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EFFECTS OF EXTRACTS, FRACTIONS AND POLYSACCHARIDES FROM *BRUCEA SUMATRANA* ROXB. (SIMAROUBACEAE) LEAVES ON STREPTOZOCIN INDUCED DIABETIC WISTAR RATS

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ABSTRACT

This study described for the first time the hypoglycemic and antidiabetic activities of aqueous extract and its soluble fractions, that of 80% methanol extract and polysaccahrides as well as the antihypergglycemic activity of aqueous extact from *Brucea sumatrana* leaves collected in Mai-Ndombe in Democratic republic of Congo. In hypoglycemic assay, results indicated that the glucose level of untreated normoglycemic rats was 88.0±0. mg/dl after 180 minutes of observation. Next, the treatment of these normoglycemic rats with Glibeclamide at oral dose of 2.5 mg/kg body weight (bw), caused significant decrease blood glucose level (BGL) of treated normoglycemic to value of 77.6±0.3 mg at the same time. In the same manner, the administration of aqueous and 80% methanol extracts at the highest oral dose of 400 mg/kg bw, also caused marked reduction of treated BGL at 80.0±0.3 and 78.5±0.1 mg after 180 min. Soluble fractions chloroform, ethylacetate, *n*-butanol and residual phase as well as polysaccharides acted the same manner by reducing treated normoglycemic BGL to values ranging from to 77.8±0.3 and 79.3±0.3 mg/dl, and 78.2±0.2 to 81.0±0.3 mg/dl after 180 min. In antidiabetic test carried out for 180 min of observation, untreated diabetic rats showed a glycemia value reaching high value of 237.3±0.3 mg/dl after 180 min. After the treatment of these diabetic rats with Glibenclamide at oral dose of 2.5 mg bw, this compound provoked marked reduction of treated diabetic rats to value 96.8±0.2 mg/dl. The same effect was also observed with the administration of aqueous and 80% methanol extracts at the highest oral dose of 400 mg/kg bw which reached the value of treated diabetic rats to values of 127.2.±0.2 and 120.0±0.3 mg/dl compared to untreated diabetic rats with glycemia of 237.3±0.3 mg/dl. Soluble fractions chloroform ethylacateate, *n*-butanol and residual phase displayed also the same effect by causing reduction of treated diabetic glymia to values ranging from 134.8±0.1 and 141.8±0.3 mg/dl after 180 min while polysaccharide brought about this glycemia of treated diabetic rats to values between 130.3±0.1 to 140.3±0.2 at the same time of observation. The obtained results at thisperiod of 180 min, indicated that the glycemia values of treated diabetic rats remained high and need new treatment. With this instituted treatment, it was observed that the administration of Glibenclamide to the same oral dose of 2.5 mg/kg bw caused marked diminishing of treated diabetic glycemia to value of 98.8±0.2 mg/dl on Day-21 and continue to dercease very significatntly to low value of 84.8±0.2 on Day-30. The same results were also obtained with the administration of aqueous and 80% methanol extracts reducing very significantly the treated diabetic glycemia to values of 88.5±0.3 and 86.3±0.3 mg/dl. on Days-21 and -28 respectively. Soluble fractions and polysaccharides produced also the same effect in bringing back the treated diabetic glycemia value to values from 132.2±0.3 and 152.3±0.1, and 91.9±0.4 to 97.5±0.4 mg/ml, and 107.6±0.2 and 132.6±90.6±0.1 to 97.3±0.2 mg/dl on Days-7 and -21 and known continual decrease until to values ranging from 90.9±.4 and 97.5±0.4 mg/dl, and 83.7±0.1 and 87.6±0.3 mg/dl respectively on Day-28. These results clearly showed that Glibenclamide, both aqueous and 80% methanol extracts, soluble fractions and polysaccharides exhibited and possessed interesting hypoglycemic and antidiabetic properties. In addition, Glibenclamide and aqueous extract were found to exhihibit antihyperglycemic activity by reducing the treated hyperglycemic BGL to low level of 83.7±0.1 and 87.6±0.3 mg/dl compared to untreated hyperglycemic normoglycenic rat presenting BGL of 166.0±0.0 mg/dl.

KEYWORDS: *Brucea sumatrana*, ethylacateate, *n*-butanol.

1. INTRODUCTION

Type 2 diabetes (T2D), characterized by hyperglycemia and abnormal carbohydrates metabolism, is a cause leading to morbidity and mortality worldwide and is considered as a major economic burden (Upadyay et al., 2018). According to the International Diabetes Federation (IDF), about 382 million people had diabetes in the year 2013, and the numbers are expected to get double by 2035 (Guariguata et al., 2014). The number of patients is now predicted to grow to 642 million in the year 2040, with the greatest increase expected in low and middle-income countries (Ogurtsova et al., 2017, Alema et al., 2019).

T2D is developed due to insulin resistance and pancreatic β-cells dysfunction, and lead to hyperglycemia state (Lacroix et al., 2014; Sekhon-Loodu and Vasantha Rupasinghe, 2019). The insulin resistance and pancreatic β-cells apoptosis can be traced back other complications such as obesity and also provide the link between T2D and this disorder (Kawser et al., 2016; Sekhon-Loodu and Vasantha Rupasinghe., 2019). The complications of the disease can be enumerated as Heart and blood vessel disease, Nerve damage (neuropathy) in limbs, Other nerve damage, Kidney disease, Eye damage. Skin conditions, Slow healing and Hearing impairment.

Moreover, the postprandial blood glucose levels has been found to play an important role in the onset and developing complications of T2D. One of the therapeutic strategies for managing postprandial hyperglycemia, involves mainly the inhibition of α-amylase and α-glucosidase (Chang et al., 2004; Sekhon-Loodu and Vasantha Rupasinghe, 2019).

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both at the same time (Alberti et al., 1998; Alema NM et al., 2020). Abnormalities in carbohydrates, fats, and proteins metabolism are common feature of DM, which are caused by inefficient action of insulin on target tissues primarily on skeletal muscle, body fats and liver (Deshmukh and Jain, 2015). It is associated with acute and chronic complications, which are accountable for the majority of DM-related morbidity and mortality, financial burden and poor quality of life. Moreover, the persistently elevated sugar level induced diabetic complications results in damage of various organs mainly the eyes, kidneys, nerves, and blood vessels (Soumya and Srilatha, 2011, Alema et al., 2019). The global social, economic and health burden of diabetes are rising at alarming rate with its devastating complications. DM has become one of the leading causes of morbidity and mortality worldwide as all ready mentioned above (Hall et al., 2011; Alema et al., 2019). Diabetes mellitus (DM) is also a metabolic disorder characterized by a persistent rise in blood glucose level (BGL) caused by ineffective insulin function, secretion and release, or both on target tissues. Chronic hyperglycemia is associated with lifelong microvascular (retinopathy, neuropathy, and neuphropathy) and macrovascular (coronary and peripheral arterial diseases and stroke) complexities, which are the typical features in all forms of DM (Yikna et al., 2021).

In spite of the fact that several antidiabetic agents from natural and synthetic sources, have been introduced to the market, diabetes and its micro and macro complications, continue to be a major medical and health problem worldwide (Piero et al., 2015).

The currently available modern drugs used for the treatment of diabetes are often associated with limitations such as inadequate efficacy, high cost, and various side effects (Bastaki, 2005, Alema, 2019). These medicines include Alpha-Glucosidase Inhibitors. Acarbose, Miglitol. Incretin-Based Drugs. Dipeptidyl Peptidase-4 (DPP-4) Inhibitors. Alogliptin, Insulin, Metformin, Metiglinide Analogues, Aldomet, Nateglinide, Pramlintide, Sodium Glucose Cotransporter-2 (SGLT-2) Inhibitors, Canagliflozin, Sulfonylureas, First Generation Sulfonylureas, Glibenclamide, etc. And their side effects per category can be mentioned for Sulfonylureas: low blood sugar, upset stomach, skin rash or itching, weight gain, Biguanides/Metformin: sickness with alcohol, kidney complications, upset stomach, tiredness or dizziness, metal taste, Alpha-glucosidase inhibitors: gas, bloating and diarrhoea, Thiazolidinediones: weight gain, risk of liver disease, anaemia risk, swelling of legs or ankles, Meglitinides: weight gain, low blood sugar for example.

In view of the aforesaid drawbacks of conventional medicines, many medicinal plants with empiracally claimed antidiabetic activity can be used as an alternative approach in the management of diabetes especially in developing countries due to their cost effectiveness, accessibility, far-reaching cultural acceptability, and lower side effects. (Sakthiswary et al., 2014). More than 1200 species of medicinal plants are used throughout the world by different ethnic people in traditional medicine for their supposed and claimed antidiabetic activity (Piero et al., 2015) and consummers found some prompt alleviations. Some of these traditional medicines may be advocated to be formulated as active ingredients that can potentially be used and exerted health benefits like antidiabetic effects, yet considerations of possible variations as a result of difference from geographic and climatic environmental and extraction techniques should be taken into account (Mollica et al., 2017; Stefanucciet al., 2018).

Brucea sumatrana Roxb. is a medicinal plant belonging to the Simaroubaceae family. Its is found in some African countries scientifically called *Brucea sumatrana* Roxb. It is widely used in traditional medicine to treat various ailments such as diarrhea, amoebiasis, diabetes, rheumatism, various bacterial infections, fever and

malaria, divers pains using leaves, stem bark or root bark in decoction and macerate forms. Seeds are mainly eaten for the same medical purposes especially to treat and fever and malaria (Newinger, (2000).

