Pharmacognosy Communications
Volume 4 | Issue 3 | July–Sep 2014
www.phcogcommn.org

Original Article

Comparative Proximate Composition and Hypoglycemic Properties of Three Medicinal Plants (*Veronoia amygdalina, Azadirachta indica and Moringa oleifera*).

Ojiako, A.Okey[1] and Chikezie and Paul Chidoka[2]

[1]*Department of Biochemistry, Federal University of Technology, Owerri, Nigeria*
[2]*Department of Biochemistry, Imo State University, Owerri, Nigeria*

ABSTRACT: The present study sought to investigate proximate composition in relation to the short and long term hypoglycemic effects of ethanol/water leaf extracts of *Veronoia amygdalina, Azadirachta indica* and *Moringa oleifera* on hyperglycemic rats. Hyperglycemia was induced by a single intra-peritoneal injection of 0.1 mol/L alloxan monohydrate in phosphate buffer saline (PBS) solution (pH =7.4) dosage = 140 mg/kg; *b/w.* Individual hyperglycemic rats received separate doses of either 400 mg/kg bw/12 h of *V. amygdalina, A. indica* or *M. oleifera* for 14 days. Blood samples were drawn from the rats at regular intervals of 12 h for 48h duration; for short-term study, and subsequently, on days 3, 7, 10 and 14; for long term study, and measured for fasting blood glucose (FBG). Determination of FBG was by the glucose oxidase method. *V. amygdalina, A. indica* and *M. oleifera* showed wide variation in crude protein content (CPC), which was within the range of 13.42–37.01%. Total carbohydrate content (TCC) of *V. amygdalina* and *A. indica* was the single highest component with values: $TCC_{V.\ amygdalina}$ = 40.65% and $TCC_{A.\ indica}$ = 50.14%. In contrast, the CPC content was higher than theTCC contentfor *M. oleifera* ($TCC_{M.\ oleifera}$ = 36.65%; $CPC_{M.\ oleifera}$ = 37.01%). The administration of *V. amygdalina, A. indica* and *M. oleifera* to the rats (0 h " *t* " 48 h) did not cause significant ($p > 0.05$) reduction in FBG of hyperglycemic rats compared to the control group. Conversely, the administration of the three leaf extracts caused reduction in FBG in a time dependent manner in the order: $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$ = 2.73 folds > $H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$ = 2.15 folds > $H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$ = 1.75 folds; $p < 0.05$. Comparative proximate composition of *V. amygdalina, A. indica* and *M. oleifera* and corresponding capacity of the three leaf extracts to reduce FBG appears to suggest that biomolecules responsible for promoting FBG lowering effects are for the most part the TCC and CPC elements.

KEYWORDS: Proximate composition, fasting blood glucose, *Veronoia amygdalina, Azadirachta indica* and *Moringa oleifera.*

INTRODUCTION

Veronoia amygdalina, Azadirachta indica and *Moringa oleifera* are edible and among the readily available medicinal plants grown in the South-Eastern Nigeria. *V. amygdalina* grows throughout tropical Africa and has been domesticated in some parts of West Africa, particularly, South Eastern Nigeria, where it is locally known as bitter leaf.[1] *A. indica* belongs to the family-Meliaceae and has a long history of use in folk medicine for treatment of various ailments.[2] The utilization of *M. oleifera* in the treatment of several ailments such as diarrhea, epilepsy, hysteria and as diuretics has been reported.[3]

Hyperglycemia is often associated with diabetes mellitus (DM); an endocrine disorder with multiple etiologies. DM has been classified into three categories: type I, insulin-dependent diabetes mellitus (IDDM); type II, insulin resistance by peripheral cells (non-insulin-dependent diabetes mellitus (NIDDM), with sub-type II, maturity onset diabetes of the young (MODY); type III,

*Correspondence author:
Chikezie, Paul Chidoka,
Department of Biochemistry, Imo State University,
Owerri, Nigeria
Phone: +2348038935327
E-mail: p_chikezie@yahoo.com
DOI: *10.5530/pc.2014.3.4*

gestational diabetes mellitus (GDM).[4] The IDDM or juvenile-onset DM is more common amongst children/adolescence,[5] arising from absolute absence or low circulating levels of insulin. Insulin insufficiency could be elicited by autoimmune/idiopathic disease,[6,7] viral infection,[6–8] pancreatic carcinoma[9,10] and other complex interactions of both genetic and environmental factors. Type II/NIDDM is characterized by resistance or insensitivity of peripheral cells to insulin[11] typified by hyperinsulinism. In addition to genetic factors, insulin resistance can be caused by acquired factors such as obesity, sedentary life style, pregnancy and hormone exchange. GDM often develops during pregnancy and may improve or disappear after delivery.[12] The majority of the complications of DM are linked to oxidative stress, induced by hyperglycemia, which overwhelms the antioxidant defense systems.[13–15] In the later stage of the disease condition, lipid metabolism is compromised engendering hyperlipidemia. Hypercholesterolemia is particularly pronounced, which is a risk factor in arthrosclerosis.[16,17] Furthermore, distortions in metabolism of protein and amino acids are affected.[18,19] Derangements in general metabolism engender several anatomic distortions, among which are weight loss, ketosis, polyuria, polydipsia, gangrene, cataract, neuropathy and renal dysfunction.[11,20–22] However, distortions in metabolism are restored to normalcy after good glycemic control is obtained.

The global prevalence of DM is on the rise and was estimated to be over 171 million in 2000 with a projection of 366 million in 2030.[23] Type II DM is rapidly emerging as a major public health challenge in Nigeria.[24] In 2003, the International Diabetes Federation (IDF) reported a prevalence of 3.4% for 24 million Nigerians between the ages of 20 and 79 years with a projection of 3.9% by 2025.[24]

Available drugs and insulin injection used for the management of hyperglycemia associated with the diabetic state come with undesirable side effects.[22,25–27] The undesirable side effects and high cost of anti-diabetic drugs have led to the search for plants with hypoglycemic properties for the management of DM.[28,29] Several plant species of medicinal values have been previously investigated for hypoglycemic properties.[11–22,30–34] Therefore, the present study sought to investigate the short and long term hypoglycemic effects of ethanolic extracts of *V. amygdalina, A. indica* and *M. oleifera* on alloxan induced hyperglycemic rats.

