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Biochemical changes in experimental diabetes before and after treatment with *mangifera indica* and *psidium guava* extracts

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Male adult albino rats were used to study the hypoglycemic effect of *Mangifera indica* and *Psidium guajava* aqueous extracts either used individually or in combination of as well as the effect of glibenclamide as reference sulfonylureas drug at the dose level of 0.5 mg/kg body weight in streptozotocin-diabetic rats. Preliminary test using different doses of each plant indicated that the most effective doses were 250 mg/kg body weight for each plant. Our studies was extended to include the effect of the tested doses on different biochemical parameters including serum insulin concentration, hepatic glycogen content, total proteins, total lipids and transaminases activities in serum and liver. The obtained data of the above mentioned investigations revealed great alleviation of the impaired glucose tolerance, serum insulin and hepatic glycogen content, also serum and hepatic total protein contents were increased as a result of treatment. In STZ-diabetic rats, the activities of (ALT, AST and ALP) either detected in sera or hepatic tissues were increased, then the activities were improved as a result of treatments.

Key words: Hypoglycemic, Streptozotocin, *Mangifera indica* and *Psidium guajava*

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1. INTRODUCTION

Diabetes is a complex and a multivarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein [1,2] characterized by increased fasting and postprandial blood sugar levels. Based on [3] and [4] diabetes mellitus is classified into two major subtypes: type I (insulin dependent diabetes mellitus, IDDM) and type II (non-insulin dependent diabetes mellitus, NIDDM). IDDM or juvenile-onset diabetes results from a cellular mediated autoimmune destruction of the β-cells of the pancreas [5,6] However, NIDDM or adult-onset diabetes results from the development of insulin resistance and the affected individuals usually have insulin deficiency [7]. Patients suffering from type I are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes can be treated with dietary changes, exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population. In 1995 it was estimated that around 135 million people were affected from this condition and it was expected to affect 300 million by the year 2025 [8]. Management of diabetes without any side effect is still a challenge to the medical community. For treatment of diabetes, several drugs such as biguanides, sulfonylurea and thiazolidenediones are presently available to reduce hyperglycemia in diabetes mellitus [9-11]. The use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects [12,13]. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs [14]. The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies [15-17] Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants have become more important. The ethnobotanical information reports state that about 800 plants may possess antidiabetic potential [18]. Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research [13,19].

Guava leaf "*Psidium guajava L.*" belonging to Myrtacea family has a long history of folk medicinal uses in Egypt and worldwide as a cough sedative, in the management of hypertension, obesity and in the control of diabetes mellitus [20-22]. During the screening of several plants, [23,24] reported that the leaves of *P. guajava* inhibit the increase of plasma sugar level in alloxan- induced diabetic rats during glucose tolerance test. Various parts of *P. guajava* have been used for the treatment of diabetes mellitus

[25]. The fruit extract showed hypoglycemic effect in alloxan treated mice and patients with diabetes [26]. *P. guajava* fruit has also been shown as a source of antioxidant due to the presence of polyphenols, ascorbic acids and carotenoids [27,28].

Mangifera indica L. (Family-Anacardiaceae) is also a medicinal plant widely distributed in tropical regions and used to cure a range of diseases [29-31]. The natural C-glucoside xanthone mangiferin has been reported in various parts of *M. indica*: heartwood [32], roots [33], leaves [34], stem bark [35] and fruits [36]. Muruganandan *et al.* [37], investigated the effects of mangiferin on hyperglycemia, atherogenicity and oxidative damage to cardiac and renal tissues in streptozotocin- induced diabetic rats.

The reported pharmacological activities of mangiferin include antioxidant [38,39], radioprotective[40], antitumor [41], anti-inflammatory [42], antidiabetic [31,37], lipolytic [43], which may support the numerous traditional uses of the plant. This antidiabetic activity of mangiferin could involve extrapancreatic actions [44,45] other than pancreatic β-cell insulin release/secretion.

The aim of the present work is to investigate the hypoglycemic effect of water extracts of *M. indica* and *P. guajava* leaves either used individually or in combination on streptozotocin diabetic rats and also to investigate their effects on various biochemical parameters in serum and liver compared to the effect of glibenclamide.

2. MATERIALS AND METHODS

2.1 Experimental animals

For performing the present work white male albino rats "Rattus rattus" weighing about 120 -150 g were used. The animals were obtained from animal house of the National Research Center (Cairo, Egypt). They were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center (Cairo, Egypt), which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Animals described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water.

2.2 Medicinal plants and chemical compounds

A) Streptozotocin

Streptozotocin (STZ) (Batch No.126k1174) was purchased from Sigma-Aldrich, Germany and was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 50 mg/kg B.W. [46] and injected intraperitoneally within 15 minutes of dissolution. Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. Rats with glucose levels between 180-300 mg/dl were considered to be diabetic and chosen for the present experiments, while those with glucose levels outside this range were excluded.

B) Glibenclamide

5) Chloro N (4[N (cyclohexyl carbamoyl) sulfamoyl] phenethyl) -2-methoxy benzamide known as daonil was purchased from Hoechst pharm. Co. (Germany).
The tablets were crushed, suspended in distilled water and given to diabetic rats at the dose level 0.5 mg/kg body weight, daily by gastric intubation, however, the glibenclamide was used as a standard drug. The administered dose was calculated equivalent to the human therapeutic dose according to the FAD Guidance for Industry and Reviewers (2000).

C) Tested plants

Psidium guajava belongs to family Myrtaceae and *Mangifera indica* belonging to family Anacardiaceae. The leaves were obtained from experimental station of faculty of pharmacy, Cairo University. Leaves of the tested plants were air dried and then powdered with an electric grinder. The infusion (water extract) was prepared according to the method described by [47]. Powdered plant material was added to already boiling water and infused for 15 minutes. The infusion was filtered and the filtrate was freshly used.

