Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(6):376-385

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vivo hypoglycemic activity and acute oral toxicity of ethanolic and aqueous leaves extract of *Momordica charantia Linn* (*Cucurbitaceae*) from Benin

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ABSTRACT

Momordica charantia or Bitter Melon, a tropical vegetable has been used extensively in traditional medicine as a remedy for many diseases. Momordica charantia Linn belongs to the Cucurbitaceae family. Momordica charantia is reported to have been successfully used in the treatment of diabetes mellitus in Benin. The present study aims: (i) evaluating the phytochemical, antiradical and hypoglycemic activities of ethanolic and aqueous extracts of Momordica charantia leaves; (ii) determining the acute oral toxicity of ethanolic and aqueous extracts of Momordica charantia. Qualitative phytochemical tests were used to detect the presence of bioactive molecules. Wistar rats were administrated Momordica charantia extracts (250 mg/kg and 500mg/kg) orally for 14 days and blood glucose was measured once a day for about 2 weeks. Antiradical activity was made by using the DPPH method. Toxicological evaluation of aqueous extract of Momordica charantia on some liver function parameters of wistar rats (150-200g) was critically examined. Results from phytochemical screening indicated the presence of triterpenoids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, anthocyanes, leucoanthocyanes, reductor sugar, and mucilage and Cyanogenic compound in Momordica charantia leaves. Ethanolic extract showed a high potential antioxidant capacity (676, 4 mmolEqAA/g ± 26,195) followed by aqueous extract (42, 23 mmolEqAA/g ± 10, 6). Moreover, an increase of conc entration to 2000 mg/kg was non-toxic for the rats. No toxicity activity was observed. Blood biochemical parameters and hematological parameters remain constant after administration of the plant ethanolic and aqueous extracts. Administration of ethanolic and aqueous extracts daily for two weeks resulted in decrease in blood glucose levels of rats. The important results obtained from this study justify the use of this plant in traditional medicine for treatment of diabetes.

Key words: *Momordica charantia*, ethanolic and aqueous extract, bioactive molecules, hypoglycemic activity, toxicity, Benin.

INTRODUCTION

Diabetes mellitus is the most common endocrine disease. The world population of diabetic mellitus in the year 2008 was approximately 150 million and the population of this pandemic was expected to double by the year 2025[1]. In Benin, the prevalence of the diabetes is 2.9 % in 2011[2].

Diabetes mellitus leads to metabolic abnormalities and is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [3]. The use of orthodox drugs in the management of diabetes mellitus has not improved the situation. Plants are well known in traditional medicine for their hypoglycemic activities. Available literature indicates that there are more than 800 plant species showing hypoglycemic activity [4]. There has been an increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. Therefore, plants are being continuously explored for their possible effect as hypoglycemic agents [5].

The ethnobotanical survey conducted through different villages and markets in Benin revealed 211 anti-diabetic plant species belonging to 78 taxonomic families groups. *Momordica charantia Linn* is among these plants very often cited. *Momordica charantia* (MC), also referred to as bitter gourd or karela, is a member of the Cucurbitaceae family and is commonly used as a traditional remedy for diabetes in India, Asia, Africa and South America. It is commonly consumed as a vegetable in India. The fruit, leaves, seeds and roots of MC have been used in the Indian system of medicine for a number of diseases, in addition to diabetes [6, 7]. The aqueous extract from MC was demonstrated to be a potent stimulator of insulin release from β cell rich pancreatic islets isolated from obese-hyperglycaemic mice [8]. It has also been reported that the oral administration of different MC extracts shows varying patterns of antihyperglycemic effect without altering the insulin response, suggesting a mechanism of action which is independent of intestinal glucose absorption and probably involves an extra pancreatic effect [9]. Charantin, a peptide resembling insulin isolated from *M. charantia* lowered fasting blood sugar in rabbits gradually beginning from 1st and lasting till the 4th hour and slowly recovering to the initial level [10].

Until now no scientific investigation has been carried out to shed light on the anti-diabetic, hypoglycemic property of *Momordica charantia in Benin* .Thus, in order to validate the tribal use of the plant as an anti-diabetic in Benin, the objective of the present survey was to study the aqueous and ethanolic extract from leaves of *Momordica charantia* on body weight, fasting blood glucose level in normoglycemic rats.

EXPERIMENTAL SECTION

Plant material

Fresh leaves from *Momordica charantia* were collected at Agbangnizoun located in the center of Benin (Zou department). The material was authenticated by a botanist at the National herbarium from University of Abomey-Calavi (Benin). A voucher specimen was deposited at the herbarium under the number: AA6531/HNB. The leaves were air-dried in the laboratory before powdering.