MATERIALS AND METHODS

Collection of plant samples and preparation of extract

Fresh leaves of *V. amygdalina, A. indica* and *M. oleifera* were harvested in October, 2012 from the native community of Uke, Idemimiri-North Local Government Area, Anambra State, Nigeria. The plant species were authenticated by Mr. P.C. Nwaiwu, Department of Crop Science, Federal University of Technology, Owerri, Nigeria. The leaves were washed under a continuous current of distilled water for 15 min and air-dried at room temperature (25 ± 5°C) for 2 weeks. The dried leaves were ground separately with a ceramic mortar and pestle. Fifty grams (50 g) of each specimen was suspended in 1.0 L of ethanol/water mixture (2:1; *v/v*) in stoppered flasks and allowed to stand in a water bath at thermostatically controlled temperature of 25 ± 5°C under continuous agitation for 24 h. The suspensions were filtered with Whatman No 24 filter paper. The separate filtrates were concentrated in a rotary evaporator at 50°C and dried in vacuum desiccator. The yields were calculated to be *V. amygdalina* (2.90%; *w/w*), *A. indica* (3.40%; *w/w*) and *M. oleifera* (3.19%; *w/w*). The extracts were finally suspended in phosphate buffered saline (PBS) solution osmotically equivalent to 0.9 g/100 mL NaCI {NaCI (9.0 g), $Na_2HPO_4.2H_2O$ (1.71 g) and $NaH_2PO_4.2H_2O$ (0.243 g)/100 mL; pH =7.4}[33] and stored at 4°C for 24 h before being administered to the rats.

Proximate composition of plant specimen

Dried samples (5 g) of the leaves were measured for moisture, ash, crude fat, crude protein, crude fiber and soluble sugar contents. The determinations were in accordance with the official methods of the Association of Official Analyses Chemist.[35] Measurement of moisture content (MC) was by weighing the samples in crucible and drying in oven at 60°C until constant weight was obtained. The ash content (AC) was measured by incinerating the samples in oven at 105°C for 3 h and the weight of the residue was determined after cooling in a desiccator. The crude fat content (CFC) was measured by the soxhlet extraction methods, whereas crude protein content (CPC) was by the Kjedahl methods. Crude fiber content (CFBC) was determined by digestion methods. Total carbohydrate content (TCC) was calculated by difference of the sum of all the other proximate composition from 100%.

Experimental animals

Male albino Wister rats weighting between 170-220g were obtained from the Animal Unit of the Faculty of Pharmaceutical Science, University of Nigeria, Nsukka, Nigeria. The rats were fed with standard commercial feed (SCF) (Ewu Feed Mill, Edo State, Nigeria) and water, in a well-ventilated stainless steel cages. After randomization on weight bases, the rats were allowed to acclimatize for 7 days at an ambient room temperature of 25 ± 5°C, 30–55% of relative humidity on a 12-h light/12-h dark cycle. Animal handling was in accordance with the stan-

dard principle of laboratory animal care of the United State National Institute of Health.

Induction of hyperglycemia and study design

Hyperglycemia was induced in 12 h fasted animals by intra-peritoneal injection of 0.1 mol/L alloxan monohydrate (Sigma, St. Louis, USA) in phosphate buffer saline (PBS) solution (pH =7.4) at a dose of 140 mg/kg; *b/w*. Hyperglycemia was confirmed in rats 72 h after alloxan treatment. The rats with fasting blood glucose (FBG) >11.0 mmol/L were considered to be hyperglycemic and selected for the study.

A total of thirty (30) rats were divided into five (5) groups of six ($n = 6$) each as follows:

- Group C1; Control-Normal (C/N): Normal rats received only PBS + SCF (Vehicle; 1.0 mL/kg; *b/w*/12 h, i. p.) for 14 days.
- Group C2; Control-Hyperglycemic (C/H): Hyperglycemic rats received PBS + SCF (Vehicle; 1.0 mL/kg; *bw*/12 h, i. p.) for 14 days.
- Group T1; $H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$: Hyperglycemic rats received *V. amygdalina* + SCF (400 mg/kg; *bw*/12 h, i. p.) for 14 days.
- Group T2; $H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$: Hyperglycemic rats received *A. indica* + SCF (400 mg/kg; *bw*/12 h, i. p.) for 14 days.
- Group T3; $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$: Hyperglycemic rats received *M. oleifera* + SCF (400 mg/kg; *bw*/12 h, i. p.) for 14 days.

Measurement of fasting blood glucose

Blood samples were drawn from the tip of the tail of the rats at regular intervals of 12 h for 48h duration; for short-term study, and subsequently, on days 3, 7, 10 and 14; for long term study, and measured for fasting blood glucose (FBG). Determination of FBG was by the glucose oxidase method according to the Randox® kit manufacturer's procedure (Randox® Laboratories Ltd. Ardmore, United Kingdom).

CALCULATIONS

Cumulative FBS per h (%FBS h^{-1}) within the short- and long-term treatments were evaluated using the Simpson's Rule. Thus:

$$f(X_1)h_1 + f(X_2)h_2 + \ldots + f(X_n)h_n$$

Area under the curve (AUC) of the plot of %FBS versus time (h) is given by:

$$\text{AUC(\%FBS } h^{-1}) = \frac{h}{(2)(y_2 + 2y_{n-1} + 2y_{n-2} + 2y_{n-3} + \ldots)}$$

Equation 1

Where:

h = time intervals in hours.

y = %FBS at corresponding time interval.

Measurement of body weight

The weights of the rats in the various experimental groups were measured with electronic weighing balance at days 0, 3, 7, 10 and 14.

Statistical analyses

The data collected were expressed in means (X) ± SD and analyzed in one-way ANOVA and Least Significance Difference (LSD). The comparison was made between groups and significance was established by ANOVA at 95% confidence level. Difference of $p < 0.05$ was considered statistically significant.

RESULTS

Figure 1 showed the comparative proximate composition of dried samples of *V. amygdalina, A. indica* and *M. oleifera*. MC of *V. amygdalina, A. indica* and *M. oleifera* was within the range of 9.21–10.29% ($p > 0.05$). Leaf samples of *A. indica* gave the highest AC, representing 10.93% of dried matter. The lowest was $AC_{M.\ oleifera}$ =7.93%, whereas $AC_{A.\ indica}$ = 10.93% gave the highest value among the three samples. CFC of the three plant samples were in the order: $CFC_{V.\ Amygdalina}$ = 9.05% >$CFC_{A.\ indica}$ = 5.17% >$CFC_{M.\ oleifera}$ = 2.11%. *V. amygdalina, A. indica* and *M. oleifera* showed wide variation in CPC, this was within the range of 13.42–37.01%. The CFBC of *V. amygdalina* was not significantly different ($p > 0.05$) from *A. indica*; $CFBC_{V.\ amygdalina}$ = 12.08% and $CFBC_{A.\ indica}$ = 10.11%. TCC of *V. amygdalina* and *A. indica* was calculated to be the single highest component with values: $TCC_{V.\ amygdalina}$ = 40.65% and $TCC_{A.\ indica}$ = 50.14%; except $TCC_{M.\ oleifera}$ = 36.65%, which was approximately equal to $CPC_{M.\ oleifera}$ = 37.01% (Figure 1).