2.3 Dose selection

Before doing the real experiments and in order to investigate the effective dose response of the tested leaves-infusion, Preliminary experiments were carried out. The selected tested doses, however was mainly dependent on the collected literatures as [48] who demonstrated that the highest decrease in plasma glucose level was obtained with 250 mg/kg (37.73%) of ethanol extract of *M. indica* leaves and [24] who showed that the water extract of *P. guajava*, at an oral dose of 250 mg/kg give significant hypoglycemic activity.

2.4 Experimental design

After the determination of the effective hypoglycemic doses of the tested plants extracts on STZ-diabetic rats. The animals were divided into the following groups:-
Group I (normal control): This group consisted of five normal rats which served as normal control and were given only distilled water daily by gastric intubation throughout the experimental period (four week). At the end of the experiment, the animals were sacrified while fasting.
Group II (diabetic control): The five animals of this group were received STZ as a single injection of 50 mg/kg body weight. Intraperitoneally, they were designated as STZ-

diabetic control and were given distilled water daily by gastric intubation throughout the experimental period.

Group III (diabetic treated): The animals of this group were STZ-diabetic and subdivided into the following subgroups:

Subgroup I: Rats of this subgroup were STZ-diabetic, received *M. indica* leaves water extract daily at the most effective dose of 250 mg/kg body weight given orally by gastric intubation throughout the experimental period.

Subgroup II: Rats of this subgroups were STZ-diabetic, received *P. guajava* leaves water extract daily at the most effective dose of 250 mg/kg B.W. given orally by gastric intubation.

Subgroup III: Rats of this subgroup were STZ-diabetic; received daily a mixture of two plant water extracts (250 mg/kg B.W. *M. indica* and 250 mg/kg body weight *P. guajava* given orally by gastric intubation for four weeks.

Group IV: Rats of this subgroup were STZ-diabetic, treated with 0.5 mg/kg/day B.W. of glibenclamide given orally by gastric intubation dissolved in water throughout the experimental period. Weekly the oral glucose tolerance test (OGTT) was performed. Blood samples were drawn from the retro-orbital venous plexus according to the method of [49]. At the end of the fourth week of treatment, the animals were sacrified while fasting. Blood samples were collected and left to coagulate at the room temperature, then centrifuge at 3000 r.p.m. for 30 minutes. The clear non hemolyzed supernatent serum was quickly removed, and was divided into two portions, one for insulin assay and the other for biochemical analysis. The obtained samples were kept at -20°C till used. The liver was quickly removed and kept in deep freezer at -20°C till used.

2.5 Biochemical examination

2.5.1 Oral glucose tolerance test (OGTT)

This test was performed on normal, diabetic and diabetic-treated rats at the end of 1st, 2nd, 3rd and 4th week of treatment. At fixed time intervals successive blood samples were taken at 0, 30, 60, 90 and 120 minutes, following the administration of glucose solution (3 g/kg body weight of 50%) by gastric intubation. Blood samples were centrifuged and the serum obtained was used for the determination of glucose concentration according to the enzymatic method described by [50] using kits purchased from Biocon Chemical Company (Burbach-Germany).

2.5.2 Insulin estimation

Insulin was assayed in the Medical Service Unit of the National Research Center (Dokky-Giza, Egypt) by ELISA kits according to the method of [51] and based on the sandwich principle.

2.5.3 Hepatic glycogen content

Liver glycogen content was determined according to the method of [52].

Principle: Glycogen was extracted by boiling of a definite weight of fresh liver tissue in a known volume of 30% KOH solution. Then, glycogen was precipitated from the extract by 95% ethyl alcohol. After separation by centrifugation, the glycogen precipitate was treated with 95% sulfuric acid containing 0.2% anthrone to give a green color compared colourimetrically with that produced with a standard glucose at 620 nm. Glycogen concentration was calculated by application of the formula,

Concentration = [(Optical density of unknown X Volume of extract)/(Optical density of standard X gm. of tissue)] X Concentration of standard x100 x0.9

Where, 0.9 = factor for converting glucose value to glycogen value.

2.5.4 Total proteins

Total proteins were determined in serum and liver according to Biuret method [53] using kits purchased From Biodiagnostic, Egypt.

2.5.5 Total lipids

Total lipids in serum and liver were determined according to the method of [54] using kits purchased From Biodiagnostic, Egypt.

2.5.6 Enzyme assay

a) Alkaline phosphatase (ALP): Alkaline phosphatase activity was determined in serum and liver according to [55] using kits purchased from Biodiagnostic, Egypt.

b) Alanine aminotransferase (ALT): Alanine aminotransferase activity was determined in serum and liver according to the method of [56] using reagent kits purchased from Spectrum, Egypt.

c) Aspartate aminotransferase (AST): Aspartate aminotransferase was determined in serum and liver colorimetrically according to the method of [56], using reagent kits purchased from Spectrum, Egypt.

2.6 Statistical analysis

The statistical analysis was applied in the present study by SPSS version 17. The present data were analyzed on the basis of one way analysis of variance (ANOVA) followed by Fisher's LSD Multiple-Comparison test to evaluate the effect in between groups and give a chance of multiple comparisons between groups. Results are expressed as mean ± standard error and values of $p < 0.05$ were considered statistically significant.

Table 1
Concentration of various biochemical parameters in sera and liver of normal and STZ-diabetic male albino rats "*Rattus rattus*"

Parameters under investigation	Organs					
	Serum		% of change	Liver		% of change
	Normal	Diabetic		Normal	Diabetic	
Insulin	68.69±2.33 ng/ml	37.44±1.66**	- 45.49%	_	_	
Glycogen	_	_		7.66 ±1.03 mg/g	1.138 ±0.06**	-85.14%
Total lipids	2.37 ±0.07 g/L	4.43 ±0.22**	86.92%	25.11 ±0.93 mg/g	38.25 ±0.98**	52.33%
Total proteins	89.81±2.20 g/L	69.50±0.29**	-22.61%	264.99±3.60 mg/g	181.05±2.42**	-31.68%
ALT activity	10.55±0.48 U/L	16.18±0.45**	53.36%	86.04 ±5.76 U/100g	187.21±1.95**	117.58%
AST activity	23.04±1.60 U/L	42.48±0.52**	84.38%	98.07 ±1.70 U/100g	224.46±3.77**	128.88%
ALP activity	27.57±0.67 U/L	51.67±1.03**	87.41%	86.59 ±3.51 U/100g	104.48±1.55**	20.66%