Preparation of aqueous extract *Momordica charantia*

100 g of dry powder added to 500 ml of distilled water are mixed in a ball warmed with a heating skullcap. During the preparation the ball mixture was subjected to a permanent agitation for 30 minutes. Then after cooling, the macerate was filtered under paper filter. The extraction was made three (03) times on the macerate. The filtrate obtained was concentrated under vacuum by using a rotary evaporator at 80°C until a dry extract is obtained. After extraction the yield was determined.

Preparation of 95% ethanolic extract of *Momordica charantia*

The fresh vegetable (100 g) was thoroughly washed in tap water, cut and the seeds were removed manually. The seedless vegetable was blended with 2 liters of 95% (v/v) ethanol in a blender and kept at room temperature in a flat bottom flask for 24 hours with occasional shaking. The suspension was then filtered and the residue discarded. The alcoholic portion of the filtrate was evaporated in a rotavapor at 40°-45°C under reduced pressure. Water was removed by lyophilizer.

Animal material

Male and female albino Wistar rats weighing 150-200 g at the age of months were used for this study. Animals were housed in polypropylene cages and maintained under standard conditions with an alternated cycle of 12 hours light and 12 hours dark cycles with free access to food and water. Room' temperature was maintained at 25^{o} C with a relative humidity of 35-60%.

Drugs and chemicals

Gallic acid, Ethanol, Methanol, Hydrochloric acid (HCl), Folinciocalteau reagent, sodium carbonate (Na_2CO_3), Sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), Phosphomolybdenum, Catechin, Rutin, Potassium ferricyanide($K_3Fe(CN)_6$), DPPH (2, 2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich

Hypoglycemic study in normal rats
It was done to evaluate the impact of different extracts in normoglycemic rats glucose level using the method according to author [11], with slight modifications and lipidic profils in daily administration for two weeks.

The normoglycemic rats were randomly assigned into five groups (1-6) of six rats (n = 5) each as follows, namely:
Group 1: Normal, treated with 250 mg/Kg aqueous extract
Group 2: Normal treated with 500 mg/kg aqueous extract
Group 3: Normal, treated with 250 mg/kg ethanolic extract
Group 4: Normal, treated with 500 mg/kg ethanolic extract
Group 5: Normal, treated with distilled water (10 mL/kg)
Group 6: Normal, treated with glibenclamid (5 mL/kg)

The blood glucose was estimated on days 1, 5, 10, and 15 using the glucometer (SD Check). At the end of 14 days, biochemical and hematological analyses were done.

Phytochemical screening
Photochemical screening which is a qualitative chemical analysis based on color reactions and precipitation of the major groups of chemical compounds in plants [12] was carried out to find out the phytoconstituants present in *Momordica charantia* leaves.

Quantification of the bioactive molecules
Polyphenolic content
Total polyphenol content (soluble and bound) was determined by the method described by author [13]. Known volume of extract was taken and made up to 250µl with distilled water and the sample was mixed with 625 µl of folinciocalteau reagent and 500 µl of 20% Na_2Co_3. The final reaction mixture volume was adjusted to 5ml using distilled water. The reaction mixture was incubated in darkness for 30 minutes and samples were then centrifuged at 2000 rpm for 5 minutes and then the supernatant absorbance was measured at 760 nm. A calibration curve was constructed with different concentrations of gallic acid as standard. The results were expressed as mg of gallic acid equivalent/g of sample.

Flavonoids content
This was assayed following the method described by authors [14; 15] with slight modifications. 400 µL of extract was mixed with 500µL of distilled water. Then, 120µl of 5% of sodium nitrite was added to the mixture and allowed to stand for 5 minutes. 120 µL of 10% of AlCl3 was added and whole solution mixed using a vortex. After 6 minutes, 800 µL of NaOH 1M were added and the mixture was incubated in darkness for 15 minutes. Solution of rutin was used as reference. The concentration values are directly read from the calibration curve established using the reference solution. The flavanoid content was expressed as mg of Rutin equivalent/1mg of sample.

Condensed tannins
Condensed tannins were estimated using the method of author [16] modified by author [17]. A volume of 500µL of extract was added to 1.5 mL of vanillin solution initially dissolved in methanol for a final concentration at 4%; 1.5 mL of concentrated hydrochloric acid and 2 mL of methanol. The mixture was then incubated for 15 minutes and the absorbance taken at a wavelength of 500 nm. Condensed tannins were expressed as mg of tannic acid equivalent/mg of extract.

Total antioxidant capacity assay (TAO)
Antioxidant activity was related to the capacity of plant extract to trap the free radical molecules. The technique applied for determination used the DPPH (2,2 –diphenyl-1-picrylhydrazyl) method described by author [18]. 1.5 mL of DPPH solution (4mg of DPPH dissolved in 10 mL of methanol) was added to 0.75 mL of leaves extract at differents concentration (from 0.5 mg/mL to 3.5 mg/mL). The mixture was then incubated in a dry bath room at room temperature for 15 minutes. The absorbance was measured at the wavelength of 517 nm using ascorbic acid as blank and standard. The total antioxidant activity was expressed as Equivalent of Ascorbic acid per gram of aqueous extract (mmolEqAA/mg).