Short-term investigation of FBG, i.e., within experimental time of 48 h (0 h ≤ t ≤ 48 h), showed that FBG of group C/N (C/N_{FBG}) was relatively constant (Table 1). Also, Table 1 showed that C/H_{FBG} was elevated and significantly different ($p < 0.05$) from C/N_{FBG} with values within the range of 14.83 ± 3.00–16.95 ± 0.05 mmol/L. In addition, Table 1 indicates that C/H_{FBG} showed a marginal increment with progression of experimental time

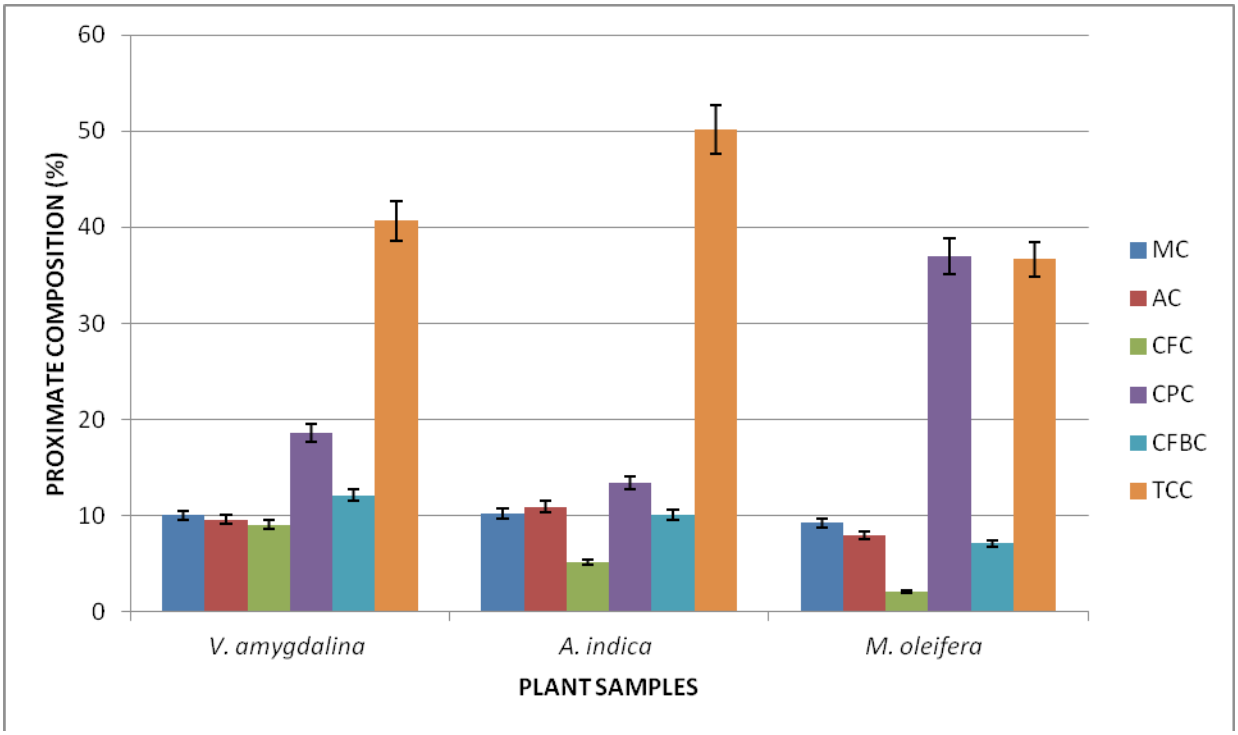

Figure 1. Proximate composition (%) of *V. amygdalina, A. indica* and *M. oleifera*. MC: moisture content; AC: ash content; CFC: crude fat content; CPC: crude protein content; CFBC: crude fiber content; TCC: Total carbohydrate content.

Table 1: Short-term effect of *V. amygdalina, A. indica and M. oleifera* on FBG.

	FBG (mmol/L)				
Time (h)	C/N	C/H	$H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$	$H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$	$H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$
0	5.00 ± 0.25^{a}	14.83 ± 3.00^{b}	$14.73 \pm 1.49^{b,c}$	$14.98 \pm 1.53^{b,c,d}$	$14.86 \pm 0.83^{b,c,d,e}$
12	5.02 ± 0.03^{a}	15.56 ± 2.88^{b}	$15.32 \pm 0.51^{b,c}$	$15.03 \pm 1.35^{b,c,d}$	$13.91 \pm 0.44^{b,c,d,e}$
24	5.07 ± 0.26^{a}	15.37 ± 3.12^{b}	$14.99 \pm 0.29^{b,c}$	$14.23 \pm 1.67^{b,c,d}$	$13.58 \pm 0.46^{b,c,d,e}$
36	5.01 ± 0.19^{a}	16.65 ± 2.31^{b}	$13.52 \pm 0.87^{b,c}$	$13.39 \pm 0.97^{b,c,d}$	$12.15 \pm 0.70^{b,c,d,e}$
48	5.00 ± 0.20^{a}	16.95 ± 0.05^{b}	$12.42 \pm 0.72^{b,c}$	$12.97 \pm 0.48^{b,c,d}$	$11.81 \pm 0.51^{b,c,d,e}$

The results are means (X) ± SD of six (n = 6) determination; Means in the row with the same letter are not significantly different at $p > 0.05$ according to LSD.

($p > 0.05$), representing 14.30% increase in FBG. The administration of *V. amygdalina, A. indica* and *M. oleifera* to the rats ($0\ h \leq t \leq 48\ h$) did not cause significant ($p > 0.05$) reduction in FBG of hyperglycemic rats compared to the group C/H. Thus, FBG of groups $H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$, $H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$ and $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$ were significantly ($p < 0.05$) elevated compared to C/N_{FBG}. It is noteworthy that within the experimental time ($0\ h \leq t \leq 48\ h$), FBG of groups C/H, $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$, $H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$ and $H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$ were comparatively over 2 folds $>C/N_{FBG}$ (Table 1 and Figure 2). However, the capacities of the three leaf extracts to cause slight reduction in FBG, as shown in Figure 2, was in the order: $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw} > H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw} > H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$; $p > 0.05$.

Figure 2 showed that at $t = 48$ h, C/H_{FBG} increased by 14.30%, whereas hyperglycemic rats treated with extracts of *V. amygdalina, A. indica* and *M. oleifera* showed reduced FBG by 15.68%, 13.42% and 20.52% respectively, compared to their FBG at $t = 0$ h. Comparatively, the capacity of extracts of the three medicinal plants to lower FBS in hyperglycemic rats was in the order: $AUC_{H[M.\ oleifera]\ =\ 400\ mg/kg;\ bw} = 78.50 \times 10^{2}\ \%FBS\ h^{-1} > AUC_{H[A.\ indica]\ =\ 400\ mg/kg;\ bw} = 87.14 \times 10^{2}\ \%FBS\ h^{-1} > AUC_{H[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw} = 89.08 \times 10^{2}\ \%FBS\ h^{-1}$; $p > 0.05$, within the 48 h experimental time.