Values are mean ±S.E, n=5 in each group, *P<0.05: significant, **P<0.01: highly significant, ˉ as compared with normal non- diabetic group

3. RESULTS

3.1 Effect on oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) of normal non–diabetic and diabetic rats were shown in **Fig.1**, the serum glucose level of normal non–diabetic rats had a fasting serum glucose level 68.56 ± 2.85 mg/dl that was much lower than that of the diabetic non- treated ones, reached its peak value at 60 minutes following glucose intake (3g/kg B.W.) and began to decrease during the next 60 minutes to reach 81.43 ± 3.84 mg/dl after 2 hours of glucose administration.

In the diabetic non-treated male albino rats, serum glucose also attained its maximal level after 60 minutes of glucose administration recording 365.25 ± 7.20, 355.77 ± 4.51, 356.09 ± 3.67 and 369.08 ± 4.05 mg /dl after the first, second, third and fourth weeks of daily treatment respectively. Subsequently, these values begin to decline during the next 60 minutes but in slower rate and still elevated than that of the normal ones.

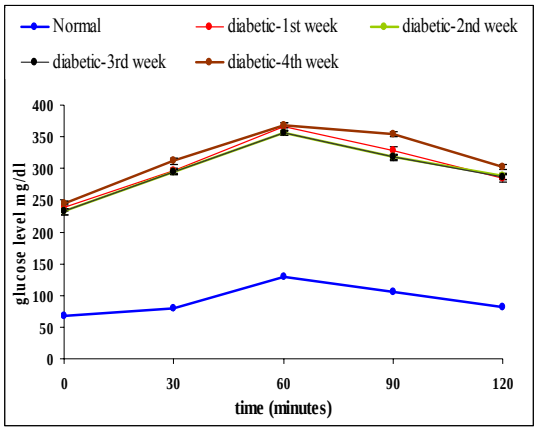


Fig.1. The oral glucose tolerance curve of normal and diabetic male albino rats at different time interval

Fig.2 represented the oral glucose tolerance test (OGTT) of diabetic and diabetic treated rats. There was a noticeable hypoglycemic effect in diabetic treated animals compared with the diabetic non–treated group, after *M. indica* extract, *P. guajava* extract, mixture extract and glibenclamide treatment respectively after one week of treatment.

Continuous treatment with the tested materials for two weeks had a beneficial effect on OGTT values alleviating hyperglycemia. *P. guajava* exhibited, a milde hypoglycemic effect, while glibenclamide treatment showed -40.11% percentage differences as compared with the diabetic non-treated group.

Prolonged treatment of the diabetic rats with each of the tested extracts as well as with glibenclamide for three weeks showed a more beneficial effect on OGTT. All changes were statistically highly significant. Fasting glucose was lower than that of the first and second weeks treated groups. At the end of the experiment, the decrease in glucose concentration was continued to reach -58.40%, - 47.97%, -51.08% and – 42.02% respectively for glibenclamide, *M. indica*, the mixture extract and *P.guajava*. However, all values recorded were highly significant ($p<0.01$).

In addition Fisher's LSD Multiple-Comparison test indicated that after four weeks of treatment, the diabetic group treated with glibenclamide was significantly different with all the treated groups at the fasting level. After 120 minutes of glucose administration, all the treated groups were significantly different when compared with each others after the first week of treatment.

3.2 Concentration of various biochemical parameters in serum and liver of normal and diabetic male albino rats "Rattus rattus"

Data of the different hematological and hepatic biochemical parameters of normal and diabetic male albino rats were shown in Table 1. As compared to the normal

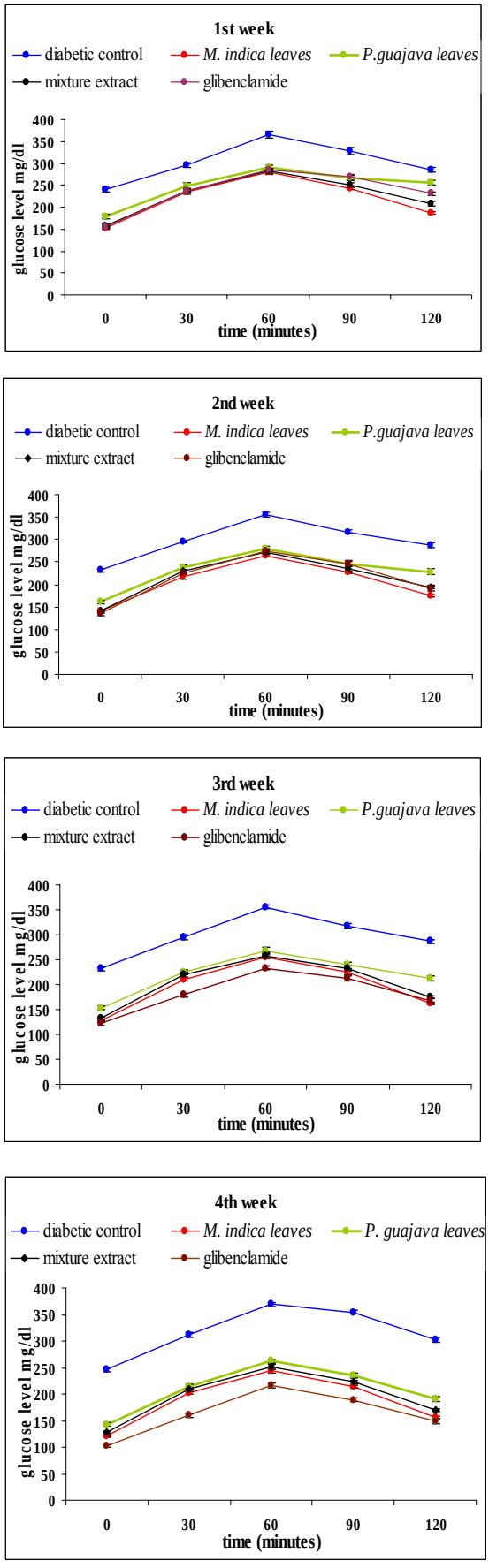


Fig.2 Effect of different plants extracts and glibenclamide on glucose tolerance curve of diabetic and diabetic-treated male albino rats at the 1st, 2nd, 3rd and 4th weeks of treatment

