World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Antidiabetic activity of hydroalcoholic extract of the root of *Croton macrostachys* in Streptozotocin induced diabetic mice

Laychiluh Bantie and Endalew Gebeyehu

Department of Pharmacology, College of Medicine & Health Sciences, Bahir Dar University, P.O, Box 79, Bahir Dar, Ethiopia

Received: 09-01-2015 / Revised: 26-01-2015 / Accepted: 29-01-2015

ABSTRACT

Diabetes mellitus is major endocrine metabolic disorder of multiple etiologies characterized by chronic hyperglycemia; with disturbances of carbohydrate, protein and fat metabolism. Despite the introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem. Therefore, research for new antidiabetic drugs continues to be an area of concern. Streptozotocin induced diabetic model was used to evaluate oral antidiabetic activity of hydroalcoholic root extract of *Croton macrostachys.* Oral administration of the root extract of the plant did not exhibit toxicity at dose of 5 g/kg. The extract had hypoglycemic activity and improved glucose tolerance of the mice after acute oral glucose solution load. The extract had very significant antidiabetic activity compared to control groups in dose dependent manner. The highest dose, *Croton macrostachys* at 300 mg/kg produced antidiabetic activity comparable to the standard drug, Glibenclamide. Hydroalcoholic root extract of plant possess very significant antidiabetic activity. Hypoglycemic activity of the extract may be due to insulin mimetic or insulin secretagogue components present in the extract. It is recommended to further investigate the plant with solvent and bioguided fractionations to identify possible lead compounds for drug development.

Key Words: *Croton macrostachys*, Streptozotocin, Antidiabetic activity

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion, insulin action or both. It has now become an epidemic with a worldwide incidence of 5% in the general population and the prevalence is expected to double in the year 2025. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025 [1]. In Ethiopia, even though there are no population based prevalence studies, hospital based studies showed that the prevalence of DM admission has increased from 1.9% in 1970 to 9.5% in 1999 of all medical admissions [2]. According to WHO estimate, the number of DM cases in Ethiopia in 2000 was 796,000 and is expected to increase to 1.82 million by 2030 [3].

DM is classified on the basis of the pathogenic process that leads to hyperglycemia, in to two broad categories designated as type I and type II. Type I DM majorly results from autoimmune beta cell distraction, which leads to insulin deficiency. Some develop insulin deficiency by unknown mechanism. Type II DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. Clinical features of DM include: weakness or fatigue, polyphagia, polydepsia, polyurea, recurrent blurred vision, peripheral neuropathy, etc [4]. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. It is frequently associated with the development of microvascular and macrovascular diseases such as neuropathy, nephropathy, cardiovascular and cerebrovascular diseases [5].

Pharmacological treatment (insulin and oral hypoglycemic agents) as well as non-pharmacological treatment (life style modification & diet control) may be used in the management of

***Corresponding Author Address:** Laychiluh Bantie, Department of Pharmacology, College of Medicine & Health Sciences, Bahir Dar University, P.O, Box 79, Bahir Dar, Ethiopia, E-mal: Laychiluh.bantie@gmail.com*

diabetes mellitus. For type I DM the only treatment option is administration of insulin. Management of Type II DM starts from the non-pharmacologic means and then to the oral antidiabetic agents such as sulfonylureas, biguanides, thiazolidinediones etc according to the stage of the DM. Combination of these agents with one another or insulin is also an option for management of DM especially at the later stages of the disease [6].

Since the time of immemorial, oral traditional medicinal plants have been used to treat patients with diabetes mellitus especially type II. In many countries of the world, a mention was made on good number of plants for the cure of diabetes and some of them have been experimentally evaluated and the active principles were isolated [7]. More than 400 plant species having hypoglycemic activity have been available in literature [8]. However, searching for new antidiabetic drugs form natural plants is still attractive because they contain substances which make alternative and safe effect on DM [9].

These plants have been used in the treatment of DM traditionally without any scientific proof for safety and efficacy. Thus, investigating the safety and efficacy of these plants in animal model could give valuable information in this regard and the society at large could be benefited from the use of these plants if the safety and efficacy is proved. A proper scientific evaluation and screening of plants by pharmacological and chemical investigations are necessary for the discovery of potential antidiabetic agents [10].

Croton macrostachyus belongs to the family of Euphorbiaceae, heterogeneous family of flowering plants with 322 genera and around 8,900 species in the world [11]. It is commonly found on forest edges along rivers, around lakes, woodlands, wooded grasslands and along roadsides. It is native to Eritrea, Ethiopia, Kenya, Nigeria, Tanzania and Uganda [12, 13].