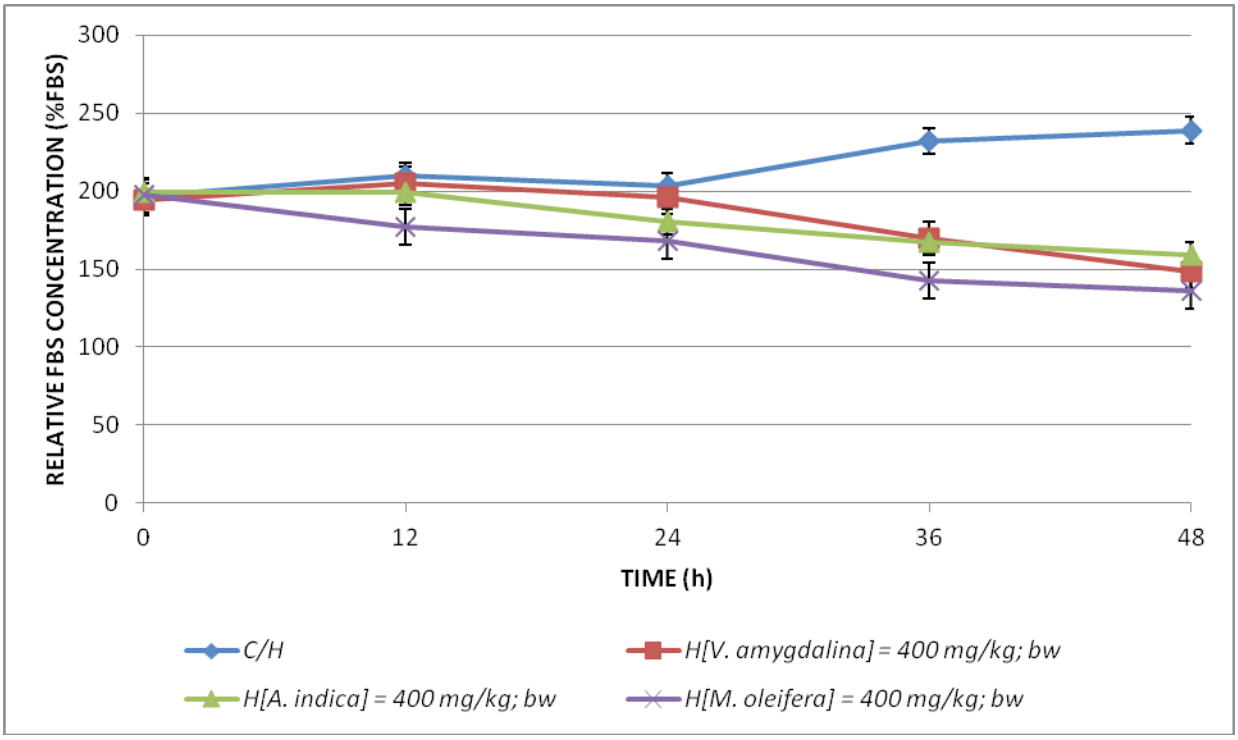

Figure 2. Comparative FBG levels of hyperglycemic rats on short-term administration of *V. amygdalina, A. indica* and *M. oleifera;* (0 h ≤ t ≤ 48 h): $AUC_{C/H}$ = 103.60 × 10^2 %FBS h^{-1}; $AUC_{H[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$ = 89.08 × 10^2 %FBS h^{-1}; $AUC_{H[A.\ indica]\ =\ 400\ mg/kg;\ bw}$ = 87.14 × 10^2 %FBS h^{-1}; $AUC_{H[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$ = 78.50 × 10^2 %FBS h^{-1} (Equation 1).

Table 2: Long-term effect of *V. amygdalina, A. indica* and *M. oleifera* on FBG.

	FBG (mmol/L)				
Time (day)	C/N	C/H	$H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$	$H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$	$H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$
0	5.00 ± 0.25^{a}	14.83 ± 3.00^{b}	14.73 ± 1.49^{b,c}	14.98 ± 1.53^{b,c,d}	14.86 ± 0.83^{b,c,d,e}
3	4.99 ± 0.40^{a}	18.58 ± 1.19^{b}	12.57 ± 0.53^{b,c}	12.53 ± 0.50^{b,c,d}	10.93 ± 0.78^{c,d,e}
7	5.00 ± 0.19^{a}	19.83 ± 0.36^{b}	10.46 ± 0.64^{c}	10.23 ± 0.22^{c,d}	8.48 ± 0.54^{c,d,e}
10	4.94 ± 0.13^{a}	21.42 ± 0.82^{b}	9.03 ± 0.71^{c}	9.74 ± 0.34^{c,d}	6.96 ± 0.32^{a,c,d,e}
14	4.99 ± 0.11^{a}	27.64 ± 0.94^{b}	6.86 ± 1.16^{a,c}	8.56 ± 1.04^{c,d}	5.44 ± 0.20^{a,c,d,e}

The results are means (X) ± SD of six (n = 6) determination; Means in the row with the same letter are not significantly different at p > 0.05 according to LSD.

Long-term investigation of FBG i.e., within experimental time of 14 days, showed that C/N_{FBG} was relatively stable with values between 4.94 ± 0.13–5.00 ± 0.25 mmol/L (Table 2). C/H_{FBG} was significantly (p < 0.05) elevated compared to C/N_{FBG}. Within the 14-day duration, C/H_{FBG} increased by 2 folds approx. Conversely, the administration of the three leaf extracts caused reduction in FBG in a time dependent manner, which was in the order: $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$ = 2.73 folds >$H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$ = 2.15 folds >$H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$ = 1.75 folds; p < 0.05 (Table 2). FBG of hyperglycemic rats was restored to values comparable to C/N_{FBG} in groups $H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$; at t = 14th day (p > 0.05) and $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw}$; at t = 10th and 14th day (p < 0.05), whereas FBG of $H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$ was 71.5% above C/N_{FBG} at t = 14th day (p < 0.05) (Figure 3).

(Figure 4) showed that group C/N gained relatively maximum weight at the end of experimental time of 14 days by 6.58%. In contrast, group C/H exhibited weight loss by 6.74%. Hyperglycemic rats administered with the three leaf extracts exhibited an increase in relative body weight but with values lower than the group C/N. Notably, $H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw}$ and $H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw}$ showed weight loss on day 10, after which maximum weight gain occurred on day 14. Specifically, the weight gained by hyperglycemic rats treated