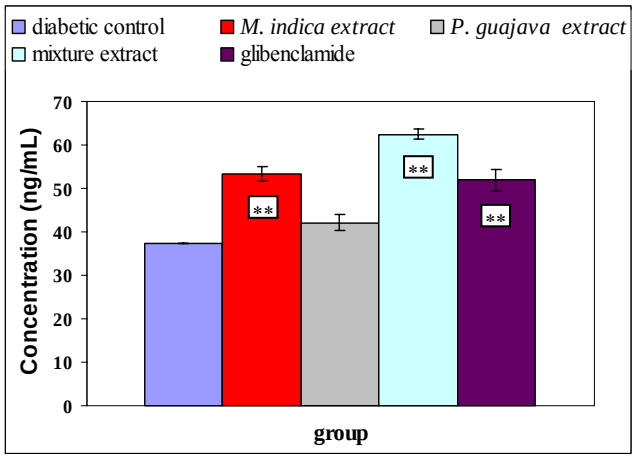


Fig.3. Insulin concentration in serum of diabetic and diabetic-treated rats after four weeks of treatment with different plants extracts and glibenclamide

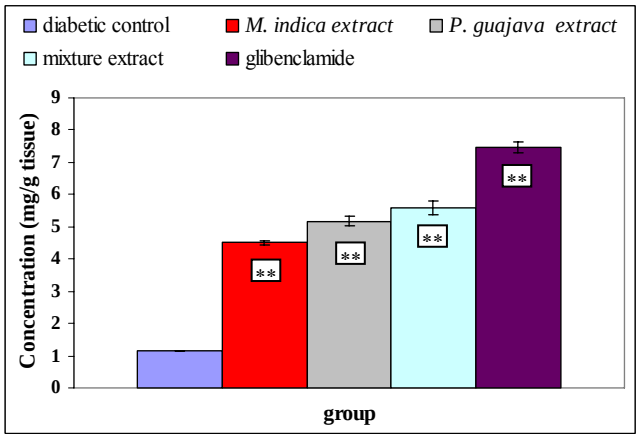


Fig.4. Glycogen concentration in liver of diabetic and diabetic-treated rats after four weeks of treatment with different plants extracts and glibenclamide

values of male albino rats, diabetic condition caused very highly significant decrements in serum insulin, hepatic glycogen contents and total protein concentration in both serum and hepatic tissues. On the other hand, there was a significant increment in the values of total lipid and activities of ALT, AST and ALP. The values detected being increased by 86.92%, 53.36%, 84.38% and 87.41% respectively in serum, also values increased by 52.33%, 117.58%, 128.88 and 20.66% for the previous hepatic parameters.

3.3 Effect of treatment with the tested materials and glibenclamide on various biochemical parameters in sera and liver of STZ-diabetic male albino rats "Rattus rattus"

3.3.1 Serum insulin concentration

Comparing with the control level of diabetic non-treated rats, the serum insulin concentration showed a highly significant ($p<0.01$) increase after the treatment with *M. indica* extract, the mixture of both tested plant and glibenclamide. The maximal elevated level was recorded after treatment with the mixture of both plants; with

percentage difference 66.77% as compared with diabetic-non treated animals (**Fig.3**).

3.3.2 Liver glycogen concentration

Fig.4 showed a highly significant ($p<0.01$) increase in liver glycogen content of diabetic rats after treatment with the tested plants extracts as well as with glibenclamide. The maximal elevation was recorded post treatment with glibenclamide followed by the tested plant mixture, *P. guajava* extract and *M. indica* extract in a decreasing order respectively.

3.3.3 Total protein concentration

Data represented an increase in serum total proteins; with the maximal percentage changes 25.32% for glibenclamide as indicated in **Fig 5**. With regards to the hepatic change, there was a noticeable significant increase in hepatic total protein concentration after four weeks of treatment. Glibenclamide seemed to be the most effective.

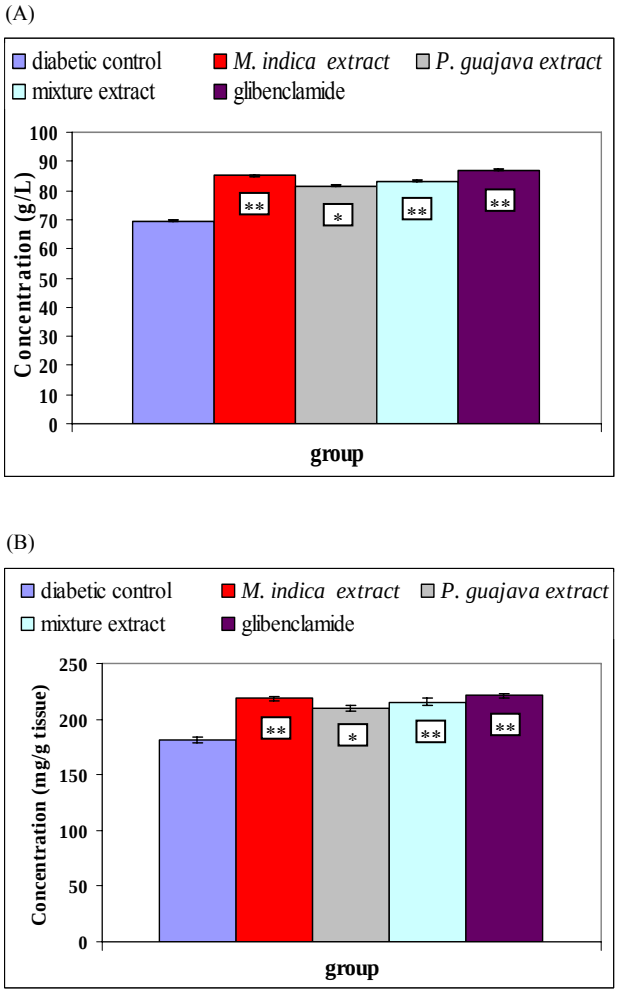


Fig.5. Total protein concentration in (A) serum and (B) liver of diabetic and diabetic-treated rats after four weeks of treatment with different plants extracts and glibenclamide