Acute Toxicity Studies
Oral acute toxicity study in wistar rats was carried out for aqueous extract of *Momordica charantia in* accordance with OCDE (Organization for Economic Cooperation and development) guideline[19]. Wistar rats (3 males +3 females) weighing 150-200 g were used for evaluation of acute toxicity test. Animals were kept out on fasting overnight prior to aqueous extract of *Momordica charantia* administration. After the period of fasting, animals were weighed and plant extract was administered at the dose of 2000 mg / kg by gavage. Following administration of

extract , food was held for further 3- 4 hours followed by observation after 30 min , 1h , 2 h , 3 h , 4 h , 24 h and once a day till the day 14 . Rats were examined for various observations like weight change, tremors, convulsion, salivation, diarrhoea, lethargy, sleep, coma, and death. Along with that the cage side observations like changes in the skin, fur, eyes and behavioral pattern were studied.

The essay of toxicity was done according to the guidelines of the organization of Cooperation and Economic development 423 (OECD, 2002) related to chemicals. The substance was tested by using a sequential process in which three females and non gravid wistar rats weighting 150 g - 200 g were used at every stage. Five (05) lots of three (03) rats were established with a total of fifteen rats. One lot used as control received only distilled water and the rest received the extract at the concentration of 2g/kg. After force-feeding, the animals were observed during the first four hours and then daily for fourteen (14) days. The animals were weighted at the beginning of the experience and afterwards every seven (07) day. At the end of 14 days, the biochemical and hematological analyses were done.

Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from anaesthetized rats into heparinised bottles for haematological studies. Blood samples collected into non-heparinised bottles were allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Hematological Measurements

Blood samples were collected from retro-orbital of the experimental rats in capillary tubes coated with ethylene diamine tetra-acetic acid (EDTA). The tubes were immediately capped, kept at – 4 °C and were immediately analyzed for blood parameters using automated Coagulating Sysmex apparatus Type 8999. The parameters included: hemoglobin (Hb), mean cell volume (MCV), red blood cells count (RBCs), white blood cells count (WBCs), mean cell hemoglobin concentration (MCHC), platelets (PLT) ; Hematocrit (THE) Aspartate aminotransferase (AST), and alanineaminotransferase (ALT) were determined using a photoelectric colorimeter (Gallenkamp® and Sons Ltd.; England) [21,22]. Serum urea and creatinine levels were determined using photoelectric colorimeter (Gallenkamp® and Sons Ltd. England) [20, 22].

Statistical Analysis

All the data obtained were subjected to statistical analysis using Minitab software Version 1.0. The results were expressed as mean. One way analysis of variance (ANOVA) was performed and differences were pointed out using Kruskal-Wallis'test. Results were considered statistically significant for $p < 0.05$. Correlations between different values were expressed as graph using Graph Pad PRISM software version 5.

RESULTS AND DISCUSSION

Momordica charantia leaves have been used for thousands of years for their medicinal properties [23]. In Benin, the main methods of preparation of this plant were decoction, infusion, maceration and trituration. That the reason why we chose to prepare aqueous and ethanolic extract.

Aqueous and ethanolic extraction made respectively with the powdered (100g) of the whole plant of *M. charantia* L enabled us to obtain the following yields (Table1)

Table 1 : Percentage yield of different extract and showed that ethanol extract yield is significant that water extract

Plant material powder (g)	Solvant (mL)	Weight of Extracts (g)	Yield (%)
100	Aqueous	3.6	7.2
100	Et-OH	7.7	15.54

The results of the extraction with water and ethanol showed that ethanol was more efficient solvent with a yield of 15.54% followed by water with 7.2% (table 1).This result agrees with that obtained by author [24]. It proves that the solvent system plays an important role in the solubility of some chemical constituents [24]

Results from phytochemical analysis of leaves extract of *M. charantia* were presented in table 2 below. Out of the ten important families of compound searched into the extract, only one compound family is absent .That is cyanogenic glycoside.

Table 2: Phytochemical analysis of *M. charantia Linn* leaves extract

chemical compounds	*M. charantia* leaves extract
Tannins	+
Flavonoids	+
Saponosids	+
Alkaloids	+
Anthocyans	+
Leucoanthocyans	+
Coumarins	+
Triterpenoids	+
reducing sugars	+
Cyanogenic glycosides	-

+ Present - Absent

Phytochemical screening of the leaves extract of *M. charantia* revealed the presence of saponins, tannins, reducing, sugars, flavonoids, alkaloids, anthocyans, leucoanthocyan, coumarins, triterpenoïdes. This result is in agreement with data reported by other investigators [24, 25, 26]. On the other hand, author [27] reported the presence of alkaloids, tannins, terpens but noted the absence of flavonoids, coumarins and quinines. These differences could be related to the age of plant, time of harvest, climate, type of soil culture and the method of extraction. The presence of these secondary metabolites in the leaves of *M. charantia* has justified the claim by traditional medicine for the use of this plant in the treatment various diseases.