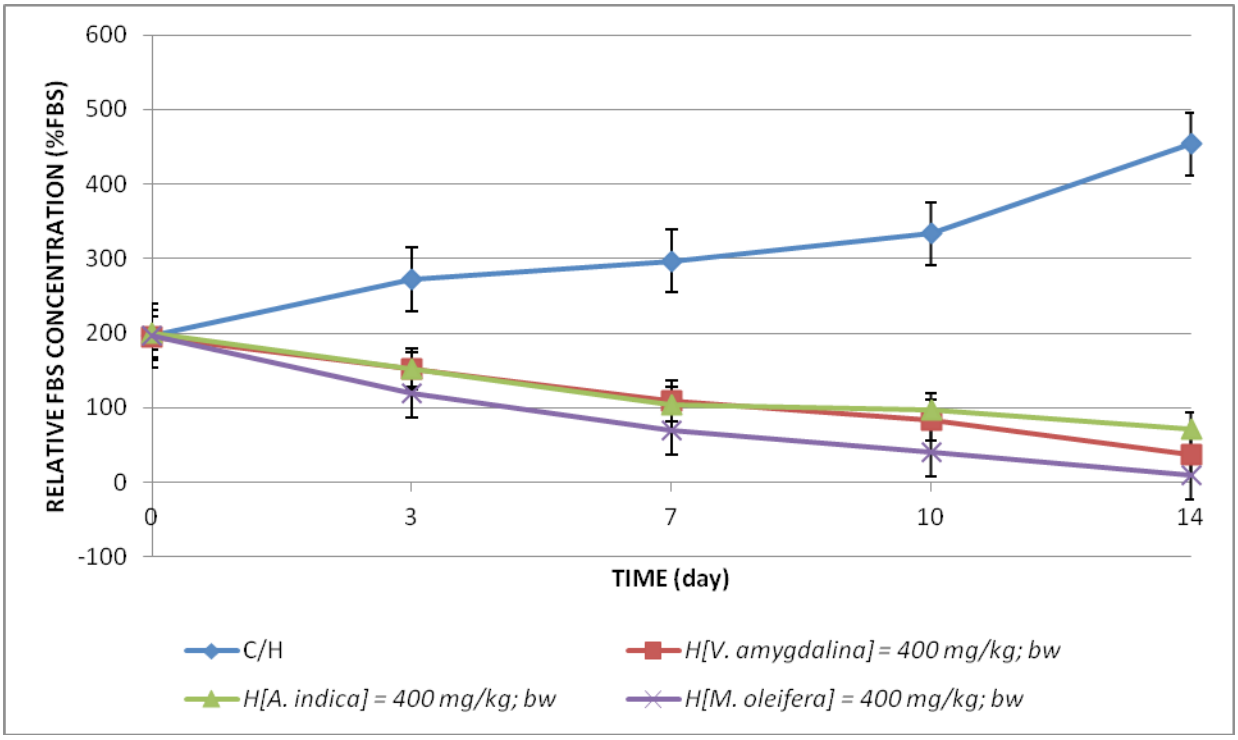

Figure 3. Comparative FBG levels of hyperglycemic rats on long-term administration of *V. amygdalina, A. indica* and *M. oleifera.* (0 h ≤ t ≤ 336 h): $AUC_{C/H}$ = 104.67 × 10^3 %FBS h^{-1}; $AUC_{H[V.\ amygdalina] = 400\ mg/kg;\ bw}$ = 37.69 × 10^3 %FBS h^{-1}; $AUC_{H[A.\ indica] = 400\ mg/kg;\ bw}$ = 40.25 × 10^3 %FBS h^{-1}; $AUC_{H[M.\ oleifera] = 400\ mg/kg;\ bw}$ = 26.81 × 10^3 %FBS h^{-1} (Equation 1).

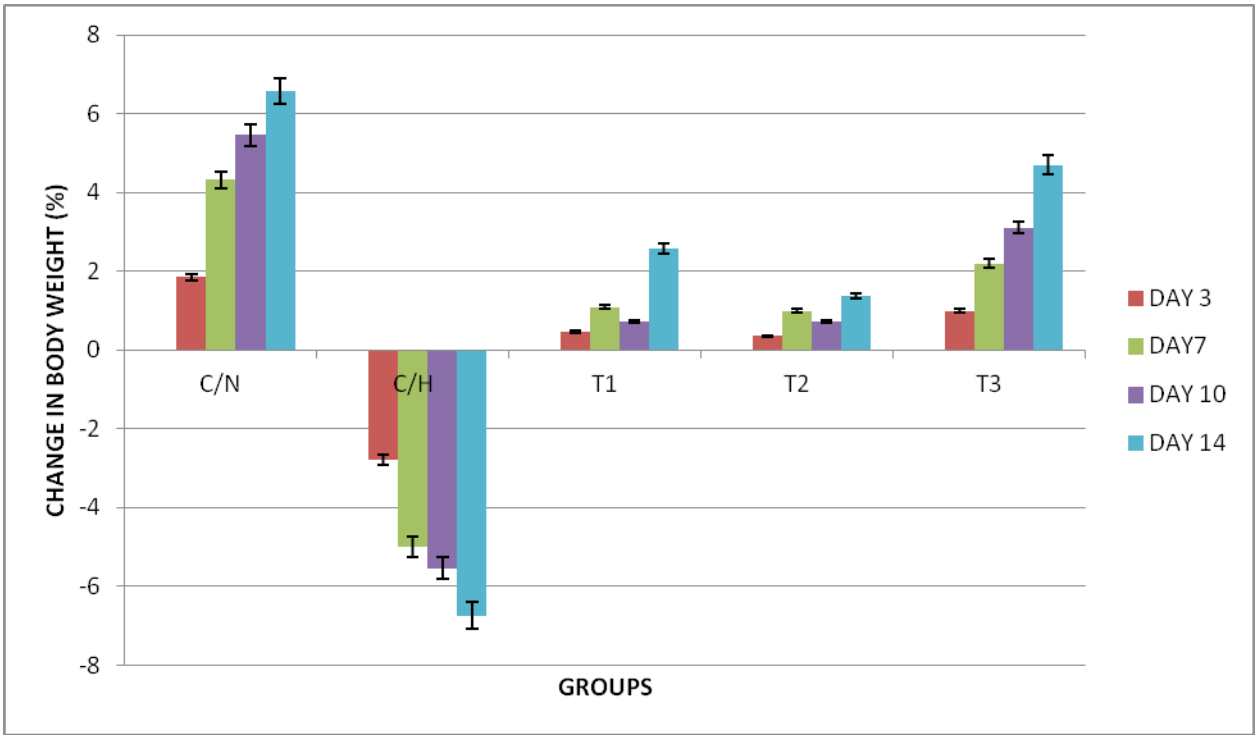

Figure 4. Comparative body weights of normal and hyperglycemic rats on long-term administration of *V. amygdalina, A. indica* and *M. oleifera.*

with the three leaf extracts at the end of the experimental time were as follows: $H_{[M.\ oleifera]\ =\ 400\ mg/kg;\ bw} = 4.70\% > H_{[V.\ amygdalina]\ =\ 400\ mg/kg;\ bw} = 2.59\% > H_{[A.\ indica]\ =\ 400\ mg/kg;\ bw} = 1.38\%$.

DISCUSSION

Although certain plants contain noxious compounds, there are several therapeutic benefits derivable from the application of plant matter for medicinal purposes and general well-being. Previous reports have attributed the \ hypoglycemic properties of the leaf extracts to polysaccharides, proteins, alkaloids, flavonoids and related compounds.[36] Other nutritive compounds and phytochemicals which have been implicated in possessing hypoglycemic properties include terpenoids, phenolics, amino acids and related nitrogenous compounds.[37–40] The hypoglycemic properties of these compounds have been evaluated and demonstrated in animal models[41,42] as well as in humans.[43] For instance, the amino acid derivative, S-methyl cysteine sulphoxide and S-alkyl cysteine sulphoxide content in *Allium cepa* (Onion),[44] *Allium sativum* (Garlic)[45] stimulates insulin secretion, whereas Charantin (a peptide)[46] inhibits hepatic glycogenolysis. Lipid components such as phytosterols[47] stimulate insulin biosynthesis and secretion; β-sitosterol[6–48] suppresses glucose-6-phosphatase and promotes glycogen biosynthesis with reduced phosphorylase activity. Other phytochemicals such as tannins,[49,50] saponins glycosidic component[38] and pectin materials[51] act by suppressing gluconeogenic enzymes and stimulate insulin secretion. Proximate compositions of the three plant specimen (Figure 1) indicated that *V. amygdalina, A. indica* and *M. oleifera* are comparatively good sources of these bioactive agents that are capable of exerting glycemic control. Specifically, comparative analyses showed remarkable high TCC in *V. amygdalina*, *A. indica* and *M. oleifera* in combination with CPC in *M. oleifera*.