3.3.4 Total lipid concentration

Concerning the treatment of diabetic animals with all of the tested materials, there was a significant decrease in serum total lipids after the oral administration of *M. indica*, glibenclamide, *P. guajava* extract and the mixture of both plants extracts respectively.

Also, **Fig.6** showed a noticeable decrease in hepatic total lipid contents at the end of the tested period. However, glibenclamide caused a maximal effect followed by *M. indica* extract as compared with the diabetic non-treated groups.

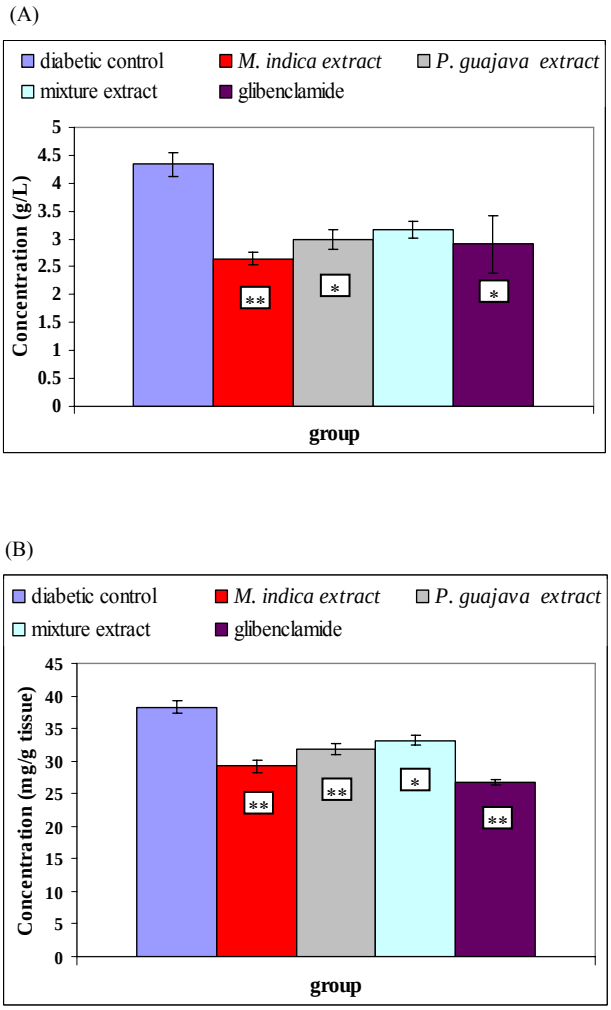


Fig.6. Total lipid concentration in (A) serum and (B) liver of diabetic and diabetic-treated rats after four weeks of treatment with different plants extracts and glibenclamide

3.3.5 Alkaline phosphatase activity (ALP)

The activity of serum and liver ALP of diabetic treated rats after four weeks of continuous administration of the different tested materials was represented in **Fig.7**.There was a highly significant decrease in serum and hepatic tissues after the effect of glibenclamide, *M. indica*, *P. guajava* and the mixture of both tested plants respectively as compared with diabetic control.

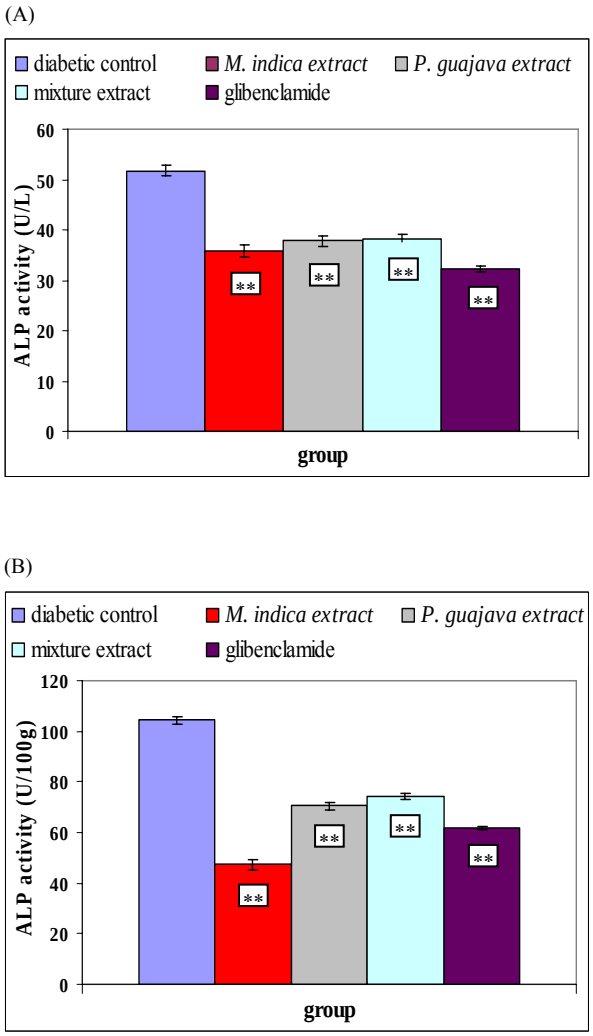


Fig.7. Alkaline phosphatase (ALP) activity in (A) serum and (B) liver of diabetic and diabetic-treated rats after four weeks of treatment with different plants extracts and glibenclamide

3.3.6 Alanine aminotransferase (ALT)

Data recorded in **Fig.8**, showed a highly significant ($p<0.01$) decrease in serum (ALT) activity after the continuous administration of glibenclamide, the mixture of both tested plants, *M. indica and P. guajava* extracts. As regards to hepatic (ALT) activity, also there was a highly significant ($p < 0.01$) decrease after the continuous treatment with the tested materials. The maximal decrease was detected with glibenclamide treatment.