The same plant is also found in same Asiatic countries under the scientific name *Bucea javanica* with synonyms *Brucea amarissima* DesV. Ex Gomes, *Gonus amarissima* Lour, *Lour amarissima* O. Kze. The Asiatic species is more studied. More biological activities of seed extracts and isolated principles maimly quassinoids were previously reported. These biological activities included antiplasmodial (Wright et al., 1988; O'Meill et al, 1995; Pavanand et al., 1986; Kim et al., 2000; Camacho et al., 2003; Nguyen-Poupli et al., 20007; Sriwilaijaroen et al., 2010), cytotoxicity and antileukemic (Lee et al., 1979; 1984; Sakadi et al., 1986), antiamoebic (Wright et al., 1993). anti-inflammatory, antitrypanosomal (Phillipson et al., 2003; Bawm et al., 2008) and antidiabetic and antioxidant (Ablat et al., 2014, 2017) activity. Differents chemical constituents like qaussinoids as major constistuents (Lee et al., 1979; Sakadi et al, 1986, Kim et al, 2004; Pan et al., 2009), aklaloids from suspension cultures of seeds (Anderson et al., 1991; Wagih et al., 2008) and lignans (Luyengi et al., 1986) were reported to be present in the seeds. The plant is considered to be antiperiodic and febrifuge.

The first study on the seeds of *B. sumatrana* growing in a African country particularly in Democratic Republic of Congo (RDR-Congo) was conducted by Tshodi et al. (2012) on its phytochemical composition and antiprotozoal activity of lyophilized aqueous extract and its soluble fractions, 80% methanol and total alkaloids s extracts. Antiparasitic and cytotoxic activity of extracts and fractions from *B. sumstrana* leaves was also reported (Tshodi et al., 2016). Recently, antiprotozoal and cytotoxic activities and the acute toxicity of extracts from *Brucea sumatrana* Roxb.(Simaroubaceae) leaves collected in Mai-Ndombe in Democratic Republic of Congo (Cimanga et al., 2015) and the *in vitro* antioxidant of extracts, fractions and polysaccharides, and subchronic and subacute toxicity of lyophilized aqueous extract from *B. sumatrana* Roxb. were also investigated (Cimanga et al., 2020). In all these studies, reported results indicated that all extracts, fractions and polysaccharide exhibited these evaluated biological activities with different magnitudes.

The present study was initiated to assess in amimal model the hyoglycemic, antidiabetic of extracts and fractions as well as antihyperglycemic of aqueous extract form *Brucea sumastrana* leaves collected in Mai-Nombe in Democratic Republic of Congo.

2. MATERIALS AND METHODS

2.1. Plant material

Leaves of *B. sumatrana* Roxb. (Simaroubaceae) were collected in Mai-Ndombe in Democratic Republic of Congo (DR-Congo). It was identified at the National Institute of Studies and Researches in Agronomy (NISRA), Departement of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of the plant had been deposited in the herbarium of this institute and in Laboratory of Pharmacognosy and Phytochemistry of the same university. Leaves were dried at room temperature and reduced to powder using an electronic blender and were kept in a brown bottle hermetically closed before use.

Figure 1: *Brucea sumatrana* leaves, immature and mature fruits.

2.2. Preparation of extracts and partition

60 g of powdered leaves were soaked with 400 ml distilled water and boiled on a hotplate at 100°C for 15 minutes. Next, the mixture was cooled and filtered on a paper filter Watman N° 1. The filtrate was evaporated in vacuum using rotary evaporator yielding dried extract named as Bsl-1 (53.87 g). An amount of 20 g of Bsl-1 extract were dissolved in 200 ml distilled water and filtered. The resulting filtrate was successively and exhaustively extracted with solvents of different polarities as chloroform, ethylacetate, *n*-butamol. All fractions including residual aqueous phase were

evaporated in vacuum as described above yielding corresponding dried extracts denoted as Bsl-1.1 (4.75 g), Bsl-1.2 (5.05 g), Bsl-1.3 (3.25 g) and Bsl-1.4 (6.15 g) respectively. On the other hand, 30 g of the plant material were macerated with 80% methanol for 24 h. After filtration, a 80% methanol macerate was obtained and the marc was exhaustively percolated with the same solvent. The macerate and percolate were combined and treated as described above yielding dried 80% methanolic extract named as Bsl-2 (27.71 g) (Cimanga et al., 2015, 2019). Bsl; *Brucea sumatrana* leaves.

2.3. Extraction and purification of polysaccharides

2.3.1. Water soluble polysaccharides

The methods proposed by Liu et al., (2014), Wang et al., (2018) and Tang et al., (2019) were used for extraction, separation and purification of polysaccharides. About 20 g of aqueous extract Bsl-1 were dissolved in 100 ml distilled water, filtered, and the filtrate was reduced to 10 ml with rotary rotavapor. After, about a five-fold volume of ethanol 95% was added into the filtrate for polysaccharides precipitation. The mixture was then placed in a refrigerator at 4°C for 24 hours giving a white precipitate after this period. The precipitate was filtrated and dried in hot at 50°C to give dried white extract denoted as CP-Bsl (crude polysaccharides: 16.63 g). This extract gave positive test for polysaccharides (Molish's regent: alpha-naphtol + H_2SO_4, purple color and with phenol/H_2SO_4, violet color) (Wang et al., 2018; Tang et al., 2019). The polysaccharide concentration was determined with the phenol-sulfuric acid method at 481 nm (Jiang et al., 2010, Wang et al., 2018).

2.3.2. Purification of polysaccharides

The crude polysaccharide CP-Bsl (15 g) was purified by gel column chromatography on DEAE (dietylaminoethyl)-cellulose put in deionized water and dumped the clarity supernatant liquid. After, 500 ml of NaOH 0.5 mol/L were added for 30 minutes, bathing the cellulose with water until neutral. It was then soaked in 500 ml of HCl 0.5 mol/L for 30 minutes and treated as described above for bathing and the process was repeated three times. Now, the DEAE-cellulose (Diethylaminoethyl cellulose) was ready for use. The crude polysaccharide CP-Bsl was dissolved in deionized water and centrifuged, and the supernatant was loaded onto a new DEAE-cellulose column (40g, 60 × 2.5 cm, internal diameter), which was eluted with deionized water and NaCl 0.1 M solution in order. The elution (3 ml) was collected and carbohydrate content was determined based on the phenol-sulfuric acid method at 481 nm absorbance (Jiang et al., 2010). The crude polysaccharide CP-Bsl was separated on Sephadex LH-20 (40g, 60 x 2.5 cm) (Liu et al., 2014; Zhang and Row., 2015) into chromatographically pure 4 fractions, which were then dried, and coded as PPF-Bsl-1 (2.75 g), PPF-Bsl-2 (2.50 g), PPF-Bsl-3 (3.38 g) and PPF-Bsl-4 (2.67 g).

2.4. Evaluation of hypoglycemic activity in normoglycemic Wistar rats

This test was conducted on normoglycemic Wistar rats weighing 160 to 180 g body weight (bw). They were grouped in 6 groups of 5 rats for each oral dose and were orally administered respective doses of 2.5 mg/kg for Glibenclamide, and 200 and 400 mg/kg bw for extracts, fractions and polysaccharides:

●- Group I: was administered 5 ml distilled water as negative control,
●- Goup II received Glibenclamide as positive control,
●- Groups IIIa and b received aqueous extract Bsl-1,
●- Groups IVa and b received 80% methanol extract Bsl-2,
●- Groups Va and b to IXa and b were administered chloroform Bsl-1.1, ethylacetate Bsl-1.2, *n*-butanol Bsl-1.3 and residual aqueous Bsl-1.4 soluble fractions respectively,
●- Groups Xa and b to XIIIa and b received crude and pure polysaccharide fractions PPF-Bsl-1 to -4 respectively, After administration of tested samples, each group was placed in individual plastic cage and glucose of all group was collected from tail tip and the glucose level measured after all 30 min until 3h and compared to untreated animals group (Arya et al., 2012).

2.5. Evaluation of antidiabetic activity in diabetic Wistar rats

2.5.1. Induction of diabetes mellitus in Wistar rats

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dose of 50 mg/kg dissolved in 0.1 M of cold citrate buffer; (pH=4.5) to overnight fasted either Wistar rats. The animals were fed with commercial pellet diet (Hindustan Lever, Bangalore, India) and water ad *libitum* after 30 min of administration of STZ. The animals were acclimatized to laboratory hygienic conditions for 3 days before starting the experiment in individual plastic cage under strict observation. In these conditions, STPZ destroyed β-cell pancreatics with consequence the secretion of insulin in low amount and treated animals were considered as in diabetic state (Padee et al. 2010).

Diabetic rats and negative control blood was collected from tail tip and measured at Day-4 as the beginimg of antidiabetic test. Animals with fasting BGL >190 mg/dL (blood glucose level:BGL) were considered as diabetic, selected and included in the study (Ahmad et al., 2014; Radenković et al., 2016).

