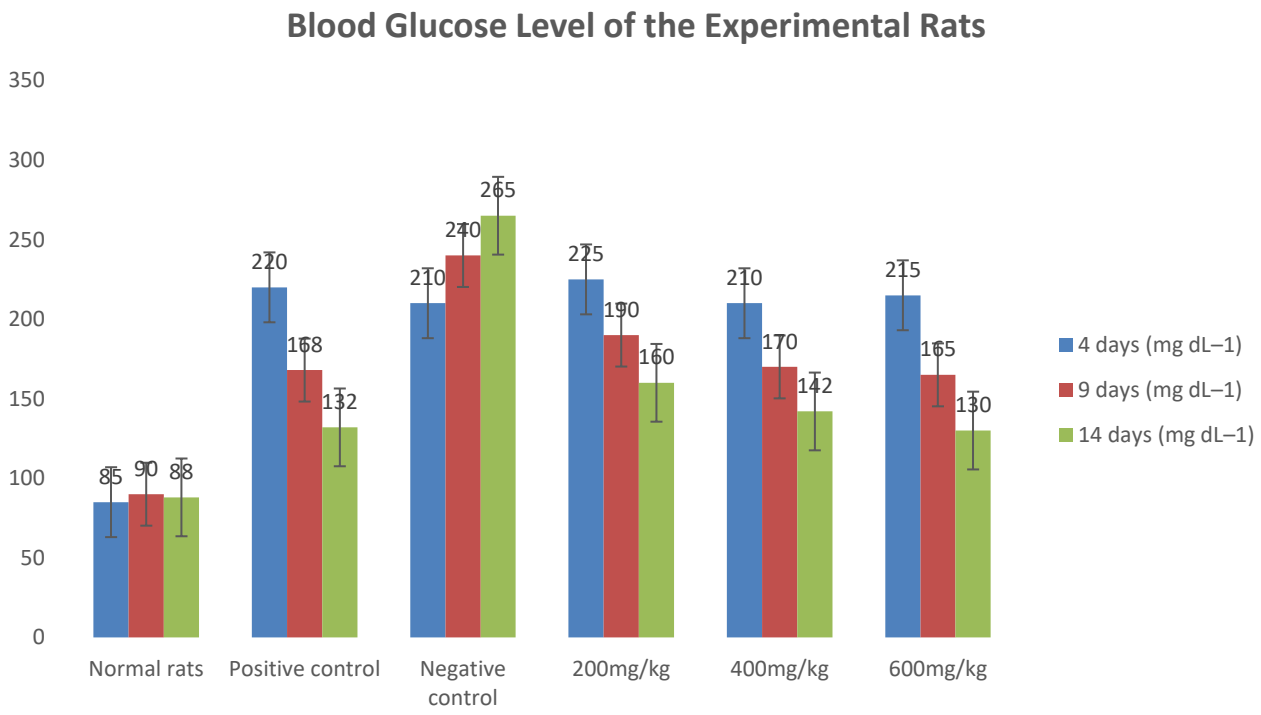

Fig 1 : effect of flavonoid fraction of *M puriens* on the blood sugar level of alloxan induced diabetic rats

The FFMU significantly ($p<0.05$) reduced the elevated blood glucose level when compared to the negative control as shown in Fig 1. .

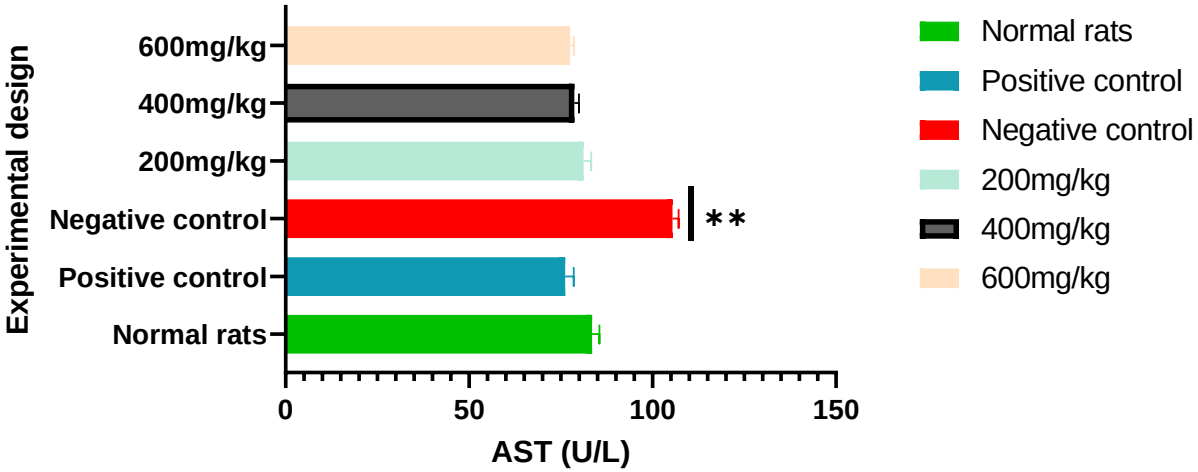

Fig 2: effect of *flavonoid fraction of M puriens* on the AST level of alloxan induced diabetic rats

The result in figure 2 shows significant ($p<0.05$) decrease in the AST of FFMU treated groups when compared with the negative control. There was non-significant ($p<0.05$) increase in the AST of the group treated with FFMU when compared with the positive control. There was significant ($p<0.05$) increase in the AST of the negative control when compared with the normal rats.