3.3.7 Aspartate aminotransferase (AST)

The data recorded that, there was a highly significant ($p<0.01$) decrease in serum AST activity with the effect of the tested materials; the minimal effect was recorded with *P. guajava* extract with percentage change -32.48% as represented in **Fig.9**.

As well as, the continuous administration of the tested materials for four weeks caused a highly significant ($p<0.01$) decrease in liver AST activity after treatment with glibenclamide, mixture of plants extracts, *M. indica* and *P. guajava* extracts, respectively with percentage changes -41.22%, -32.09%, -30.62% and -28.48%.

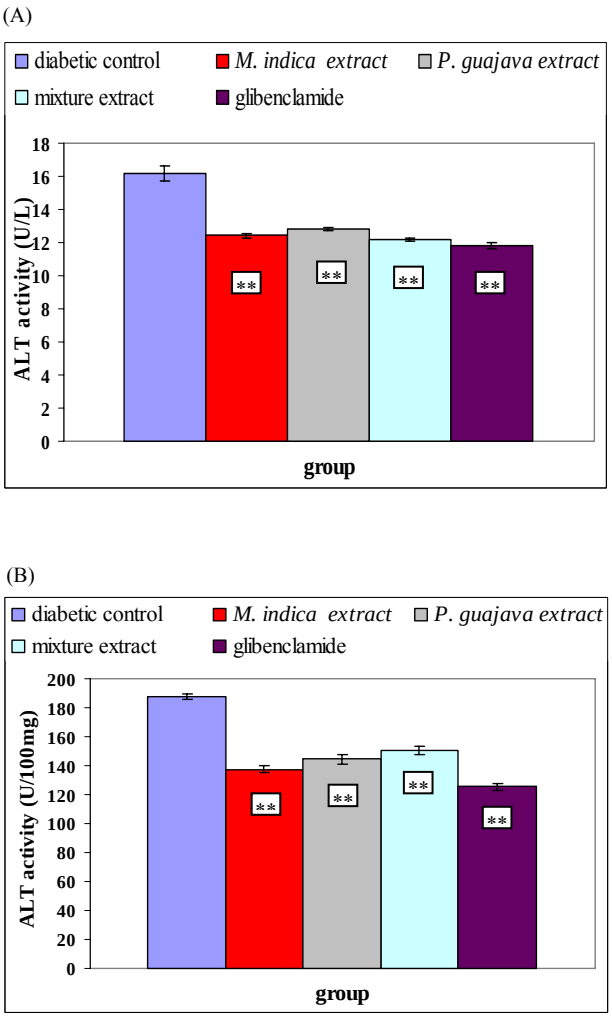


Fig.8. ALT activity in (A) serum and (B) liver of diabetic and diabetic-treated rats after four weeks of treatment with different plants extracts and glibenclamide

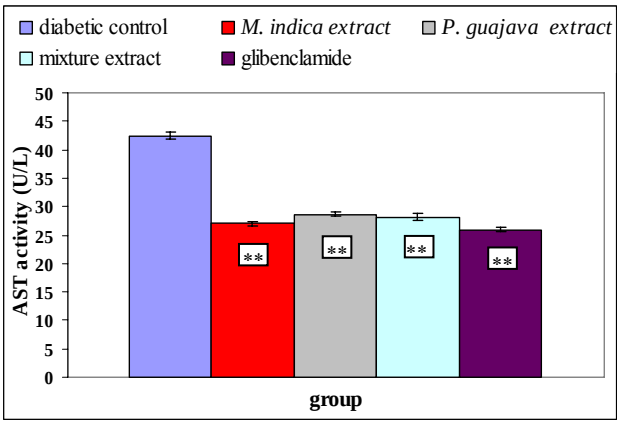
4. DISCUSSION

Diabetes mellitus is a metabolic disease characterized by multivarious groups of disorders that disturbs the metabolism of carbohydrates, fat and protein [1,2]. Its syndrome characterized by the loss of glucose homeostasis and shortage or lack of insulin secretion [57,58].

In spite of introduction of various hypoglycemic agents, diabetes and its complications continue to be a major problem in the world populations [59]. The disease complication is mainly associated with a high risk of atherosclerosis [60]; coronary heart disease [61,62], stroke and peripheral vascular disease [63.64].

Modern medicines like biguanides, sulphonylureas and thiozolidinediones are available for the treatment of diabetes.

(A)



(B)

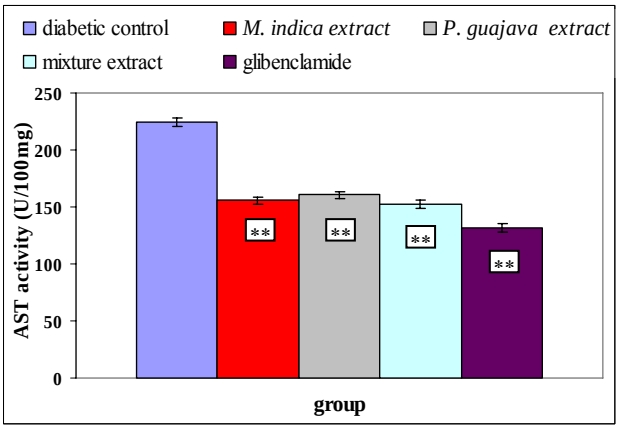


Fig.9. AST activity in (A) serum and (B) liver of diabetic and diabetic-treated rats after four weeks of treatment with different plants extracts and glibenclamide

But they also have undesired effects associated with their uses and fail to give a long term glycemic control [65]. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability [66]. Medicinal Plants are a rich source of natural products and their products have been widely used for treatment of diabetes all around the world with less known scientific basis of their functioning [67,68].