Another hypoglycemic potential of medicinal plants are attributable to trace elements present in them. Previous studies have shown that administration/intake of micronutrients are effective preventive and treatment measures for both type I and type II DM and common associated complications. Narendhirakannan *et al.,*[52] reported the roles of inorganic trace elements present in four traditional medicinal plants (*Murraya koenigii, Mentha piperitae, Ocimum sanctum*, and *Aegle marmelos)* used in the treatment of DM. Also, Rajasekaran *et al.,*[53] noted that inorganic elements like vanadium, zinc, sodium, potassium, calcium, copper, manganese, and traces of chromium zinc and chromium are required to correct glucometabolic disorders associated with DM. Trace elements are integral components of enzymatic reactions where they act as cofactors. Notable enzymatic reactions are those involving chromium dependent activation of insulin receptor signaling and the cofactor antioxidant activity of selenium in glutathione peroxidase[54] involve in scavenging multiple reactive oxygen species associated with the diabetic states.[55–56] In addition, low magnesium levels have been associated with increased type II diabetes.[57–59] Results of comparative proximate composition of the three plants showed appreciable AC (Figure 1), which suggest that *V. amygdalina*, *A. indica* and *M. oleifera* contain relatively rich quantity of inorganic trace elements posited to possessing glycemic control property. In similar manner, CFBC of *V. amygdalina*, *A. indica* and *M. oleifera* advance the view that the plants products are agents of glycemic control. A high fiber content in diet engendered profound lowering of plasma glucose concentration in DM.[60] Likewise, dietary fiber increases viscosity of gastrointestinal tract, thereby suppressing carbohydrate absorption.[61] This mechanism is selectively advantageous in that the risk of hyperglycemia and repercussive hyperinsulinism is controlled. Based on the inferences of the present study, it is worthwhile to note that the mixtures and combinations of these bioactive principles and essential elements act in synergy to exert glycemic control.

Short-term administration of some herbal extracts have been previously reported to exhibit little or marginal efficacy in hyperglycemic rats.[33,62–63] In another study, a methanol/water extract of *Phaleria macrocarpa* fruits caused non-significant ($p > 0.05$) blood glucose lowering effect between 15 and 120 min, which was in contrast with the application of methanol extract of the same fruit.[64] This observation may not be unconnected with the type and nature of the bioactive principles present in the two extracts used in their study. Different bioactive principles in leaf extracts possess different mode of blood glucose lowering activity that determines the potency and duration required to restore therapeutic benefits. From the present study, the observed low efficacy (Table 1) following the administration of ethanol/water extracts of *V. amygdalina*, *A. indica* and *M. oleifera* to hyperglycemic rats for 48 h, was an obvious indication that the extracts were not fast acting anti-hyperglycemic preparations. However, the results as presented in Table 1indicate that the three extracts elicited slight short-term efficacy in reducing elevated levels of FBG. Therefore, the capacity of extracts of *V. amygdalina, A. indica* and *M. oleifera* to induce reduction in levels of FBG was not comparable to the fast acting subcutaneous insulin injection and the oral sulfonylureas, biguanides and glinides.[34–65] The mechanisms of action of these drugs include: reduction in hepatic glucose release, enhancement of insulin secretion and sensitivity as well as inhibition of intestinal glucose absorp-

tion.[66] Although the present study has shown that extracts of *V. amygdalina, A. indica* and *M. oleifera* caused marginal short-term efficacy, oral administration of ethanolic extract of *Berberis lyceum* **(**Royle) has previously been demonstrated to possessing substantial short-term acting capacity to ameliorate elevated blood glucose in alloxan induced diabetic rats within 180 min.[67] They further noted that ethanol extracts of *B. lyceum* yielded slightly better results compared to equivalent aqueous extracts. Likewise, rats fed by gavage with 500 and 1000 mg/kg/day of *Viscum album* extract for 72 h caused significant ($p < 0.05$) reduction in blood glucose and increased antioxidant power of alloxanized rats.[68] These findings indicate that the route and mode of drug administration alters the level of drug delivery and efficacy.

The present findings showed that long-term administration of extracts of *V. amygdalina, A. indica* and *M. oleifera,* in its present form, caused profound but variable capacity to reduce FBG in hyperglycemic rats at the end of 14 days treatment (Table 2), which agreed with the findings of Nagappa *et al.,*[50] on antidiabetic activity of *Terminalia catappa linn* fruits. This was an indication that the leaf extracts acted by eliciting re-adjustments in the distorted haemostasis of hyperglycemic rats through mechanisms that are relatively of the delayed type. Such mechanisms include promoting biosynthesis of enzymes and structural molecules required for regeneration and restoration of the functional integrity of insulin secreting cells (Islet of Langerhans) in concert with quick acting mechanism of insulin mimicking.[40,69–70] The leaves of *Gymnema sylvestre* (Gumer)[71] and the fruits of *T. catappa* Linn[50] induced β-cell regeneration in simultaneous with β-cell stimulation and suppression of gluconeogenic enzymes[72] that corroborates the plausible mode of action of *V. amygdalina, A. indica* and *M. oleifera* extracts. From another perspective, Ugochukwu and Babady,[73] reported that the long-term hypoglycemic property of *Gongronema latifolium* extract was mediated by activation of hepatic hexokinase, phosphofructokinase, glucose-6-phosphate dehydrogenase in diabetic rats. In furtherance to these findings, Ngozi *et al.,*[74] posited that the glycemic control mechanism of *G. latifolium* could be linked to the capability of the extract to increase cellular ratio of reduced glutathione to oxidized glutathione (GSH/GSSG). Thus, their findings were in concord with previously reported tendency of *G. latifolium*[75] and oil of *Eruca sativa* seeds[76] to ameliorate oxidative stress associated with the diabetic state.