By using absorbance of molecules, some important bioactive families were quantified. Indeed, total phenolic compound, flavonoid content and condensed tannins content were determined and gallic acid, rutin, catechin were used respectively as standard for each of family. Figure 1 presented the respective contents of total phenolic, flavonoids, condensed tannins.

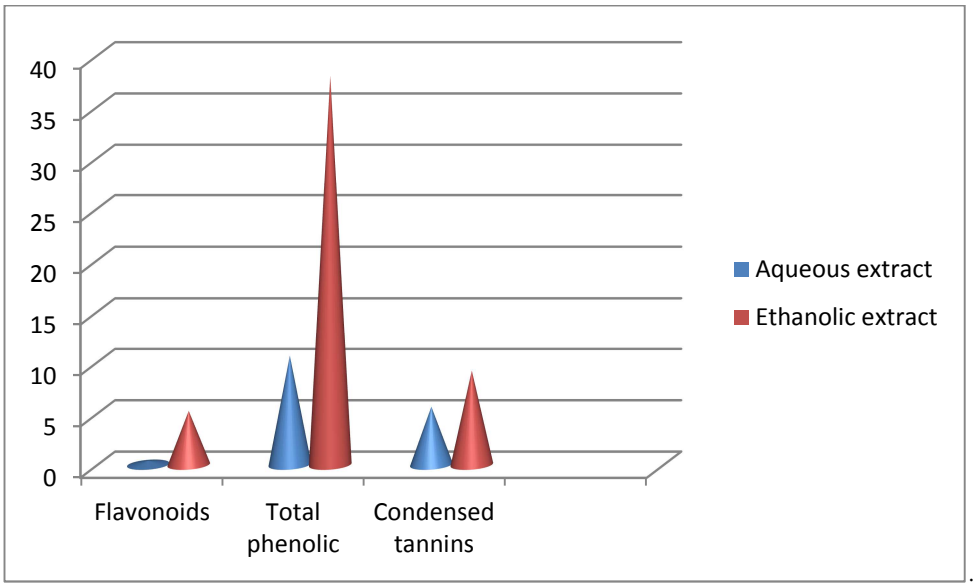

Figure1: Respective contents of total phenolic, flavonoids, condensed tannins

The contents were expressed in mg equivalent of each standard per mg of plant extract. The respective contents of total phenolic, flavonoids, condensed tannins for ethanolic extract were : 38.016±0.16 ; 5.42±0.32; 9.16±1.018 and 10.63±0,43; 0.395±0.32 ; 5.6±2.03 for aqueous extract . The results of the content of polyphenols, flavonoids, condensed tannins in ethanolic and aqueous extracts showed that ethanolic extracts have a high level of these bioactive molecules. Our findings are in agreement with the result of investigator [28] who reported that the aqueous extract of *Momordica charantia* L has a content of an order of 7.36 ± 0.86 mg EqCAT / g of extract

Total antioxidant capacity represents both oil soluble and water soluble antioxidants that are capable of scavenging reactive oxygen species and protects from chronic diseases such as cancer, diabetics and arthritics. Results from antioxidant activity using DPPH' method revealed a high potential antioxidant activity of the ethanolic extract with 676.4 mmolEqAA/g ± 26.195, followed by aqueous extract 42.23 mmolEqAA/g ± 10.6.(Figure 2)