This study highlights the comparing effects of crude aquous extract of *M. indica* and *P.guajava* leaves either used individually or in combination in addition to the effect of glibenclamide as sulfonylurea drug in STZ- diabetic male albino rats "*Rattus rattus*".

The present study revealed a significant decrease of basal serum insulin after four weeks of STZ injection. This decrease was of percentage change - 45.49% as compared with normal non-diabetic rats. Such results agree with that of [69] and may be ascribed to the diabetogenic effect of STZ which lead to destruction of β-cells and decreased number of insulin-containing secretory granules as indicated in the present study. On the other hand the results obtained for serum glucose concentration of STZ-diabetic rats showed high levels and impaired glucose tolerance as compared with the normal non-diabetic rats. These results are in accordance with the finding of several authors using STZ- diabetic animals [70-72]. As recorded by [73,74] glucose intolerance could arise from either a defect in insulin secretion as in case of insulin dependent diabetes (Type I) or a defect in insulin resistance (receptor or post-receptor defect) as in case of non insulin dependent diabetes mellitus (Type II). Our finding on serum insulin concentration confirms the previously mentioned hypothesis.

In the present study, the serial of post-loading blood glucose level for STZ-diabetic rats after the 1st, 2nd, 3rd and 4th weeks of treatment with different types of the tested materials showed marked hypoglycemic effect as compared with the diabetic-non treated ones. The hypoglycemic effect of the tested plants extracts and glibenclamide was pronounced noticeably as the treatment extends. Glibenelamide and *M. indica* extract appear highly affected. These results run parallel with [74 -77] reported a hypoglycemic effect of different plants extracts.

Furthermore, the improvement with glibenclamide administration in diabetes was evident by significant increases in insulin levels by 38.86% and in lowering glucose tolerance curves, and this correlated well with the observations of [72,78] who demonstrated that glibenclamide is able to maintain prolonged increase in serum insulin. It binds to receptors on the surface of pancreatic β-cells; as a result, the cell membrane creates an influx of calcium ions and a subsequent release of insulin [79].

Also, the improvement with *M. indica* extract administration in diabetes was evident by significant increases in and insulin levels and in lowering glucose tolerance of STZ-diabetic albino rats, and this correlates well with the observation of [80,81] who demonstrated that the *M. indica* water extract may interfere with the intestinal glucose absorption in the gut by various mechanisms when given with a simultaneous glucose load in diabetic rats. Muruganandan *et al.* [37] suggest that both pancreatic and extrapancreatic mechanism might be involved in its antidiabetic or antihyperglycemic action. However, the extrapancreatic actions [44,45] could consist of (i) a stimulation of peripheral glucose utilization; (ii) an enhancement of glycolytic and glycogenic processes [82]; and /or (iii) a glycemia reduction through the inhibition of glucose intake.

As regards to *P. guajava* aqueous extract, the tested dose level cause a highly significant decrease in serum glucose concentration, but without a noticeable change in insulin level in comparison to the non-treated diabetic rats. In general, our result are in accordance with the finding of [28,58,83]who reported that the treatment with aqueous extract of leaves of *P. guajava* has proved highly effective in causing significant antihyperglycemic response in rats. Most

of these studies indicated that the leaves of *P. guajava* inhibit the increase of plasma sugar level in STZ-induced diabetic rats during glucose tolerance test and attributed the hypoglycemic effect to the phytochemical constituents which possess antidiabetic activities.

Furthermore, the antidiabetic activity of the *P. guajava* may be possible through various mechanisms such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose uptake by peripheral tissue, or activation of gluconeogenesis in liver and muscles. Such results agree with that of [13,84]. According to the last investigator, the antidiabetic activity might be due to two possible explanations. First, by preventing the death of β-cells and/or second, it may permit recovery of partially destroyed β-cells.

The combination between *M. indica* and *P. guajava* water extract in the present study caused a highly significant decrease in the blood glucose level. This hypoglycemic effect may be due to the presence of glycosides, alkaloids, saponins, tannins, resins and triterpenes as reported by [28,85] for *M. indica*. Such components were showed to be responsible for the hypoglycemic action. Also according to our results, there are highly significant increases in serum insulin concentration of the tested mixture extracts of percentage change 66.77%. Such findings however, support the previously mentioned hypothesis.

Liver glycogen level may be considered as the best marker for assessing anti-hyperglycemic activity of any drug [86]. In the present study, the decrease in hepatic glycogen of diabetic rats are in accordance with the finding of [75,87] and support the suggestion of increased glucose output during insulin deficiency. Several investigators have attributed hepatic glycogen loss to the loss of glycogen synthetase activating system in STZ- diabetic animals [88] and/or increased activity of glycogen phosphorylase and glucose -6-phosphatase in diabetic rats [75,89]. Our results revealed a marked depletion in hepatic glycogen content reached to -85.14% at the end of the tested period. This decrease may be attributed to the enhanced glycogen breakdown, decreased glucokinase and increased glucose-6-phosphatase activity.

The present investigation also shows that daily administration of *M. indica* extract (250 mg/kg b.W.), *P. guajava* extract (250 mg/kg b.W.) either used individually or incombination as well as the tested dose level of glibenclamide induce an increase in liver glycogen concentration. This effect is in accordance with the finding of [87,90, 91] who attributed the increase in liver glycogen of diabetic treated with different plants extracts and glibenclamide to the increased insulin response which in turn promotes conversion of inactive form of glycogen synthetase to the active form and enhances conversion of blood glucose into glycogen.

In the present study, total lipids were increased in serum and liver of STZ-diabetic rats as compared with the normal ones at the end of the experiment. Our results are in accordance with the finding of [91,92] who recorded a marked increase of total lipids in serum and liver of STZ-diabetic rats. Several investigators, however, recognized that insulin deficiency in STZ -diabetic animals brings about an enhanced breakdown of fat [93,94]; increase in mobilization of free fatty acids from the peripheral depots [77,95]and consequence of the uninhibited actions of lipolytic hormones (glucagon and catecholamines) on the fat depots [96].