Comparative proximate composition of *V. amygdalina, A. indica* and *M. oleifera* and corresponding capacity of the three leaf extracts to reduce FBG appears to suggest that biomolecules responsible for promoting FBG lowering effects are for the most part the TCC and CPC elements, which might have acted in synergy with other anti-diabetogenic components. The improvement in body weight of hyperglycemic rats treated with the leaf extracts, as against weight loss in the group C/H further confirmed nutraceutical benefits as previously reported.[14,22–77]

REFERENCES

1. Igeile GO, Oleszeki W, Fafunso M. Hypoglycemic assessment of *Vernomia amygdalina* leaves in growing mice. J Agric Food Chem. 1995; 43: 2162–6.
2. National Research Council (NRC). Neem: a tree for solving global problems. National academic Press, Washington DC. 1992; 23–39.
3. Fugie IJ. The miracle tree: *Moringa oleifera*: National Institute for Tropic: Church World Service, Dakar. 1999; 68.
4. Velho G, Froguel P. Maturity-onset diabetes of the young (MODY), MODY genes and non-insulin dependent diabetes mellitus. Diabetes. 2002; 23: 34–43.
5. Pietropalo M. Pathogenesis of diabetes: our current understanding. Clinical Cornerstone Diabetes. 2001; 4(2): 1–21.
6. Kumar V, Cotran RS, Robbins J. Basic pathology.5th edition. Harcourt Brace and Company, Philadelphia. 1992; 570–3.
7. Kukreja A, Maclaren NK. Autoimmunity and diabetes. J Clin Endocrinol Metabol. 1999; 84: 4371–8.
8. Filippi CM, von Herrath MG. Viral trigger for type 1 diabetes pros and cons. Diabetes. 2008; 57: 2863–71.
9. Wakasugi H, Funakoshi A, Iguchi H. Clinical observations of pancreatic diabetes caused by pancreatic carcinoma, and survival period. Int J Clin Oncol. 2001; 6(1): 50–4.
10. Trna J, D´ıt˘e P, Adamcov´a A, Crawford BJ, Hermanov M. Diabetes mellitus in pancreatic cancer patients in the Czech Republic: sex differences. Experi Diabetes Res. 2012; Article ID 414893, 5 pages. doi:10.1155/2012/414893.
11. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Anti-diabetic agents from medicinal plants. Curr Med Chem. 2006; 13: 1203–18.
12. Mayfield J. Diagnosis and classification of diabetes mellitus. New criteria. Bowan Research Center, Indiana University, Indianapolis, Indiana. 1998.
13. Udoh EA, Iya N, Okon E, Mary N. Red cell catalase activity in diabetes. Pak J Nutri. 2007; 6: 511–15.
14. Dewanjee S, Bose SK, Sahu R, Mandal SC. Antidiabetic effect of matured fruits of *Diospyros peregrina* in alloxan induced diabetic rats. Int J Green Pharma. 2008; 2: 95–9.
15. Rajeshwari CU, Andallu B. Oxidative stress in NIDDM patients: influence of coriander (*Coriandrum sativum*) seeds. Res J Pharmaceuti Biol Chem Sci. 2011; 2(1): 31–41.
16. Ross RN. Mechanism of disease: arthrosclerosis-an inflammatory disease. New Engl J Med. 1999; 340: 115–26.
17. Schwartz SI. Diabetes and dyslipidemias. 2006; 8: 355–64.
18. Ohtsuka Y, Agishi Y. Abnormal amino acid metabolism in diabetes mellitus. Nihon Rinsho. 1992; 50(7): 1631–6.
19. Tessari P, Cecchet D, Cosma A, Puricelli L, Millioni R, Vedovato M, *et al.* Insulin resistance of amino acid and protein metabolism in type II diabetes. Clin Nutri. 2011; 30(3): 267–72.
20. Baskin DG, Lattemann DF, Seeley RJ. Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. Brain Res. 1999; 848: 114–23.
21. Tortora GJ, Derrickson B. Principal of anatomy and physiology, 11th Edition. United State. John Wiley and Sons Inc. 2006; 669.
22. Sharma US, Kumar A. Anti-diabetic effect of *Rubus ellipticus* fruits extracts in alloxan-induced diabetic rats. J Diabetol. 2011; 2: 4.
23. Wild S, Roglic G, green A. Global prevalence of diabetes: estimate for the year 2000 and projection for 2030. Diabetes Care. 2004; 27(5): 1047–53.
24. Rheeder P. Type II diabetes: the emerging epidemic. Nigerian Family Practice. 2006; 48(10): 20.
25. Peidrola G, Noro E, Escober F. White blood cell count and insulin resistance with patient with coronary disease. Ann Endocrinol. 2001; 62: 7–10.