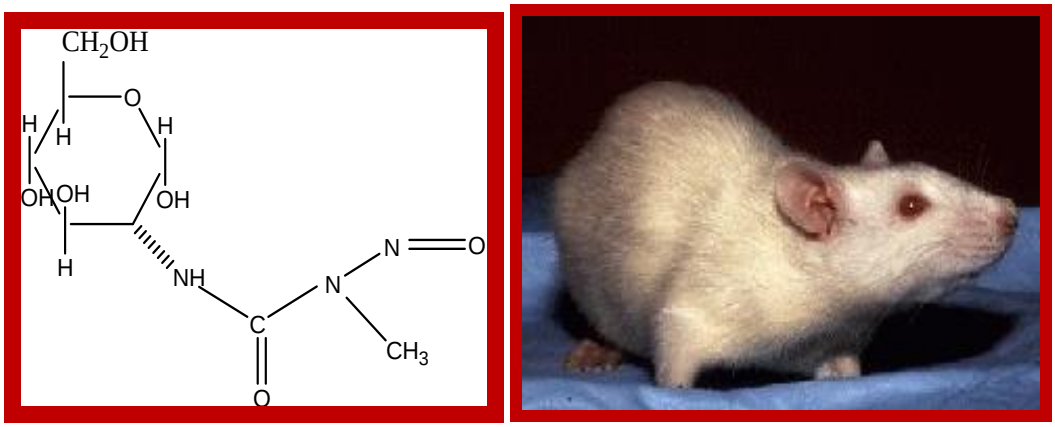

Figure 2: Structure of streptozocin [(2-deoxy-2(methylnitroamino)-carbonyl]-D-glucopyranose or 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-pyranose, STZ: nitrosoured or glycosyled antitumoral antibiotic and Wistar rat.

2.5.2. Protocol of treatment of diabetic Wistar rats

Diabetic rats were divided in following groups with 5 rats for each separately oral dose and orally administered the dose of 200 and 400 mg/kg bw respectively of each tested samples:

●- Group I: received; received 5 ml distilled water as negative control,

●- Group II received orally Glibenclamide (2.5 mg/kg bw) as positive control,

●- Group III received intraperitoneally STPZ 50 mg/kg bw in buffer as negative control,

●- Groups IVa and b were administered aqueous extract Bsl-1,

●- Groups Va and b were administered 80% methanol extract Bsl-2,

●- Groups VIa and b to IXa and b and b were given soluble fractions Bsl-1.1 to Bsl-1.4,

●- Groups Xa and b to XIIIa and b were given crude polysaccharide CP-Bsl and pure polysaccharide fractions PPF-Bsl-1 to -4.

2.6. Measurement of glycemia levels in normoglycemic and treated diabetic Wistar rats

Blood was collected from the tail tip of each treated animal and BGL was determined immediately prior to treatment (at 0 min) as baseline and then after 30, 60, 120 and 180 min of sample administrations. The determination of glycemia levels was carried out using a glucosemeter (Fig. 4) coupled with strip imbibed glucose oxydase (*Aspergilus niger* ≥ 0.08 IU), ferricyanure ≥ 22 µg), (ONETOUCH, Lifescan, Inc, Miltipas CA96036, USA). Reactive strips contained a dessicative agent (Saleem et al., 2019; Alema et al., 2020). The lowering of glycemia level in treated animals was calculated using the following formula:

$$\% \text{ Reduction of glycemia} = \frac{(Gnc - Gta) \times 100}{Gnc}$$

where **Gnc** was BGL in negative control and **Gta** BGL in treated animals.

Figure 3. Glucometer or glucosemeter (ONETOUCH, USA)

Positive and negative and positive controls (Gnc-Gta: glycemia level) and in tested animals values indicated a decrease and increase of glycemia level respectively. The glycemia levels were automatically skicked up on the apparatus. BGL was expressed in mg/dl (Karau et al., 2012; Salem et al., 2019; Alema et al., 2020). The glycemia levels in hypoglycemic and antidiabetic tests of treated groups were compared to negative control.

2.8. Determination of insulin levels in normoglycemic and diabetic rats Wistar blood

Insulin level in untreated and treated animals was performed using radio-immunologic method with appropriate kits DSL-1800 insulin (Diagnostic system laboratory, Inc.USA). Insulin values were expressed in µg/ml (Gouda et al., 2019; Alema et al., 2020).

2.9. Oral glucose tolerance test (OGTT) in hyperglycemic Wistar rats

This test was only carried out with the aqueous extract which was the principal preparation taken by human in traditional medicine. Animals were grouped with 5 rats for each oral dose of tested aqueous extract Bsl-1 as followed:

●- Group I received 5 ml distilled water as negative control group,

●- Group II was made hyperglycemic by the oral administration of glucose solution (2 g/kg bw) as negative control group.

●- Group III received Glibenclamide 2.5mg/kg as positive control group,

●- Groups IV was administered 400 mg/kg bw of aqueous extract Bsl-1.

Blood of hyperglycemic animals was collected from tail tip and measured after 30, 60, 90 et 180 minutes and the glycemia level was determined using electronic glucosemeter (Alema et al., 2020) and compared to negative control (Demmers et al., 2017; Lopa et al., 2018)

2.10. Statistical analysis

Results were expresses in mean ± SD. All data were analysed by comparing values for different treatment groups with the values of negative control group. Data were analyzed by analysis variance (ANOVA and Student's t test. p value ≤ 0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSION

3.1. Effects of Glibenclamide, aqueous Bsl-1 and its soluble fractions Bsl-1.1 to -1.4, 80%methanol Bsl-2 extracts and polysaccharides CP, PPF-Bsl-1 to -4 on glucose levels of teated normoglycemic Wistar rats

Results from the effects of Glibenclamide and aqueous Bsl-1 and 80% methanol Bsl-2 extracts from *Brucea sumatrana* leaves were presented in Table 1. They showed that glibenclamide, used as reference hypoglycemic product, at oral dose of 2.5 mg/kg body weight (bw) produced significant reduction of treated normoglcemic Wisatr rats blood glucose level (BGL) from 30 minutes (min) at 82.6±0.2 mg/dl compared to negative control presenting 84.5±0.1 mg/dl. Its effect continue to be accuented to attain low value of 77.6±0.3 m/dl compared to negative control with value of 88.0 ± 0.3 mg/dl after 180 min of obseravtion. On the other hand, the oral administration of aqueous Bsl-1 and 80% methanol extract Bsl-2 from *B. sumatrana* at 200 and 400 mg/kg bw caused reduction of treated normolycemic BGL in dose-dependent manner. At the highest oral dose of 400 mg/kg bw, they carried away also marked reduction of treated normglycemic animals BGL to 83.0±0.2 and 82.8±0.3 mg/dl respectively after 30 min and continued to significantly decrease to reach values of 79.8 ± 0.2 and 78.5±0.1 mg/dl after 180 min of observation. These results indicated that Glbenclamide and both extracts from *B. sumatrana* were endowed with interesting hypoglycemic properties after 180 min of observation.

Table 1: Effects of Glibenclamide, aqueous Bsl-1 and 80% methanol Bsl-2 extracts of *M. morinodides* on normoglycemic Wistar rats BGL after 180 minutes of observation.

Groups	Treatment (mg/kg bw)	0 min	30 min	60 min	120 min	180 min
NC I	5ml DW	85.1± 0.5	84.5±0.1	85.1±0.6	85.7 ± 0.2	88.0 ± 0.3
Glb II	2.5	85.2± 0.8	82.6±0.2	80.0±0.1	79.3± 0.4	77.6 ± 0.3
Bsl-1 IIIa	NR + 200	84.8± 0.1	83.3±0.4	82.8±0.4	81.1 ± 0.6	80.0± 0.1
IIIb	NR + 400	84.6± 2.3	83.0±0.2	82.5±0.5	81.0± 0.6	79.8 ± 0.2
Bsl-2 IVa	NR + 200	84.8±0.1	83.0±0.2	82.6±0.1	81.6±0.2	80.3±0.3
IVb	NR + 400	84.5±0.0	82.8±0.3	80.8±0.1	79.0±0.2	78.5±0.1

NC: negative control, Glb; Glibenclamide, Bsl-1 and Bsl-2: aqueous and 80% methanol extract from *B. sumatrana* leaves.

The administration all soluble fractions and polysaccharides were also found to produce the same effects observed after treatment with Glibenclamide and both extracts from *B. sumatrana* leaves. Indeed, administered at all dose of 200 and 400 mg/kg bw, they also produced significant reduction of treated normoglcemic animals BGL in dose-dependent manner. At the highest oral dose of 400 mg/kg bw, all soluble fractions brought about the treated normoglycemic animal BGL to values ranging from 77.8±0.3 to 79.5±0.3 mg/dl with ethylacetate soluble fractions as the most active. Polysaccharides had also the same effects as soluble fractions. They also carried away treated normoglycemic animals BGL to low levels as for crude polysaccharide to 78.0±0.3 mg/dl, pure polysaccharide fraction PPF-Bsl-1 to 78.6 mg/dl, PPF-Bsl-2 to 79.6±0.3 mg/dl, PPF-Bsl-3 to 81.0±0.2 mg/dl and PPF-Bsl-4 to 79.8±0.1 mg/dl. From these results, it can be concluded that Bsl-1.1 to -1.4 soluble fractions.

Table 2: Effects of soluble fractions from the partition of aqueous extract Bsl-1 and polysaccharides on BGL levels of normoglycemic Wistar rats after 180 minutes of observation.