Treatment of STZ-diabetic rats in the present study with glibenclamide produce a marked decrease in serum and liver total lipids after four weeks of treatment. The present results, however are in accordance with the finding of [96,97] and explained on the basis of sulfonylureas effect to decrease high density lipoproteins (HDL and LDL) [98,99]. Other investigators postulated that the disulphides and its monoxides which yielded from sulfonylurea compounds have the ability to oxidize NADPH, which is necessary for lipids synthesis. Thus leading to decrease in lipids synthesis and lipid level [92,93].

Our results revealed that treatment of STZ-diabetic rats with *M. indica* extract, *P. guajava* extract either used individually or incombination produced great improvement of the altered serum and hepatic lipid variables. The ability of *M. indica* extract to reduce serum total lipids by about 40.18% and liver total lipids by about 23.63% could be explained on the basis of insulin releasing capacity (42.28%). These results run parallel with [71,100] who reported that the rate of lipogenesis is normalized by fenugreek alkaloids due to insulinogenic effect on the lipid metabolism or it could be due to achievement of normoglycemia where there was no further degradation of already accumulated lipid for otherwise glucose starved cells. Furthermore, the improvement with *M. indica* administration is in agreement with[31] who reported marked decrease in lipid variables after treatment with *M. indica* and its polyphenol compound mangiferin which may be ascribed to lipid lowering activity of mangiferin or due to its influence on various lipid regulation systems.

Begum *et al*. [101], Kamath *et al*. [102] and Rai *et al*. [103] reported that the leaves of *P. guajava* are rich in flavonoids, particularly quercetin, tannins, phenols and triterpenes which responsible for antioxidant activity. The ability of quercetin to reduce plasma cholesterol and triglycerides could be explained by the insulin releasing capacity of quercetin [104]. Also the ability of scavenging free radicals and antioxidant properties of the *P. guajava* extract may also participates in the hypolipidemic activity by inactivating hepatic HMG-CoA reductase, a key enzyme, in cholesterol synthesis, also Jung *et al*. [10] stated that, flavonoids decreases liver HMG-CoA reductase activity in type II diabetic mice.

Regarding to the effect of STZ- injection on serum and liver total protein content, there was a significant decrease as compared with the normal non-diabetic one. The decreased rate of total protein may be due to several reasons like

increased rate of amino acids conversion to glucose [105], decreased amino acids uptake [106], and increased conversion rate of glucogenic amino acids to CO_2 and H_2O[89]. Another group of investigators postulated protein decrease to a decrease in the amount and availability of mRNA [75], a loss of transitional factor [107] , reduction of ribosomal protein synthesis as a result of insulin deficiency [108] and decreased defensive mechanism [109].

The present results showed that, the treatments of diabetic rats with glibenclamide, *M. indica, P. guajava* or mixture extract caused marked improvement of serum and hepatic total protein contents. These results run parallel to the study of [110] which reported that serum total protein concentration was increased in STZ-diabetic rats treated with different plants extracts. This improvement could be attributed to increased protein synthesis, increasing incorporation of certain amino acids as a result of increasing insulin secretion, increase of hepatic uptake of glucogenic amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzing amino acids transamination. Also, good correlation between protein synthesis and insulin level has been recorded by [87].

The present data indicates significant increases in the activity of AST and ALT of STZ-diabetic animals which are concomitant with the [89,111]. In the present investigation AST activity appears to be more elevated than ALT. According to [112,113] (AST) had more activity than did (ALT) in the liver of diabetic mice which is concomitant with the present finding. The higher AST levels in diabetic rats in the present study are thought to be consistent with their greater need for gluconeogenic substrate[112]. The elevation of both enzymes may also reflect the damage of the hepatic cells. [92,114] concluded that the elevation in AST and ALT levels may be due to the destructive changes in the hepatic cells as a result of toxemia.. On the other hand, other investigators have postulated that diabetes could induce defects in sarcolemmal enzymatic activities [115] which leads finally to such effects.

The present study revealed that treatment of STZ-diabetic rats with the tested plants extracts or glibenclamide caused a detectable decrease of the transaminases activity. The rate of decrease however, ranging from -28.48% in the hepatic cells after treatment with *P. guajava* extract to - 41.22% after treatment with glibenclamide for AST, and ranging from -19.41% in the hepatic cells after treatment with mixture extract to -32.98% after treatment with glibenclamide for ALT. Our results however run parallel with the finding of [76,116] who reported the inhibitory effect of different plant extracts on the transaminases activity.

Furthermore, the *P. guajava* aqueous extract (250 mg/kg) could produce a marked significant decrease of the elevated AST and ALT activities; and this correlated well with [21,28,103] attributed this decrease to the good hepatoprotective and antioxidant activity which due to the presence of a number of constituents, the major ones are flavonoids, caryophyllene oxide, caryophyllene and a number of tannins. Since antioxidants are known to reduce the development of chemically induced liver damage [117].

Concerning the effect of STZ-diabetes on ALP activity, the data obtained revealed a significant increase after the administration of the tested dose level of STZ. In agreement with this finding, [103] showed increase in ALP activity after induction of diabetes with STZ.

The data recorded in the present investigation showed a marked decrease of serum and liver ALP activity after four weeks of treatment with the tested materials as well as glibenclamide. According to [103], *P. guajava* extract reduced ALP level by 25.18 % indicating its protective effect over liver and improvement in liver function efficiency. Furthermore, the present finding are in agreement with [92,93] who reported that glibenclamide caused a significant decrease in serum and hepatic ALP activity, also [118] showed that glibenclamide cause restoration of the elevated enzyme levels to normal implying the normal function of liver.

5. CONCLUSIONS

In conclusion, our study revealed that *M. indica* water extract and *P. guajava* water extract either used individually or incombination have strong hypoglycemic and anti-diabetic effects as compared with glibenclamide.

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