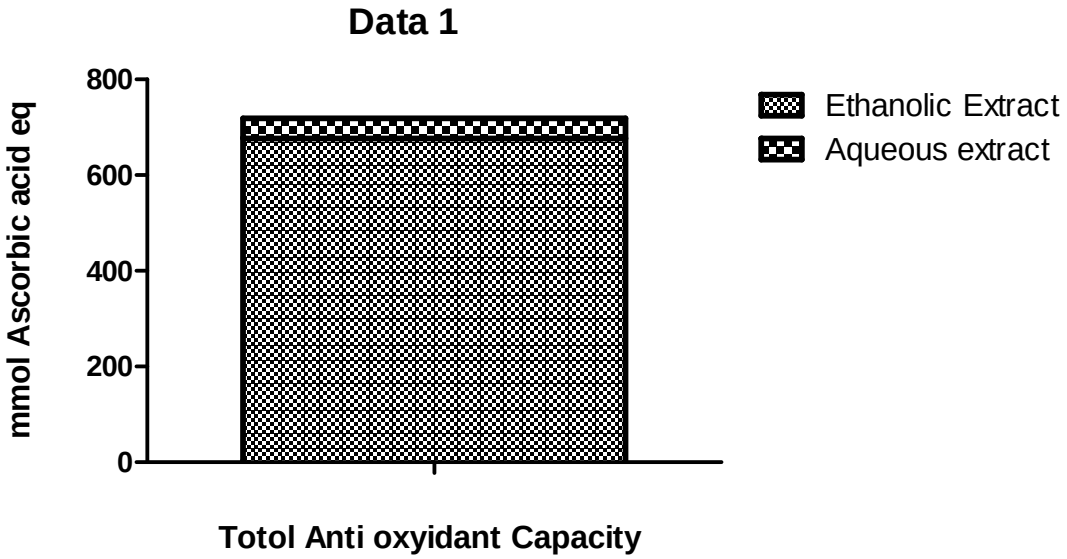

Figure 2: Total antioxidant capacity of extracts of Momordica

It can be observed that ethanolic extract of *Momordica charantia Linn* possesses the highest antioxidant capacity when compared to the aqueous extract of the same plant.

Antioxidant capacity of the plant leaves extract was 676,44±26,19 mmol equivalents of ascorbic acid per mg for the ethanolic extract and 42,23±10,16 19 mmol equivalents of ascorbic acid per mg for aqueous extract. The scavenging action of plant constituents has been found to relate to polyphenolic compounds [29, 30]. Free radicals and reactive oxygen species are involved in a variety of pathological events such as aging, inflammation, cancer, atherosclerosis, diabetes. *M. charantia* Linn would be useful for the treatment of various diseases mediated by free radicals. Overall, the plant would be useful as an antioxidant and free radical scavenging agent and thus help in treatment of many diseases mediated by Reactive oxygen species.

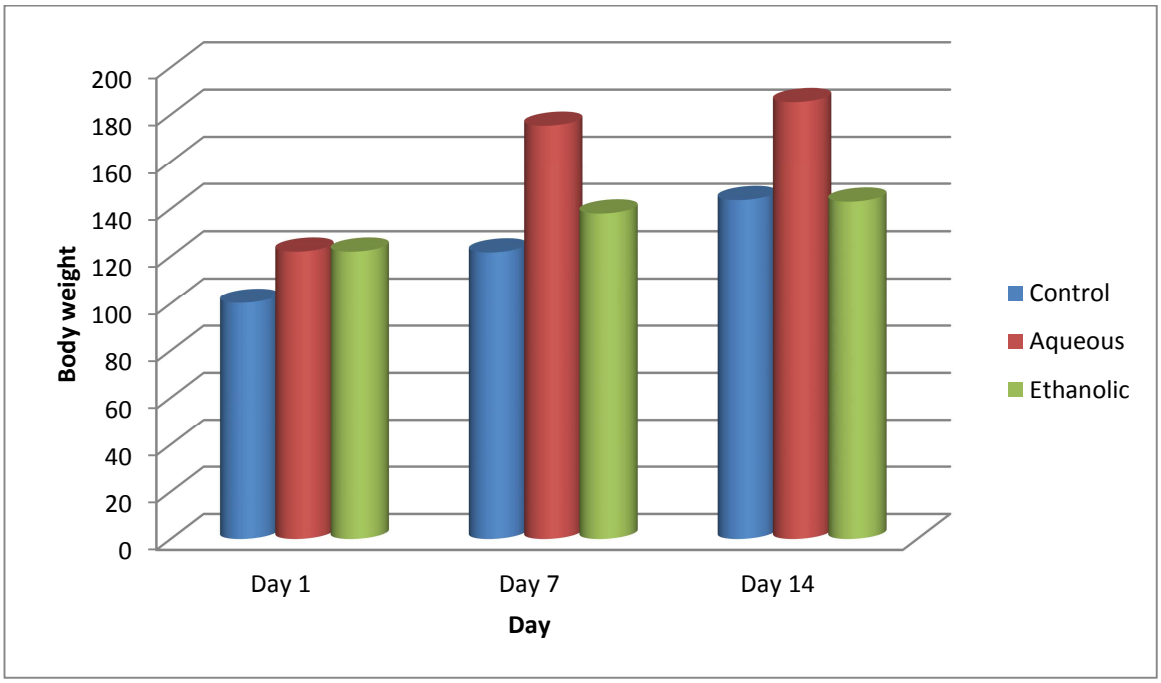

Figure 3 : Effects of *Momordica charantia* on the body weight of rats

After administration of the extracts with the dose of 2000 mg/Kg, we observed that no rats died. This result indicated that the toxicity threshold was higher than the previous concentration administrated to the rats and also the

DL50 is more than 2000 mg/Kg. Figure 3 presented below showed the evolution of animals weight during the fourteen days.