Groups	Treatment (mg/kg bw)	0 min	30 min	60 min	120 min	180 min
NC I	5 ml DW	85.1± 0.5	84.5± 0.1	84.1± 0.3	84.7 ± 0.2	87.0± 0.3
Glb II	2.5	85.2± 0.8	82.6±0.2	82.9± 0.1	82.3± 0.4	76.6± 0.1
Bsl-1.1						
VIa	200	85.0±0.2	82.0±0.2	81.5±0.4	81.2±0.3	80.0±0.2
VIb	400	83.2±0.7	81.5±0.1	81.0±0.4	79.5±0.4	79.0±0.1
Bsl-1.2						
VIIa	200	82.5±0.1	79.9±0.1	79.5±0.2	79.0±0.2	78.5±0.2
VIIb	400	84.6±0.4	81.0±0.2	80.6±0.4	78.3±0.2	77.8±0.3
Bsl-1.3						
VIIIa	200	84.1±0.9	82.6±0.1	82.0±0.6	81.00±0.3	80.5±0.2
VIIIb	400	84.8±0.3	83.5±0.3	81.7	80.2±0.1	79.5±0.3
Bsl-1.4						
XIa	200	84.3±0.5	81.6 ±0.3	80.0 ±0.1	79.6±0.2	79.2±0.0
IXb	400	85.1±0.3	81.6±0.2	81.2±0.2	79.6±0.1	78.3±0.3
CP-Bsl						
Xa	200	85.7±0.1	83.6 ±0.2	83.0±0.2	82.1±0.2	80.5±0.1
Xb	400	85.5±0.3	82.3±0.2	81.5±0.0	79.0±0.3	78.0±0.3
PPF-Bsl-1						
XIa	200	85.6±0.2	83.7±0.1	83.0±0.1	82.2±0.2	81.0±0.3
XIb	400	85.4±0.1	83.1±0.1	81.7±0.1	81.0±0.3	79.6±0.3
PPF-Bsl-2						
XIIa	200	85.7±0.3	82.1±0.3	81.4±0.1	81.0±0.1	80.2±0.1
XIIb	400	85.4±0.2	83.3±0.1	81.0±0.2	79.0±0.3	78.8±0.2
PPF-Bsl-3						
XIIIa	200	85.1±0.3	83.7±0.1	83.1±0.3	80.3±0.3	80.0±0.2
XIIIb	400	85.6±0.2	82.8±0.2	82.3±0.1	81.6±0.2	81.0±0.3
PPF-Bsl-4						
XIVa	200	85.5±0.2	83.6±0.2	81.8±0.3	81.5±0.1	81.1±0.2
XIVb	400	85.3±0.2	82.7±0.3	82.0±0.1	80.6±0.2	79.8±0.1

Bsl-1.1 to -1.4: chloroform, ethylacetate, *n*-butanol and residual phase from the partition of aqueous extract Bsl-1, CP-Bsl, PPFBsl-1 to 4: crude polysaccharide, pure polysaccharide fractions. from the treatment of CP-Bsl. and polysaccharides from *B. sumatrana* leaves possessed and exerted also interesting hypoglycemic properties.

The production of insulin by Glibenclamide, aqueous and methanol Bsl- and Bsl-2 respectively in treated normoglycemic rats was shown in Figure 2. It indicated that negative control produced high amount of 36 µg/ml of this hormone, Glb (Glibenclamide) 34 µg/ml at 2.5 mg/kg bw, aqueous and methanol Bsl extracts -1 and Bsl-2 supplied 29 and 31 µg/ml respectively at the highest oral dose of 400 mg/kg bw (Fig. 2).

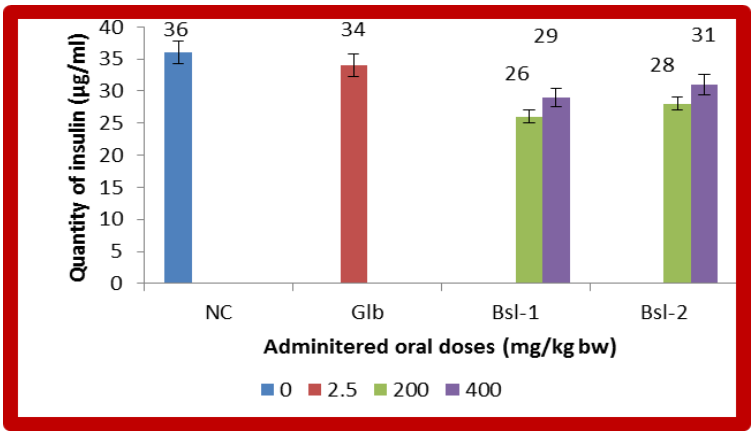

Figure 2. Production of insulin by negative control Glibenclamide (Glb), aqueous and methanol extracts Bsl-1 and Bsl-2 in treated normoglycemic rats.

Figure 3 reported the production of insulin by soluble fractions and indicated that all soluble fractions were also capable to produce the hormone insulin. Thus, at the highest administered dose of 400 mg/kg bw, chloroform soluble fraction Bsl-1.1 gave 18 µg/ml, ethylacetate Bsl-1.2 24 µ/ml as the most producer, *n*-butanol Bsl-1.3 17 µg/ml and residual aqueous phase Bsl-1.4 21 µg/ml (Fig.3). The decreasing order of the production can established Bsl-1.2 .> Bsl1.4 > Bsl-3 > Bsl-1 low compared to that of aqueous extract 29 µg/ml. Methanol extract Bsl-2 extract produced high amount compared to aqueous extract Bsl-1 extract (Fig.1 and 3).

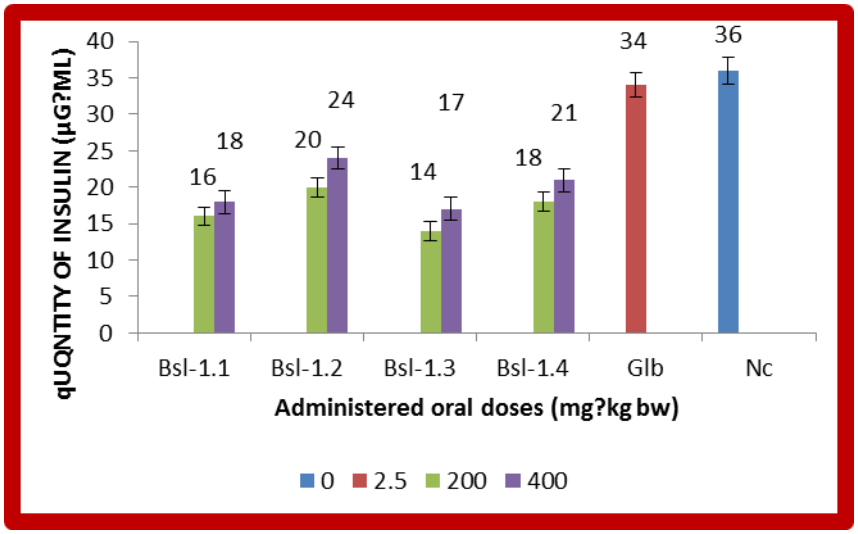

Figure 3. Production of insulin by soluble fraction Bsl-1.1 to Bsl-1.4 I normoglycemic rats.

The same results was also obtained with polysaccharides as shown in Figure 4. These metabolites produced insulin in amounts from 24 to 32 µg/ml at oral dose of 400 mg/kg bw with the crude polysaccharide CP-Bsl as the most producer 32 µg/ml, followed by PPBsl1.4 29 µg/ml PPFBsl-2 26 µg/ml, PPF-Bs-31 25 µg/ml and PPFBsl1 24 µg/ml.

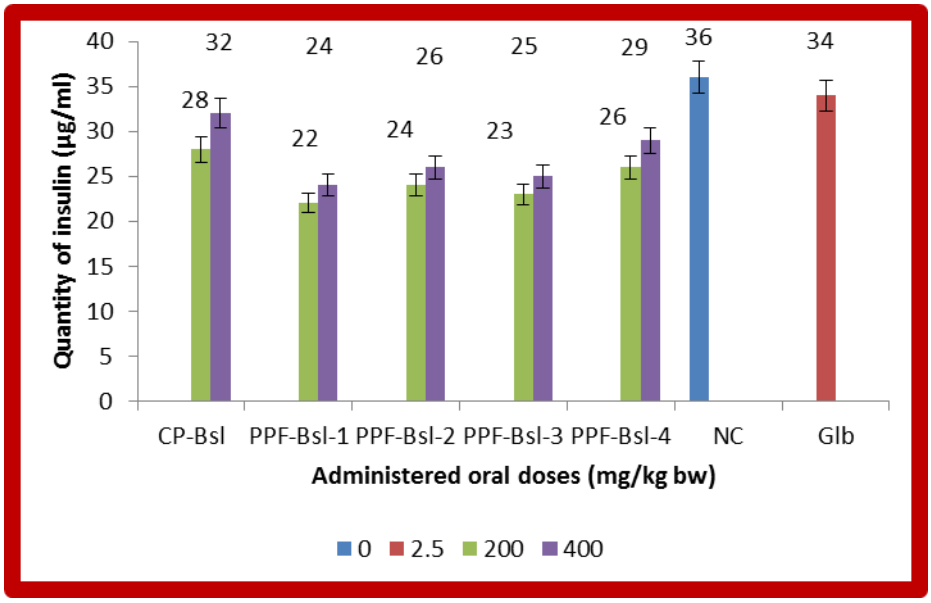

Figure 4. Production of insulin by polysaccharides CP-Bsl, PPF-Bsl-1, PPF-Bsl-2, PPF-Bsl-3 andPPF-bsl-4 in normoglycemic rats.