26. Yargina-Tobias JA, Pinto A, Neziroghlu F. Anorexia nervosa, diabetes mellitus, brain atrophy and fatty liver. Int J of Eating Disorders. 2001; 30: 350–3.
27. Gardhipuram PSK, Durairaji SK, Sorimuthu PS. Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin-induced diabetic rats. J Health Sci. 2006; 54: 283–91.
28. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medication. (Phytotherapeutic agents). Brazilian J Med Biol Res. 2000; 33(2): 179–89.
29. WHO. Traditional medicine strategy 2002–2005. WHO Publications. 2002; 66.
30. Mentreddy SR, Mohamed AI, Rimando AM. Medicinal plants with hypoglycemic/anti-hyperglycemic properties: a review. Proc Assoc Adv Industrial Crop Conf. 2005; 20: 341–53.
31. Brai BIC, Odetola AA, Agomuo PU. Anti-hyperglycemic activity of *Cassia kleinii* leaf extract in normal rats and alloxan-induced diabetic rats. Indian J Pharmacol. 2007; 34: 409–15.
32. Gondwe M, Okoro PK, Juta R. Effects of diabetes on kidney. Diabetes. 2008; 52: 283–91.
33. Ibegbulem CO, Chikezie PC. Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium*, *Aloe perryi, Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (*Rattus norvegicus*). Phcog. Commn. 2012; 3(2): 12–16.
34. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific J Trop Biomed. 2012: 320–30.
35. AOAC. Official methods of analyses. 21st Edition. Association of Official Analytical Chemist. Washington DC. USA. 1999.
36. Ivorra MD, Paya M, Villar A. A view of natural products and plants as potential anti-diabetic drugs. J Ethnopharmacol. 1989; 27: 243–75.
37. Marles RJ, Farnsworth NR. Anti-diabetic plants and their active constituents. Phytomed 1995; 2: 137–89.
38. Grover JK, Yadav S, Vates V. Medicinal plant of Indian of anti-diabetic potentials. Journal of Ethnopharmacology. 2002; 81: 81–90.
39. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity- a review of ten years of herbal medicine research (1990–2000). Int J Diabetes Metabol. 2006; 14: 1–25.
40. Prabhakar PK. Doble M. A target based therapeutic approach towards diabetes mellitus using medicinal plants. Curr Diabetic Rev. 2008; 4: 291–308.
41. Gupta R, Gabrielsen B, Forgueon FM. Nature's medicine: Traditional knowledge and intellectual management. Case study from the National Institute of Health (NIH), USA. Curr Drug Discovery Technol. 2005; 2: 203–19.
42. Kesari AN, Gupta RK, Singh SK, Diwakar S, Watal G. Hypoglycemic and anti-hyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats. J Ethnopharmacol. 2006; 107: 374–9.
43. Jayawardena MHS, de Alwis NMW, Hettigoda V, Fernando DJS. A double blind randomized placebo controlled cross over study of herbal preparation containing *Salacia reticulta* in the treatment of type II diabetes. J Ethnopharmacol. 2005; 96: 215–18.
44. Sheela CG, Kumund K, Augusti KY. Anti-diabetic effect of onions and garlic sulfoxides amino acids in rats. Planta Med. 1995; 61: 356–7.
45. Augusti KT, Sheela CG. Antiperoxidase effect of S-alkyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia. 1996; 52: 115–20.
46. Rao BK, Kasavula MM, Giri R, Appa RC. Anti-diabetic and hypoglycemic effects of *Momordica cymbalaria* Hook. Food powder in alloxan diabetic rats. J Ethnopharmacol. 1999; 67: 103–19.
47. Rajasekaran FK, Sivagnanam KR, Subramanian S. Hypoglycemic effect of *Aloe vera* gel on streptozotocin-induced diabetes in experimental rats. J Med Food. 2004; 7: 61–6.
48. Hossain MZ, Shibib BA, Rahman R. Hypoglycemic effects of *Coccina indica*: inhibition of key glycolytic enzymes, glucose-6-phosphatase. Indian J Experimental Biol. 1992; 30: 418–20.
49. Singh SN, Vats P, Suri S, Shyam R, Kumria MMI, Ranganathan S, *et al.* Effect of an anti-diabetic extract of *Catharanthus rosesus* on enzymatic activities in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2001; 76: 269–77.
50. Nagappa AN, Thakurdesai PA, Venkat Rao N, Singh J. Anti-diabetic activity of *Terminalia catappa* Linn fruits. J Ethnopharmacol. 2003; 88: 45–50.
51. Chattopadhyay RR. Possible mechanism of anti-hyperglycemic effect of *Gymnema sylvestre* leaf extract, Part I. Gen Pharmacol. 1996; 27: 431–4.
52. Narendhirakannan RT, Subramanian S, Kandaswamy M. Mineral content of some medicinal plants used in the treatment of diabetes mellitus. Biological Trace Element Res. 2005; 103(2): 109–15.
53. Rajasekaran S.; Sivagnanam, K.; Subramanian, S. Mineral contents of *Aloe vera* leaf gel and their role on streptozotocin-induced diabetic rats. Biological Trace Element Res. 2005; 108 (1–3): 185–96.
54. O'Connell BS. Select Vitamins and minerals in the management of diabetes. Diabetes Spectrum. 2001; 14(3): 133–48.
55. Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA. Role of reactive oxygen species in the progression of type II diabetes and atherosclerosis. Mediators Inflammation. 2010; 45: 38–92.
56. Cheng Z, Tseng Y, White MF. Insulin signaling meets mitochondria in metabolism. Trends Endocrinol Metabol. 2010; 21: 589–98.
57. Evangelopoulos AA, Vallianou NG, Panagiotakos DB, Georgiou A, Zacharias GA, Alevra AN, *et al.* An inverse relationship between cumulating components of the metabolic syndrome and serum magnesium levels. Nutri Res. 2008; 28: 659–63.
58. Wells IC. Evidence that the etiology of the syndrome containing type II diabetes mellitus results from abnormal magnesium metabolism. Canadian J Physiol Pharmacol. 2008; 86: 16–24.
59. Chaudhary DP, Sharma R, Bansal DD. Implications of magnesium deficiency in type II diabetes: a review. Biol Trace Element Res. 2010; 134: 119–29.
60. Miranda PM, Horwitz DL. High-fiber diets in the treatment of diabetes mellitus. Annual Intern Med. 1978; 88(4): 482–6.
61. Tiwar AK, Rao LM. Diabetes mellitus and multiple therapeutic approaches phytochemicals: present status and future prospects. Curr Sci. 2002; 83: 30–3.
62. Shrotri DS, Kelkar M, Deshmukh VK and Aiman R. Investigation of hypoglycemic Properties of *Vinca rosea, Cassig auriculata* and *E. Jambolara*. Indian J Medicinal Res. 1963; 51: 464.
63. Mungle AN, Bodhankar MM, Chandak KK. Antidiabetic potential of *Dolichandrone falcata* leaves in alloxan induced diabetic rats. Inter J Res Pharmaceut Biomed Sci. 2012; 3 (1): 319–24.
64. Ali RB, Atangwho IJ, Kuar N, Mohamed EAH, Mohamed AJ, Asmawi MZ, *et al.* Hypoglycemic and anti-hyperglycemic study of *Phaleria macrocarpa* fruits pericarp. J Medicinal Plants Res. 2012; 6(10): 1982–90.
65. DeRuiter J. Overview of the antidiabetic agents. Endocrine Pharmacotherapy Module. Spring. 2003; 1–33.
66. Cheng AYY, Fantus IG. Oral anti-hyperglycemic therapy for type II diabetes mellitus. Canadian Med Assoc J. 2005; 172: 213–26.
67. Gulfraz M, Qadir G, Nosheen F, Parveen Z. Antihyperglycemic effects of *Berberis lyceum* Royle in alloxan induced diabetic rats. Diabetol Croatica. 2007; 36–3: 49–54.
68. Shahaboddin ME, Pouramir M, Moghadamnia AA, Lakzaei M, Mirhashemi SM, Motallebi M. Anti-hyperglycemic and antioxidant activity of *Viscum album* extract. Afri J Pharm Pharmacol. 2011; 5(3): 432–6.
69. Jelodar GA, Maleki M, Motadayen MH, Sirus S. Effect of fungi reek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. Indian J Med. 2005; 59: 64–9.
70. Adewole SO, Ojewole JA, Caxton-Martins EA. Protective effects of quercetin on the morphology of pancreatic β cells of streptozotocin treated diabetic rats. Afri J Trad Compl Alternative Med. 2007; 4: 64–74.
71. Chaltopadhyey RR. Possible mechanism of anti-hyperglycemic effect of *Gymnema sylvestre* leaf extract, Part IV. Gen Pharmacol. 1999; 31: 495–6.
72. Shanmugesundaram ER, Rajeswari G, Baskaran K, Kumar BR, Shanmugesundaram K, Kizar AB. Use of *Gymnema sylvestre* leaf extracts in the control of blood glucose in insulin dependent diabetes mellitus. J Ethnopharmacol. 1990; 30: 281–94.
73. Ugochukwu NH, Babady NE. Anti-hyperglycemic of effect aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin induced diabetic rats. Life Sci. 2003; 73(15): 1925–38.
74. Ngozi H, Ugochukwu HN, Makini K. Modification of renal oxidation stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with *Gongronema latofolium* leaves. Clin Chimica Acta. 2003; 336: 1–2.
75. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J Biosci. 2003; 28: 1–5.
76. El-Missiry MA, El Gindy AM. Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of *Eruca sativa* seeds. Ann Nutri Metabol. 2000; 44: 97–100.
77. Ene A.C, Nwankwo E.A, Samdi L.M. Alloxan-induced diabetes in rats and the effects of black caraway (*Carum carvi L.*) oil on their body weight. Res J Med Medical Sci. 2007; 2(2): 48–52.