The animals used for this test gained weight like control animals.

In order to appreciate the impact of the extracts on the rats during the acute oral toxicity test, we have measured hematological and biochemical parameters.

Results obtained from different measures of hematological parameters were presented in the table 3. No difference was observed among values obtained from control and experimental animals. The results indicated probably the innocuousness of the plant leaves extract on the metabolic parameters of the rats. These parameters were important in maintaining of the metabolic equilibrium.

Table 3: Hematological parameters in rats

	NGR (T/L)	Plaq (G/L)	NGB (T/L)	Hb (g/dl)	HTE (%)	MCV (fL)	MCHC (%)	TCMH (pg)
Control group	5.22±0.10	821.33±29.7	3.17±0.57	11.4±0,2	32±1.00	62.33±2,52	27.93±0,40	21.8 ±0.40
Aqueous extract group	5.173±0.189	857.33±44.79	4.20±0.30	12.47±1.0	33.00±1.73	64.33±2.52	27.70±1.22	23.20±0.43
Ethanolic extract group	5.40±0.36	818.33±26.69	2.8±0.79	12.07±0.8	33.33±2.08	61.00±1.00	28 .23±0.93	22.50±0.35
P value	5.22±0.10	821.33±29.7	3.17±0.57	11.4±0.2	32±1.00	62.33±2.52	27.93±0.40	21.8 ±0.40

Hemoglobin (Hb) ; (MCV) : mean cell volume; (MCHC); mean cell hemoglobin concentration; Red blood cells count (NGR), white blood cells count (NGB), platelets (PLT), hematocrit (HTE) .For each parameter, no significant difference was found for the dose of 2000 mg/Kg. However, P values were higher than 0.05.

Results obtained from different measures of biochemical parameters are in Table 4. It was observed no difference among values obtained from control and experimental animals

Table 4: Determination of biochemical parameters of rats after treatment with *Momordica charantia Linn* extract

	Creatinine (mg/l)	Urea (g/l)	ALAT (UI/L)	ASAT (UI/L)
Control	7.47±1.10	0.30±0.012	7.47±1.10	0,30±0,012
Aqueous extract	8.60±1.64	0.30±0.04	8.60±1.64	0.30±0.04
Ethanolic extract	7.13±0.23	0 .28±0.03	7.13±0.23	0.28±0.03
P value	P>0.05	P>0.05	P>0.05	P>0.05

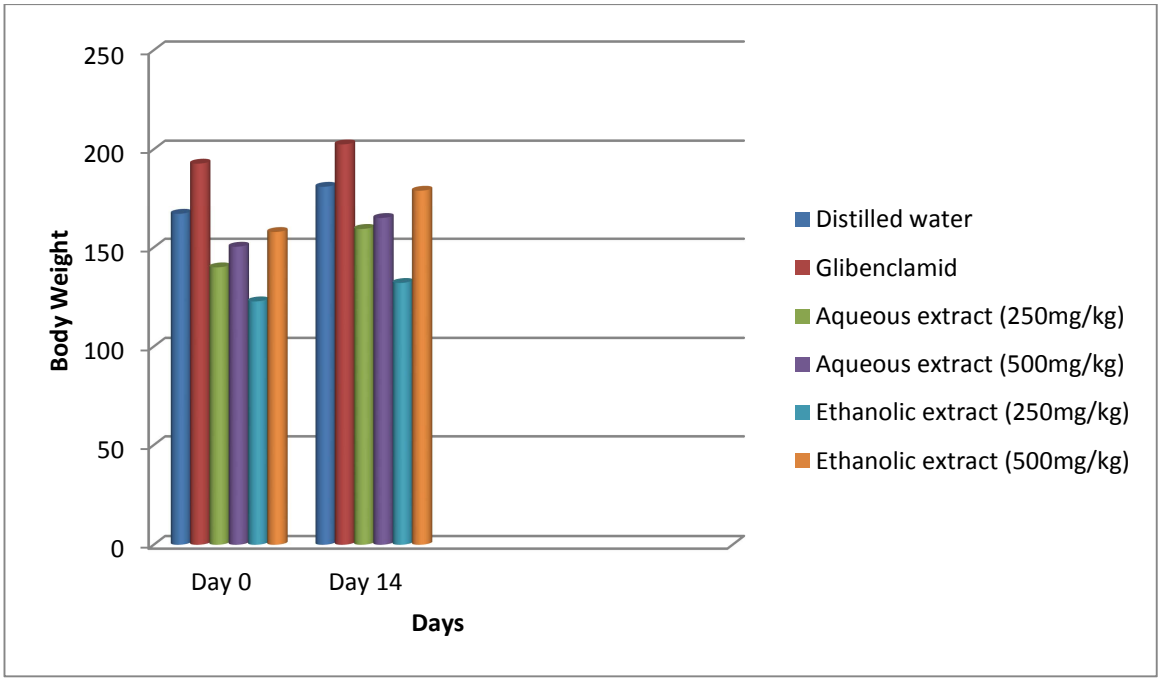

Figure 4: Effects of *Momordica charantia* on the body weights during hypoglycemic test