3.1. Effects of Glibenclamide, aqueous Bsl-1 and its soluble fractions Bsl-1.1 to -1.4, 80%methanol Bsl-2 extracts and polysaccharides CP, PPF-Bsl-1 to -4 on glycemia levels of treated normoglycemic Wistar rats after 180 min of observation

Results presented in Table 3 indicated that the oral administration of Glibenclamide at dose of 2.5 mg/kg bw cause marked diminishing of treated diabetic animals after 30 min and continue to decrease progressively to reach low value of 96.8±0.2 mg/dl after 180 min of

observation and significant difference was observed ($p < 0.05$).

Table 3: Effects of Glibenclamide, aqueous Bsl-1 extract and 80% methanol Bsl-2 extract of *B. sumatrana* leaves on the glycemia levels of treated diabetic Wistar rats after 180 minutes of observation.

Groups	Treatment (mg/kg bw)	0	30 min	60 min	120 min	180 min
NC I	5 ml DW	85.3±0.3	85.6±0.2	87.2±0.3	87.9±0.2	86.8±0.3
DR+ STPZ II	: 50	198.1±0.2	215.6±0.2	225.6±0.3	232..3±0.2	237.7±0.3
DR+Glb III	2.5	182.2±0.1	153.2±0.3	141.7±0.3	110.8±0.1	96.8±0.2
DR + Bsl-1						
IVa	DR + 200	196.2±0.3	184.2±0.2	175±0.3	165.8±0.1	140.2±0.1
IVb	DR + 400	195.3±0.2	182.5±0.1	164.5±0.1	150.2±0.2	127.2±0.3
DR + Bsl-2						
Va	DR + 200	196.5±0.1	172.2±0.1	151.6±0.2	136.2±0.3	127..5±0.2
Vb	DR + 400	196.8±0.2	168.3±0.2	142.8±0.2	130.7±0.1	120.0±0.3

DR: diabetic rats, DW; distilled water, See Table 1

Also, the oral administration od aqueous extract Bsl-1 and 80% methanol extract Bsl-2 also provoked significant decrease of treated diabetic Wistar rats glycemia levels from 30 to 180 min in dose-dependent manner (Table 2). At the highest oral dose of 400 mg/kg bw, they brought back the glycemia of treated diabetic rats to values of 182.5±0.1 and 168.3±0.2 mg/dl compared to untreated diabetic rats showing a glycemia value of 215.6±0.2mg/dl after 30 min. This effect of both extracts continued progressively to decrease to show the glycemia values of 127.2±0.3 and 120.0±0.3 mg/dl compared to untreated diabetic rats presenting a glycemia level of 237.7±0.3 mg/dl after 180 min of observation and marked difference was also observed ($p < 0.05$).

Soluble fractions Bsl-1.1 to -1.4 from the partition of the aqueous extract Bsl-1 acted in the same manner when used in the treatment of diabetic rats. At all oral doses administered, they caused also significant reduction of treated diabetic rats glycemia in dose-dependent manner (Table 4). Administered at the highest oral dose of 400 mg/kg bw, they took back the glycemia levels of treated diabetic rats to values from153.6±0.3 to 165.3±0.3 mg/dl in 30 min compared to untreated diabetic rats with a glycemia of 215.6±0.mg/dl. This effect os solube fractions continued gradually to decrease to reach values from 134.8±0.1 to 140.1±0.3 mg/dl in 180 min of observation compared to untreated diabetic rat group presenting high glycemia value of 237.7±0.3 mg/dl after 180 min. Significant difference was deduced ($p < 0.05$).

On the other hand, the same effect was also obtained with the oral administration of polysaccharides at dose of 200 and 400 mg/kg bw showing a reduction of treated diabetic rats glycemia in dose-dependent manner (Table 4). At the highest oral dose of 400 mg/kg bw, administered polysaccharides including crude polysaccharide and pure polysaccharide fractions, induced marked reduction of treated diabetic rats glycemia levels to values ranging from 152.3±0.3 to 162.3±0.3 mg/dl in 30 min, and reaching at last, glycemia values ranging between 130.3±0.1 to 140.3±0.1 mg/dl after 180 min of observation compared to untreated diabetic rats with a glycemia of 237.7±0.3 mg/dl.

Table 4: Effects of soluble fractions Bsl-1.1 to-1.4 and polysaccharides on the glycemia of treated diabetic Wistar rats after 180 min of observation.

Groups	Treatment (mg/kg pc)	0	30 min	60 min	120 min	180 min
NC I	5 ml DW	85.3±0.3	85.6±0.2	87.2±0.3	87.9±0.2	86.8±0.3
DR II		198.1±0.2	2015.6±0.2	225.6±0.3	232..3±0.2	237.7±0.3
Glb III	2.5	182.2±0.1	153.2±0.3	141.7±0.3	110.8±0.1	96.8±0.2
Mm-1.1						
VIIa	DR + 200	197.3±0.3	168.95±0.2	152.3±0.2	150.6±0.3	148.6±0.1
VIIb	DR +400	196.3±0.2	165.3±0.3	152.6±0.2	150.3±0.1	140.1±0.3
Mm-1.2						
VIIIa	DR +200	199.5±0.4	167.2±0.1	157.3±0.3	153.6±0.2	145.3±0.3
VIIIb	DR + 400	191.3±0.2	153.6±0.3	150.6±0.2	147.2±0.3	134.8±0.1
Mm-1.3						
XIa	DR + 200	195.6±0.3	188.5±0.4	1766±0.1	167.6±0.2	160.3±0.3
XIb	DR + 400	194.6±0.2	177.3±0.3	162.3±0.3	15.3±0.2	142.8±0.3
Mm-1.4	9					

Xa	DR + 200	198.6±0.2	166.6±0.1	176.3±0.3	169.6±0.2	147.5±0.2
Xb	DR + 400	197.3±0.1	162.3±0.4	166.3±0.4	158.3±0.2	136.6±0.4
CP-Bsl						
XIa	DR + 200	197.3±0.3	160.95±0.2	157.3±0.2	140.6±0.3	133.6±0.1
XIb	DR + 400	196.3±0.2	152.3±0.1	147.6±0.1	139.3±0.3	130.3±0.1
PPF-Ms-1						
XIIa	DR + 200	197.3±0.3	164±0.2	160.3±0.2	148.6±0.3	143.2±0.3
XIIb	DR + 400	196.3±0.2	157.3±0.3	150.6±0.3	140.3±0.1	138.3±0.1
PPF-Ms-2						
XIIIa	DR + 200	197.3±0.3	160.8±0.2	160.3±0.2	155.6±0.3	145.6±0.1
XIIIb	DR + 400	196.3±0.2	156.3±0.3	152.6±0.3	140.3±0.3	135.3±0.1
PPF-Ms-3						
XIVa	DR + 200	197.3±0.3	164.5±0.2	160.3±0.2	150.6±0.3	143.6±0.1
XIVb	DR + 400	196.3±0.2	162.3±0.0	153.1±0.3	144.3±0.3	140.3±0.2
PPF-Ms-4						
XVa	DR + 200	197.3±0.3	164.7±0.2	158.3±0.2	147.6±0.3	145.6±0.1
XVb	DR + 400	197.±0.2	160.3±0.3	155.2±0.3	140.3±0.3	136.7±0.1

See Tables 1 and 2, DW : distilled water, DR: diabetic rats, Glb : Glibenclamide

Figure 5 showed the production of the hormone insulin in treated diabetic Wistar rats after 180 min. Results indicated that negative control (untreated diabetic rats group) only supplied very low amount of this hormone at 11 µg/ml. Glibenclamide as reference product gave 38 µg/ml at oral dose of 2.5 mg/kg bw as the most producer while aqueous and methanol extracts Bsl-1 and Bsl-2 produced 31 and 34 µg/ml at the highest oral dose of 400 mg/kg bw with Bsl-2 extract as the better producer compared to Bsl-1 extract (Fig.5).

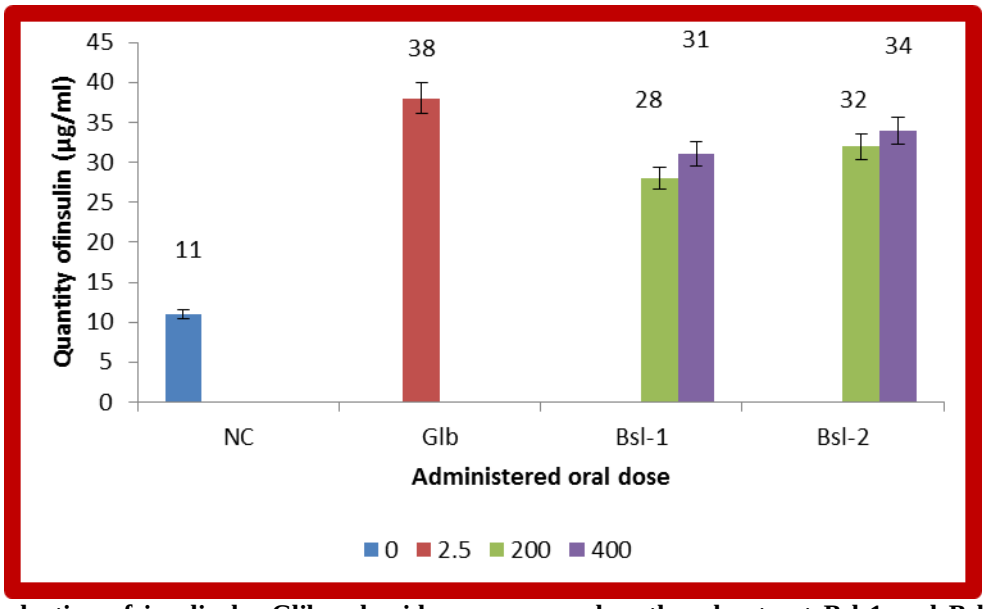

Figure 5. Production of insulin by Glibenclamide, aqueous and methanol extract Bsl-1 and Bsl-2 in treated diabetic rats after 180 min.