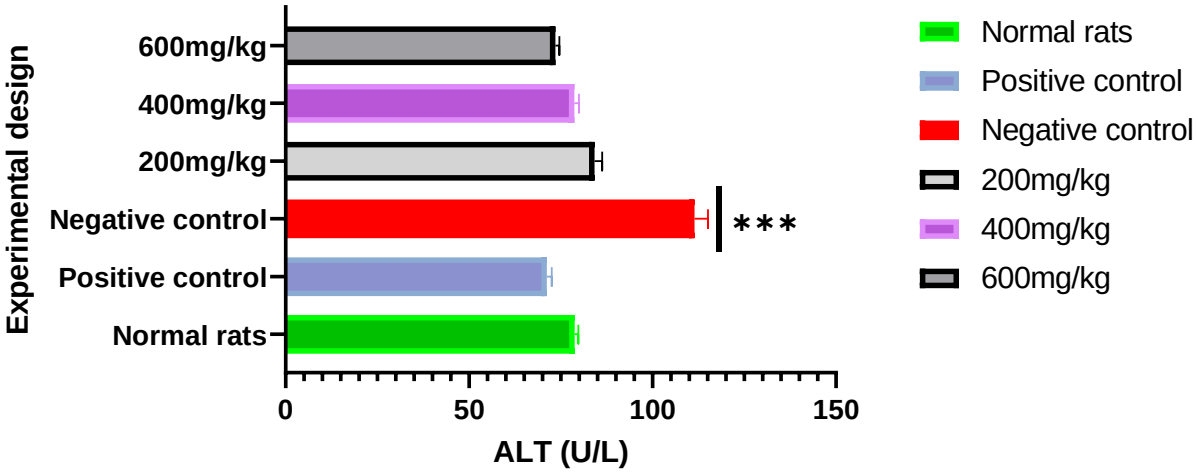

Fig 3: effect of *flavonoid fraction of M puriens* on the ALT level of alloxan induced diabetic rats

The result in figure 3 shows significant ($p<0.05$) decrease in the ALT of FFMU treated groups when compared with the negative control. There was non-significant ($p<0.05$) increase in the ALT of the group treated with FFMU when compared with the positive control. There was significant ($p<0.05$) increase in the ALT of the negative control when compared with the normal rats.

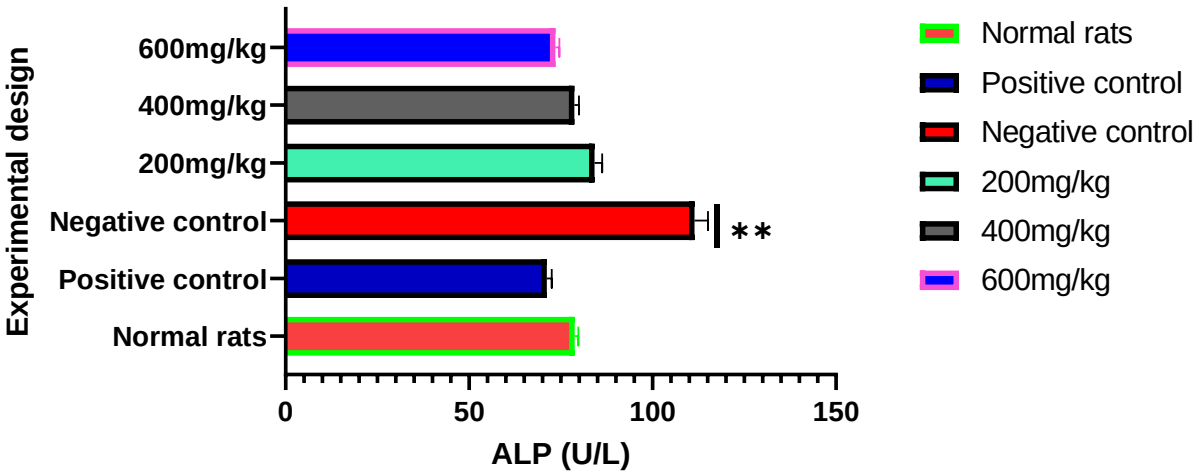

Fig 4: effect of *flavonoid fraction of M puriens* on the ALP level of alloxan induced diabetic rats

The result in figure 4 shows significant ($p<0.05$) decrease in the ALP of FFMU treated groups when compared with the negative control. There was non-significant ($p<0.05$) increase in the ALP of the group treated with FFMU when compared with the positive control. There was significant ($p<0.05$) increase in the ALP of the negative control when compared with the normal rats.