With a dose of 2000 mg/kg, ethanolic and aqueous extracts showed a non toxicity effect on rats. LD50 of both is higher than 2000 mg/kg. No significant difference was observed as well on biochemical parameters as on hematological parameters. This result could suggest the non destructive effect of this plant on the liver and the kidneys, which can be confirmed by histological dissection of these organs. More other, the findings from this study

aid in providing more informations on the safety level of recommended dosage of ethanolic and aqueous extracts of *M. charantia leaves* for further applications.

Regarding hypoglycemic activity, it was showed that experimental rats body weight gained during the fourteen days of administration of aqueous and ethanolic *M. charantia* leaves extracts (Figure 4).

Decreasing of blood glucose level was observed after administration of different extracts of *Momordica charantia* in two concentrations (500mg/Kg and 1000mg/Kg). The high blood glucose level is observed with distilled water when the low glucose blood level is obtained with ethanolic extract (500mg/kg). (fig 5)

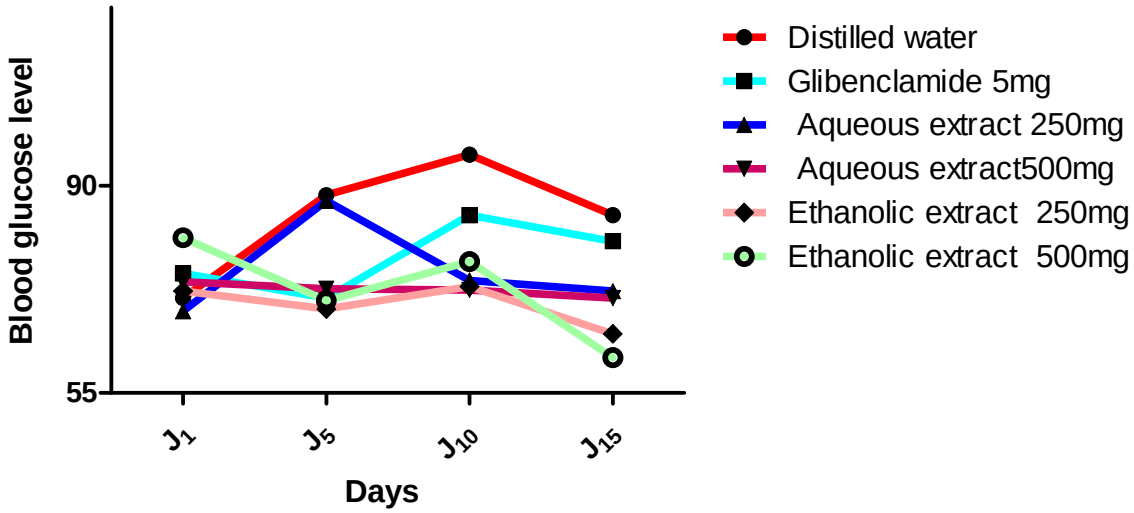

Figure 5: Effect of *Momordica charantia* leaves extracts of blood glucose level (p< 0.05)

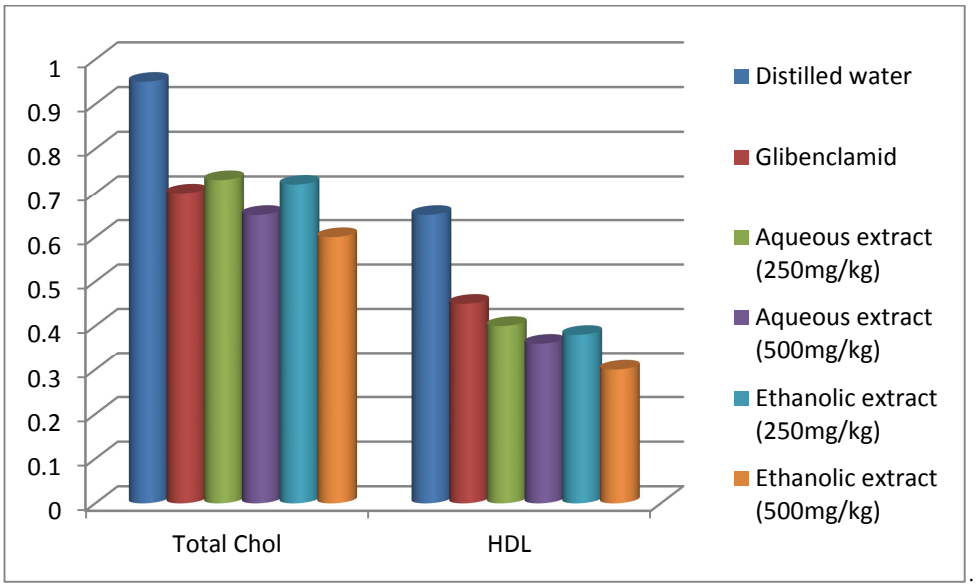

Figure 6: Effect of different extracts and glibenclamid on serum total cholesterol, HDL normal rats (p<0.05)