Figure 6 reported tha amount of insulin supplied by the administration of soluble fractions at oral doses of 200 and 400 mg/kg bw. Their effect was dose-dependent (Fig.5). They supplied different amount of this hormone accoording the nature of the administered fraction. At the highest oral dose of 400 mg/kg bw, chloroform soluble fraction Bsl-1.1 rich in steroids sn terepenoids caused a productoion of 21 µg/ml, ethylacetate Bsl-1.2 rich in flavooids 25 µg/ml, *n*-butanol Bsl-1.3 in saponins 20 µg/ml and residual aqueous phase Bsl-1.4 rich in other phenolic compounds tha flavonoids 23 µg/ml, with ethylacetate Bsl-1.2 as the better producer compared to other (Fig 6).

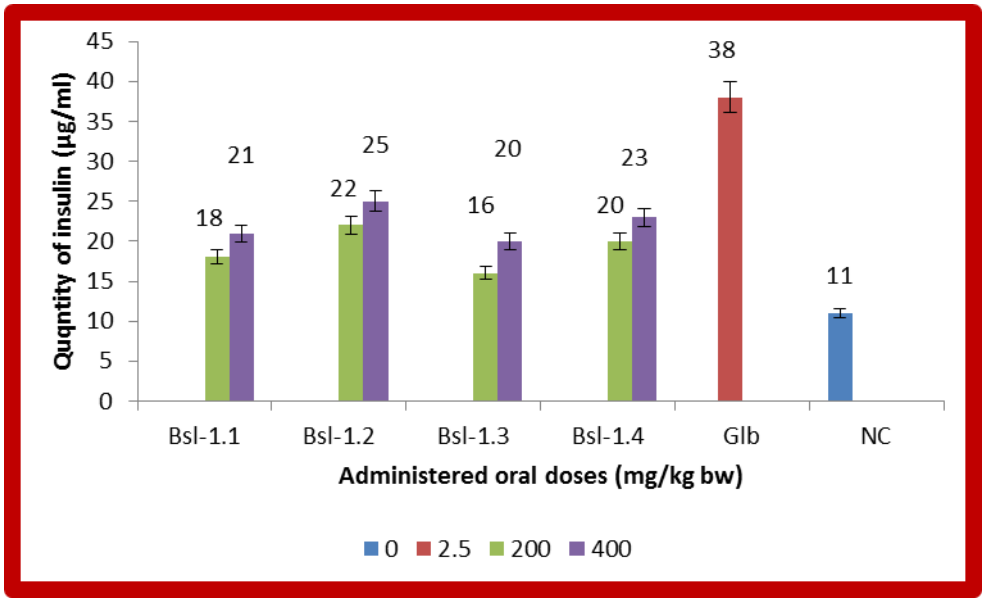

Figure 6. Production of insulin by soluble fractions Bsl-1.1 to-1.4 in treated diabetic rats after 180 min.

Figure 7 shown the amount of the hormone insulin produced in treated diabetic rats after 180 min in dose-dependent manner. At the highest oral dose of 400 g/kg bw, crude polysaccharide supplied 34 µg/ml, PPF-Bsl-1 27 µg/ml, PPF-Bsl-2 29 µg/ml, PPF-Bsl-3 27.

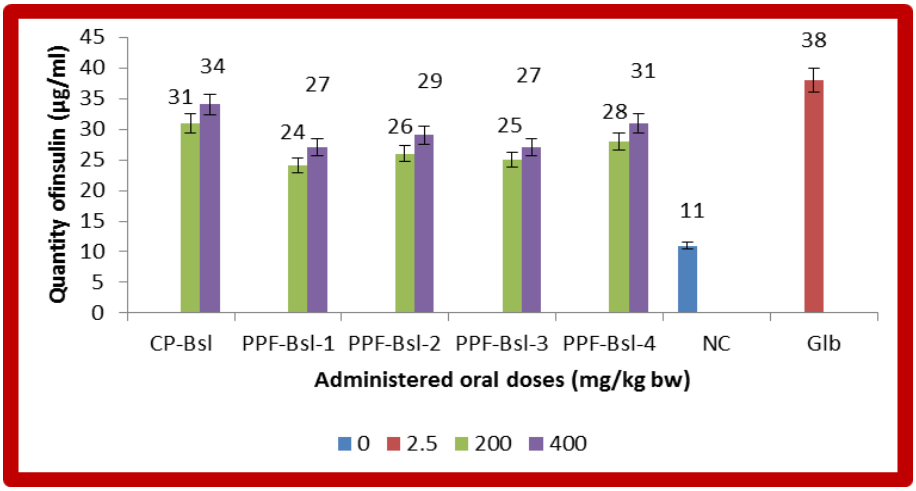

Figure 7. Production of insulin by polysaccharides from *Brucea sumatrana* leaves after 180 min of observation.

µg/ml and PPF-4 31 µg/ml with crude polysaccshride as the most producer and PPF-Bsl-4 as the better producer pure polysaccharide compared to other. This difference production may be due to the difference in the number and nature of monosaccharides that constituted their structure although they were well known to be macromolecules from their monosaccharide building blocks.

After this first time of treating of diabetic Wistar rats after 180 min of observation, it was observed that the oral administration of Glibeclamide, extracts, fractions and polysaccharides from *B. sumatrana* caused in irreversible manner the reduction of treated diabetic rats glycemia compared to untreated diabetic animals. But the obtained results showed the levels of glycemia of treated diabetic rats remained again high and these diabetic rats needed a special treatment aiming to reduce as soon as possible their glycemia near or under 100 mg/dl. The instituted new treatment consisted in the administration the same oral doses twice per day and to measure the glycemia at the end of each week.

3.3. Effects of Glibenclamide, aqueous and 80% methanol extracts Bsl-1 and Bsl-2 on glycemia of treated diabetic rats during 21 days

With this special treatment applicated on diabetic rats already treated before for 180 min, the oral administration of Glibenclamide, aqueous and 80% methanol extracts Bsl-1 and Bsl-2 carried away

significant reduction of treated diabetic rats to values of 93.5±0.2, 135.3±0.2 and 116.6±0.3 mg/dl respectively after administration oral doses of 2.5 and 400 mg/kg bw respectively on Day-7. Their effect continued to decrease until showing treated diabetic rats glycemia values of 87.3±0.0, 118.3±0.0 and 98.3±0.3 mg/dl compared to untreated diabetic rats with glycemia value of high 238.6±0.3 mg/dl on Day-21 and at last on Day-28, with the glycemia values of 84.8±0.2, 88.3±0.3 and 86.3±0.3 mg/dl respectively compared the untreated diabetic rats with glycemia level o 257.3±0.3 on Day-28. All gycemia levels recorded mainly on Day-28 were under 100 mg/dl with consistent objective attained.

Tableau 4: Diabetic rats glycemia levels produced by the administration of Glibenclamide, aqueous Bsl-1, 80% methanol Bsl-2, after 21 days de treatment.

Groups	Treatment (mg/kg pc)	Day-0	Day-7	Day-14	Day-21	Day-28
NC I	5 ml DW	86.8±0.3	85.6±0.5	87.2±0.4	87.9±0.8	86.8±0.8
DR II	STZ	237.7±0.3	240.8±0.4	246.5±0.1	250.6±0.3	253.2±0.3
Glb III	2.5	96.8±0.2	93.5±0.2	90.2±0.2	87.3±0.0	84.8±0.2
Bsl-1						
IVa	DR + 200	140.2±0.1	140.2±0.3	135.3±0.3	121.5±0.4	90.5±0.3
IVb	DR + 400	127.2±0.3	135.3±0.2	130.2±0.1	118.3±0.0	88.3±0.3
Bsl-2						
Va	DR + 200	127..5±0.2	121±0.2	114.2±0.3	101.6±0.2	93.5±0.3
Vb	DR + 400	120.0±0.3	116.6±0.3	103.3±0.1	98.3±0.3	86.3±0.3

See Table 1

Figure 8 presented the quantity of insulin hormone produced by the oral administration of Glibenclamide, aqueous and methanol extracts Bsl-1 and Bsl-2 respectively in treated diabetic rats after 21 days of treatment. Results revealed that these samples produced the amount of this hormone by Glibenclamide of 40 µ/ml, of 32 µg/ml by aqueous extract Bsl-1 and of 36 µg/ml by methanol extract Bsl-2 after 21 days of the treatment. Glibenclamide was the better producer compared to Bsl-1 and Bsl-2 extract while this last extract showed high production than the first one (Fig.8).