Effect of FFMP on Lipid profile

The result of the effect of the FFMP leave extract on lipid profile parameters of alloxan induced diabetic rats is shown in Table 1. The cholesterol and Triacyglycerol level was significantly ($p<0,05$) reduced in the extract treated rats when compared with the negative control (untreated diabetic rats). There was significant ($p<0.05$) increase in the HDL of the positive control and extract treated rats when compared with negative control.

Table 1: effect of *flavonoid fraction of M puriens* on the lipid profile of alloxan induced diabetic

GROUP	CHOL(mg/dl)	TAG(mg/dl)	HDL(mg/dl)	LDL	VLDL
Normal rats	153.05±2.58^{d}	185.73±5.57^{d}	66.50±2.93^{b}	49.40±1.89^{d}	37.15±1.11^{d}
Positive control	124.53± 2.58^{a}	135.09±5.57^{a}	78.84±2.93^{c}	18.67±1.89^{b}	27.02±1.11^{a}
Negative control	191.18±2.58^{e}	276.21±5.57^{e}	50.98±2.93^{a}	84.96±1.89^{e}	55.24±1.11^{e}
200mg/kg	141.65±2.58^{c}	171.42±5.57^{c}	72.93±5.57^{c}	72.93±2.93^{c}	34.28±1.11^{c}
400mg/kg	131.49±2.58^{b}	155.23±5.57^{b}	81.55±2.93^{c}	18.89±1.89^{b}	31.05±1.11^{b}
600mg/kg	128.10±2.58^{a}	141.03±5.57^{a}	88.34±2.93^{d}	11.56±1.89^{a}	28.20±1.11^{a}

Values are mean±SD of triplicate determination. Alphabet with different superscript differs significantly p (<0.05).

Discussion

Medicinal plants used to treat hypoglycaemic or hyperglycaemic conditions are of considerable interest for ethnobotanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of hypoglycaemic, hepatic modulation and antihyperglycaemic activity. The acute toxicity study of FFMU revealed that the fraction was not toxic because there was no mortality recorded even at the highest dose of 5000mg/kg of FFMU. The FFMU significantly reduced the elevated blood glucose level.

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are examples of liver enzymes that play crucial roles in the body. Liver damage has been determined by a large rise in serum levels of ALT, AST, ALP, and total bilirubin. According to Rajesh and Latha (2004), elevated levels of these enzymes in the blood are a sign of cellular leakage and a loss of the functional integrity of the cell membrane and have been linked to the structural integrity of the liver. The findings revealed that the ALT, AST, and ALP levels in the diabetic rats were noticeably elevated. This shows that diabetes-related liver damage has occurred. However, after 14 weeks of daily dosing, FFMU or glibenclamide caused a considerable decrease in these parameters.

Hyperglycemia and dyslipidemia coexist in diabetes subjects. Dyslipidemia which includes quantitative and qualitative abnormalities of lipoprotein plays a significant role in the proatherogenesis of vascular complications in diabetes mellitus (Rotimi et al., 2011). High cholesterol levels and hyperlipidemia are associated consequences of diabetes mellitus (Iweala and Oludare, 2011). Abnormalities of lipid profile are one of the most common metabolic complications of diabetes mellitus which is found in about 40% of diabetics (Ravi et al., 2005).

The present study showed an increase in the concentration of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and a decrease in HDL-C in diabetic rats. This results support the findings of Mendez and Balderas (2001) who have reported increased plasma cholesterol, triglycerides, LDL C and decreased HDL-C in streptozocin-induced hyperglycemia in rats. The marked hyperlipidemia that describes the diabetic state may therefore be viewed as a magnitude of unlimited actions of lipolytic hormones on the fat depots (Claudia et al., 2006). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Claudia et al., 2006). In this study, administration of all doses of the flavonoids fraction of Mucuna pruriens significantly reduced serum levels of total cholesterol, triglyceride, low-density lipoprotein and increased serum levels of high-density lipoprotein in Alloxan-induced diabetic Wistar rats. However, significantly decrease on serum lipid profile levels observed on treatment with the FFMP may presumably be mediated by a control of lipid metabolism by some of the phytochemicals present in the plant (Rotimi et al., 2011).

VI. Conclusion

The outcomes of this study evidently revealed that flavonoids fraction of Mucuna pruriens (FFMU) significant decrease on the levels of elevated blood glucose, modulated hepatic indices and lipid profile in alloxan-induced diabetes. The FFMU seemed to be effective and were comparable to the standard drug. The result obtained from this work showed that the plant may be useful in the management of secondary complications associated with diabetes mellitus.

VII. Acknowledgment

The authors of this research study wish to acknowledge the Tertiary education trust fund (TETFund) IBR for their financial support.

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Cite this article as :

Ahamefula Uchenna, Ezeibe Anderson, Ajah Obinna, Nnaoma Ikenna, "Effect Flavonoids Rich Fraction of Mucuna Pruriens Leaf on Blood Glucose, Liver Function Indices and Lipid Profile of Alloxan-Induced Diabetes in RATS", International Journal of Scientific Research in Science, Engineering and Technology (IJSRSET), Online ISSN : 2394-4099, Print ISSN : 2395-1990, Volume 10 Issue 5, pp. 159-165, September-October 2023.

Journal URL : https://ijsrset.com/IJSRSET2310522