The results of hypoglycemic test demonstrated that ethanolic and aqueous extracts has a significant hypoglycemic effect in normoglycemic rats and that, the reduction of blood glucose level in normoglycemic rats was found highest in ethanolic leaves extract of *Momordica charantia* as the standard drug (glibenclamid). The hypoglycemic property of *Momordica charantia* could be mediated by some of these active chemical constituents. Indeed, flavonoids, terpens, tannins and coumarins have been shown to possess hypoglycemic activity [31, 32]. The hypoglycemic action of flavonoids was reported to be caused by the stimulation of insulin secretion from pancreatic ß-cells or by an insulin-like effect [31]. It has also been shown that alkaloids possess anti-hyperglycaemic activity that is mediated by the inhibition of α-glucosidase [33]. Phytochemical screening showed the presence of polysaccharides, reducing sugars, and saponins. Hypoglycemic plants are found to contain polysaccharides and the various

experimental results indicate that the polysaccharides increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose [34]. Saponins [35] and flavonoids [36] are also known to possess potent hypoglycemic activity.

Figure 6 presented effect of different extract and glibenclamid on serum total cholesterol, HDL of normal rats after 2 weeks experimental period.

Figure 7 showed that aqueous and ethanolic extract decreased significantly ($p<0.05$) in serum triglycerides level of normal rats. Ethanolic and aqueous leaves extracts of *M. charantia* activity at dose 500mg/kg is better than both extracts and the reference drug activity at dose 250mg/kg.

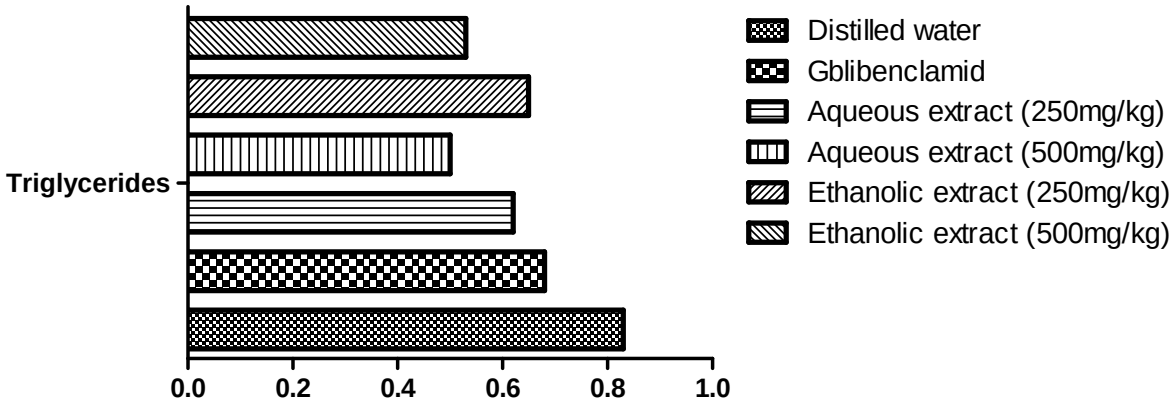

Figure7: Effect of different extracts and glibenclamid on serum triglycerides normal rats

HDL and total cholesterol level were significantly decreased in serum; these results are in agreement with author [37]. On the other hand, HDL-cholesterol level was significantly decreased in serum of rats in the present study as compared to the normal control group (distilled water). This finding parallels that of author [38], and disagrees with author [39].

CONCLUSION

This study indicates that consumption of ethanolic and aqueous extract of *M. charantia* leaves exerts significant hypoglycemic effect in normoglycemic rats. However, it's important to mention that the ethanolic extract gave the most interesting results .These findings support the traditional use of *M. charantia* leaves extracts for controlling diabetics .Therefore, future research will have to be carried out in order to study antidiabetic activity of the plant, to isolate active principle(s) of the extracts as well as to elucidate their exact mechanism(s) of action.

Acknowledgment
The authors thank sincerely the scientific council of University of Abomey-Calavi for financial support through the VPMAS Project. Mr. Abiodoun Pascal OLOUNLADE acknowledges receiving a scholarship from UAC to undergo his Post-Doc studies. This study would not have succeeded without the role played by Traditional Healers from Benin and The staff of.

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