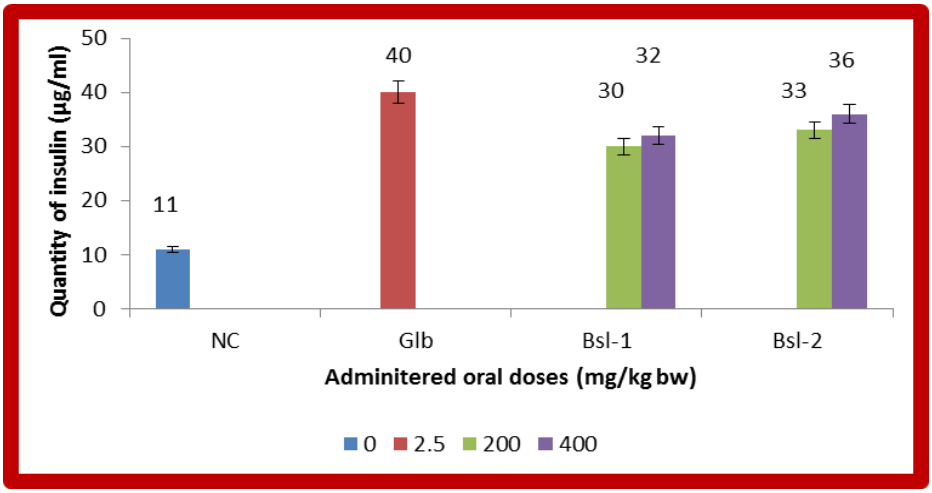

Figure 8. Production of insulin by Glibenclamide, aqueous and methanol extracts Bsl-1 and Bsl-2 respectively.

Next, the application of this special treatment of treated diabetic rats with soluble fractions and polysaccharides gave the same results as aqueous Bsl-1 and 80% methanol Bsl-2 extracts. Indeed, the administration of soluble fractions and polysaccharides at oral dose of 200 and 400 mg/kg bw caused significant diminishing of glycemia levels of treated diabetic rats in dose-dependent manner (Table 5). For soluble fractions, their reductor action was characterized by the reduction of these glycemia to values ranging from 131.3±0.2 to 140.02±0.2 on Day-7 compared to untreated diabetic group presenting glycemia level of 210.8±0.4 mg/dl. They continued to decrease progressively following days to values from 110.6±0.2 to 127.3±0.3 on Day-21 compared to untreated diabetic rats with glycemia value of high 238.2±0.3 mg/dl and to values between 92.5±04 and 98.5±0.3 compared to untreated diabetic rats with very high glycemia of 253.2±0.3 mg/dl on Day-30. Significant difference was observed.

The effects of polysaccharides were similar since they also produced marked decrease of treated diabetic glycemia levels to values from 107.6±0.3 to 132.6±0.5

on Day-7, and continue to known significant decrease in the following days to values of 94.6±0.1 to.

Tableau 5: Diabetic rats glycemia levels change by the administration of Glibenclamide, soluble fractions and polysaccharides after 21 days de treatment.

Groups	Treatment (mg/kg pc)	Day-0	Day-7	Day-14	Day-21	Day-28
NC I	5 ml DW	86.8±0.3	85.6±0.5	87.2±0.4	87.9±0.8	86.8±0.8
DR II	STZ	237.7±0.3	240.8±0.4	246.5±0.1	250.6±0.3	253.2±0.3
Glb III	2.5	96.8±0.2	93.5±0.2	90.2±0.2	87.3±0.0	84.8±0.2
Mm-1.1						
VIa	DR +200	147.3±0.1	142.3±0.3	136.2±0.3	127.3±0.3	97.3±0.3
VIIb	DR + 400	145.3±0.3	140.2±0.2	132.5±0.2	124.6±0.0	94.5±0.4
Mm-1.2						
VIIa	D R+ 200	140.6±0.1	134±0.2	129.3±0.2	115.6±0.4	94..2±0.1
VIIb	DR + 400	138.3±0.1	132.3±0.2	125.2±0.0	110.6±0.2	92.5±0.4
Mm-1.3						
VIIIa	DR + 200	163.3±0.3	158.3±0.1	148.3±0.3	138.5±0.2	103.2±0.1
VIIIb	DR + 400	159.8±0.3	152.3±0.1	145.3±0.1	122.5±0.4	98.5±0.3
Mm-1.4						
IXa	DR + 200	154.5±0.2	138.3±0.2	139.2±0.0	131.3±0.4	110.5±0.1
IXb	RD + 400	158.6±0.4	135.3±0.3	133.3±0.3	128.6±0.1	97.5±0.4
CP-Bsl						
Xa	DR +200	134.6±0.1	110.3±0.2	95.2±0.1	112.6±0.3	92.3±0.3
Xb	DR + 400	131.3±0.1	107.6±0.3	102.4±0.3	98.6±0.1	90.6±0.1
PPF-Bsl-1						
XIa	DR+ 20	144.6±0.1	134.1±0.2	118.5±0.3	109.6±0.3	101.3±0.2
XIb	DR + 400	140.3±0.1	128.6±0.5	114.2±0.1	102.5±0.1	96.3±0.0
PPF-Bsl-2						
XIIa	DR + 200	140.6±0.1	129.6±0.0	114.7±0.3	106.3±0.1	95.6±0.2
XIIb	DR + 400	134.3±0.1	125.3±0.2	106.2±0.0	99.6±0.2	94.6±0.2
PPF-Bsl-3						
XIIIa	DR + 200	146.6±0.1	132.9±0.1	125.3±0.0	113.5±0.2	104.6±0.2
XIIIb	DR + 400	144.3±0.1	132.6±0.2	120.5±0.3	108.7±0.0	97.3±0.2
PPF-Bsl-4						
XIVa	DR + 200	143.6±0.1	132.5±0.2	122.6±0.2	102.5±0.2	98.5±0.2
XIVb	DR + 400	136.3±0.1	128.6±0.3	118.5±0.1	98.2±0.3	94.0±0.0

See Table 2

108.7±0.0 on Day-21 and to values ranging from 90.6±0.1 to 97.3±0.2 on Day-30 and were found to be under the value of 100 mg/ml on mainly Day-28.

With results obtained in this special treatment, it can be assumed that Glibenclamide, both aqueous Bsl-1and its soluble fractions Bsl-1.1 to- 1.4 as well as 80% methanol extract Bsl-2 reduced very significantly the treated diabetic glycemia and thus, possessed interesting antidiabetic properties.

Figure 9 reported the quantity of insulin produced by soluble fractions in treated diabetic rats after 21 days of treatment. Results indicated that these soluble fractions were capable to provoke the production of this hormone in different quantities from 22 to 26 µg/ml attributed to 24 µg/ml for chloroform Bsl-1.1, to 28 µg/ml for ethylacetate Bsl-1.2, to 22 µg/ml for *n*-butanol Bsl-1.3 and to 26 µg/ml for residual aqueous phase Bsl-1.4 soluble fractions respectively compared ton untreated diabetic rats producing only low quantity of 11 µg/ml of this hormone and significant difference was observed (< 0.05).

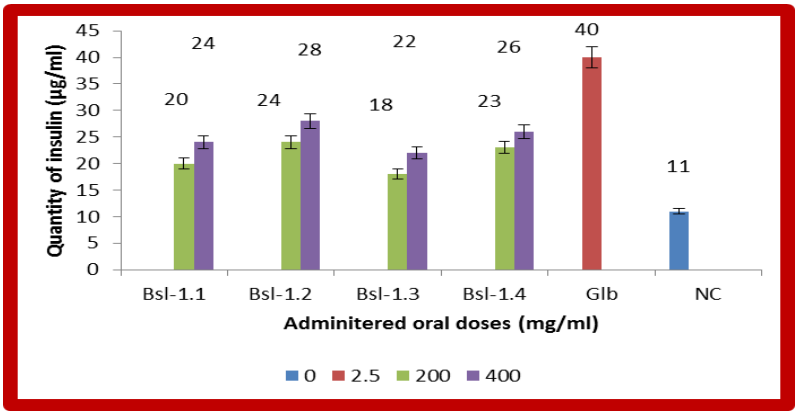

Figure 9. Production of insulin by soluble fractions Bsl-1.1 to-1.4 from the partition of aqueous extract Bsl-1.

Figure 10 showed the quantity of insulin produced by the oral administration of polysaccharides in treated diabetic rats after 21 days of treatments. Results revealed that crude polysaccharide CP-Bsl supplied 35 µg/ml of this hormone, PPF-Bsl-1 30 µg/ml, PPF-Bsl-2 32 µg/ml, PPF-Bsl-3 29 µg/ml and PPF-Bsl-4 33 µg/ml compared to untreated diabetic rats producing only 11 µg/ml. CP.Bsl was the better producer while PPF-4 was the better pure polysaccharide compared to other (Fig. 10). Significant difference can be observed ($p < 0.05$).

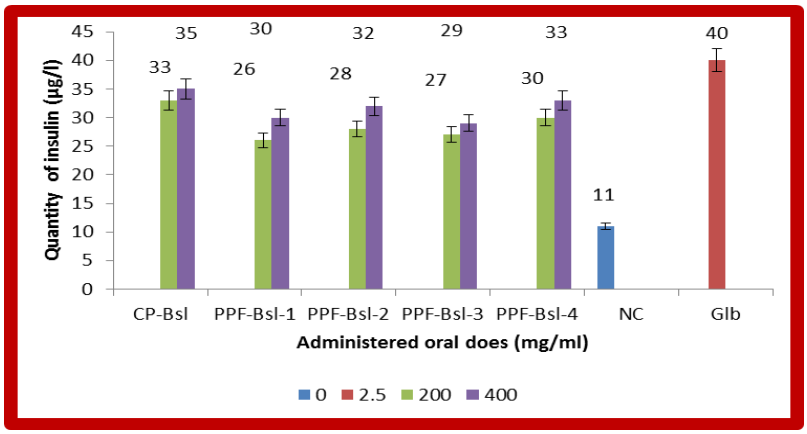

Figure 10. Production of insulin hormone by the oral administration of polysaccharides.

This hormone insulin was also previously quantitavively estimated in other medicinal plants such as *Allium sativum* (Eidi et al., 2006), *Pseuderanthemum palatiferum* (Padee eta la. 2010) and in *Myrcia bella* leaf (Vareda et al., 2014) and other.

In comparison of the production of hormone insulin, it was observed that Glibenclamide used as reference product produced high amount as better producer compared to *B. sumatrana* samples, which in turn showed high amount compared to untreated diabetic diabetic rats. This finding can be explained by the fact that in untreated diabetic rats, the organization and the amount of pancreatic β cells was not good and was in very low amount because of their permanent destruction by the administrated STPZ. On the other hand, Glibenclamide and different samples from *B. sumatrana* leaves strongly inhibited the toxic effect of STPZ leading to the regeneration of pancreatic beta cells in high amount, which in turn, stimulated the secretion and release of insulin, with consequence the decrease BGL and glycemia of treated normoglycemic and diabetic animals. (Fig 1 and 2) (Eleazu et al., 203, Cimanga et al., 2019).

In addition, Glibenclamide exerted high hypoglycemic and antiabetic activities compared to all samples from *B. sumtrana* although in sames cases, these activities were sometimes comparables mainly with 80% methanol extract. Samples of *B. sumatrana* were found to have Glibenclamide like-effects since they acted according the same mechanisms of action as hypoglycemic and antidiabetic agents.

In general, our reported results were in qaulitatively in good agreement with other previously described results from various studies on hypoglycemic and antidiabetic activity of various medicinal plants (Liu et al., 2013; Hu et al., 2016; Wang et al., 2017, 2018; Estiasb et al., 2018; Zhang et al., 2018; Anbonuva and Shsutnsya, 2020; Seedevi et al., 2020).

3.4. Effects of Glibenclamide and aqueous extract Bsl-1 on glucose level of hyperglycemic normoglycemic Wistar rats

In OGTT test, results reveleaded that the oral administration of glucose at 4 g/kg bw to normolycemic treated rats reached significant increase of their BGL

from 30 min to value of 142.0±0.1 and continued to inscrease significantly to attain the value of 161.2±0.1mg/dl after 180 min and were declared as hyperglycemic compared to negative control presenting glucose levels between 86.0±0.3 mg/dl to 87.00±0.0 mg/dl at the same times of observation and significant difference was observed ($p < 0.05$).

Table 6: Reduction by Glibenclamide and aqueous extract Bsl-1 of treated hyperglycemic rats BGL after 180 min.

Times (min)	Group I NC	Group II HR	Group II HR- Glb	Group IV HR-Bsl-1
0	85.6±0.2	136.±0.2	133±0.00	134.3±0.2
30	86.0±0.3	142.0±0.1	120.6±0.2	124.3±00
60	86.2±0.0	150.8±0.3	115.1±0.4	117.2±0.2
120	86.6±0.4	161.2±0.1	99.2±0.4	100.00±0.0
180	87.0±0.0	166.0±0.0	83.7±0.1	87.6±0.3

NC normoglycemic negative control, HR; hyperglycemic rats, Glb: glibenclamide, Bsl1; aqueous extract from *B. sumatrana* leaves.

Next, the oral admininitration of Glibenclamide (Glb) at 2.5 mg/kb bw and aqueous extract Bsl-1 at the highest oral dose of400 mg/kg bw caused significant reduction of glucose levels of treated hyperglycemic normoglycemic rats after 30 min to 120.6±0.2 mg/dl and 124.3±0.0 mg/dl respectively which continued to decrease very markedly to values of 83.7±0.1 and 87.6±0.3 mg/dl after 180 min with significant difference observed ($p < 0.05$). This observed effect clearly demonstrated that Glibenclamide and aqueous extract Bsl-1 from *B. sumtrarna* leaves possessed antihyperglycemic properties. Our results well collaborated with other reported on the antihyperglycemic activity of polysaccharides isolated from other medicinal plants (Sun et al., 2014; Fan et al., 2015; Guo et al., 2017).

The hypoglycemic and antidiabetic activity of *Brucea javanic*a, an Asiatic species were also previously reported. It concerned firstly the antidiabetic activity of the seed extracts (Ablat et al., 2014, 2017; Simamora et al., 2019). Secondly, the hypoglycemic and antidiabetic of leaf extracts and infusion from *Brucea leaves* (Handir et al., 2017) was also reported. Results reported in this last studie were only qualitatively in good accord with our because these biological activities were reported against alloxan induced-diabetic rats vesrus against streptozocin-induced-induced diabetic rats. The active principles of this medicinal were known to be quassinoids and other polyphenolic compounds from the seeds such vanillic acid, para-hydroxybenzoic acid, luteolin, protocatechuic acid and galic aid (NoorShhida et al., 2009; Abdat et al., 2014, 2017). Mainly quassinoids were detected in *Brucea sumatrana* leaves (Tshodi et al., 2012).

At last, several mechanism of action of medicinal plant extracts were previously proposed in various studies. They included acting on glycolysis, Kreb cycle, glucosynthetis and their degradation, cholesterol synthesis, metabolism and adsorption of corbohydrates, synthesis and release of insulin, aldose and reductase pathway and free radical scavenging action in human body, insulin mimetic effect (Pranav and Mukesh, 2008), regeneration of pancreatic beta cells which stimilated the secrection and release of insulin, that in turn reduced glucose and glycemia levels of treated normoglycemic and diabetic animals (Patel et al. 2012; Prashant et al., 2013; Cimanga et al., 2019), improving inhibition of neoglucogenesis and glycolysis (Andrade Ceto, 2012), muscule in an adiput tissue glucose uptake, but must by reducing hepatic glucose protection trough both insulin-dependent and insulin-independent resistances, stimulation of hepatic glycogenesis and the suppression of glucose-6-phosphatase (G6Pase), the key enzyme of hepatic gluconeogenesis, stimulation of hepatic glycogen synthase (GS), the enzyme that stores excess glucose as glycogen, positively correlated with increased phosphorylation of glycogen synthase kinase-3 (GSK-3), inhibition of glycogen synthesis in liver and muscle (Andrade Ceto, 2012; Hoda et al., 2014), decrease and maintenance of blood glucose level (BGL) by stimulating insulin secretion and release from pancreatic beta cells or increasing glucose uptake in the peripheral (Gurhickel et al., 2016), inhibition of alpha amylase and alpha glucosidase and other sugars, antioxidant properties, insulin like-effects or mimetics, inverse of the size of Langerhans istlets caused changes in STPZ diabetic animals pancreatic beta cells leading to improve Langerhans istlets release insulin remaining or replicated beta cells, the use sensitivity and the peripheral tissue usage of glucose, having insulin mimetic properties, promoting glucose transporter (GLT-2) expressing and transformation, blocking pancreatic beta cell channels, stimulating apetic adenosine monophosphate cAMP, also providing some essential elements Ca, Zn, Mg, Mn and Cu for beta cells use also some mechanisms that are possibly participated in all dysfunction found in diabete mellitus (Algahtana et al., 2020), activation of releasing insulin from ß-cells, reduction of glucose absorption, stimulation of glycogenesis, and/or enhancement of glucose use, in addition to lowering BGL, secondary metabolites obtained from MPs have the capacity to restore the impaired ß-cells and terminate oxidative stress on ß-cells, inhibiting cellular apoptosis, reducing renal glucose reabsorption, enhancing the metabolic rate of oxygen consumption, and promoting glucose transporter GLUT-2 expression and translocation of GLUT-4 are also important mechanisms illustrated with certain secondary metabolites that are responsible for antidiabetic effects, blocking pancreatic *β*-cell K^+ channel, stimulating cyclic adenosine monophosphate

(cAMP), blocking the actions of *α*-amylase and *α*-glycosidase enzymes, which are essential for carbohydrate digestion, used as an optional treatment approach for type 2 diabetes. (Berlan, 2021. Yikna et al,, 2021). With regards to experimental data, it can be assumed that extracts, fractions and polysaccharides from *B. sumastrana* leaves acted as hypogycemic and antidiabetic agents by more than one of these mechaninsm actions as also Glibenclamide because these samples had Glibenclamide like-effects as all ready mentioned above, etc.

4. CONCLUSION

This study reported for the first time the hypoglycemic, antidiabetic and antihyperglycemic activities of aqueous extract and its soluble fractions as well as for 80% methanol extract and polysaccharides from *Brucea sumtrana* leaves collected in Democratic Republic of Congo, an African countries. Both extracts, fractions and polysaccharides from this medicinal plant were found to be able to reduce considerably glucose and glycemia levels of treated normoglycemic and diabetic rats. These results can support and justify the traditional used of aqueous extract from *B. sumatrana* leaves for the treating of diabetes mellitus type 2 and the use of the plant part for the preparation of ameliorated galenic forms such tablets and sirops for simple and easy use by the population in cheaper price.

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