In Ethiopia, it is used for the treatment of malaria by the Shinasha, Agew-awi and Amhara people [14]. Ethnobotanical studies revealed that Croton macrostachys has a wide range of activities. The plant has shown a promising antidiabetic [12], purgative and anti-inflammatory effects [15]. Yirga [16] and Mesfin *et al* [17] have reported the ethnobotanical use of the plant for the treatment of skin diseases, urinary retention, intestinal parasites, hepatitis, amoebas and bronchitis. The fact that this plant is used for many human and animal disorders in Ethiopia as evidenced above makes it a good candidate for further animal studies.

MATERIALS AND METHOD

Materials: The following chemicals, reagents and drug were used in the this study: Streptozotocin, Sensocard Glucose strip, Glibenclamide, Sensocard digital glucometer, absolute methanol, petroleum ether, normal saline, Tween 80, Dragendrof's reagent, Mayer's reagent, 10% ethanolic ferric chloride, 2% lead acetate, concentrated sulfuric acid, glacial acetic acid, 5% ethanolic ferric chloride, sodium chloride, 1% gelatin, trisodium citrate, citric acid and Benedict's solution. All the chemicals were analytical grade.

Plant material: Fresh roots of Croton macrostachys were collected from Amhara region, West Gojjam zone, Shindi woreda (172 kms from Bahir dar). Identification and authentication of the plant specimens was done at the college of science, department of Biology of Addis Ababa University and plant specimen was kept there with voucher number LB001.

Experimental animals: Female Swiss albino mice (age 8 weeks and weight of 25-28g) obtained from Amhara region animal health institute were used for the study. The animals were housed in the animal house of Amhara region animal health institute under standard environmental conditions (25±1 °C, 12 h light/dark cycle). The mice were given access to standard laboratory pellet and water ad libtum before and during the experiment. The mice were maintained and cared according to the international guidelines for the use and maintenance of experimental animals [18].

Methods

Extraction: The collected roots were cleaned with tap water, air dried under shade at room temperature and coarsely powdered by using a grinding mill. The coarsely powdered plant material was macerated with 80% methanol in Erlenmeyer flask for 72 h at room temperature. After 72 h, the extract was separated from the marc using gauze and further filtered by Whatman filter paper No. 3. The marc was remacerated twice using the same volume of 80% methanol to exhaustively extract the plant material. After exhaustive extraction, the hydroalcohol was removed by evaporation in an oven drier calibrated at temperature of 40 °C to obtain the dried crude extract of the plant.

Preliminary phytochemical screening: The crude extract of C. macrostachys was screened for the presence of different chemical constituents following standard procedures [19, 20].

Acute toxicity test: Nine female Swiss albino mice were randomly divided into 3 groups of 3 mice per cage. After being fasted for 2 h [18], the mice in the first and second group were given 2 and 5 g/kg of the root extract dissolved in distilled water orally, respectively and observed for any signs of toxicity. The mice in third group were provided only distilled water. The mice were observed for gross changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhoea, mortality and other signs of overt toxicity manifestations [21].

Pharmacological screening

Effect of the extract on fasting blood glucose level (FBGL) in normal mice: 25 mice fasted overnight for 12h were grouped into 5 groups of 5 mice per group. Using aseptic precautions, blood was collected from the tails to determine fasting blood sugar levels (FBGL). Group 1 control (CON) was given distilled water 2 ml/kg while G2 (CM100), G3 (CM200) and G4 (CM300) were given 100 mg/kg, 200 mg/kg and 300 mg/kg of root extract of Croton macrostachys, respectively and G5 (GC5) was given Glibenclamide 5 mg/kg. Blood samples were collected from each mouse by tail clip at 2, 4 and 6h post-treatment and blood glucose level was determined using automatic glucometer.

Effect of the extract on oral glucose tolerance test (OGTT) in mice: 25 mice fasted overnight for 12h were grouped into 5 groups of 5 mice per group. Group 1 (CON) was given distilled water 2 ml/kg while G2 (CM100), G3 (CM200) and G4 (CM300) were given 100 mg/kg, 200 mg/kg and 300 mg/kg of root extract of Croton macrostachys, respectively and G5 (GC5) was given Glibenclamide 5 mg/kg. One hour later, all these mice were orally loaded with 5 ml/kg of 50% (w/v) glucose solution. Blood samples were collected from the tails of these mice immediately prior to commencement of treatment and at 30, 60, 90 and 120 minutes after glucose loading and blood glucose level was determined using automatic glucometer.

Effect of the extract on BGL streptozotocin induced diabetic mice: Streptozotocin was dissolved in 0.1M citrate buffer of PH 4.5. The freshly prepared streptozotocin solution was injected intraperitonially to each mouse at a dose of 45 mg/Kg. 72h later, 25 mice showing blood glucose level > 200 mg/dL (day 0) grouped into 5 groups of 5 mice per group. Group 1 (CON) was given distilled water 2 ml/kg while G2 (CM100), G3 (CM200) and G4 (CM300) were given 100 mg/kg, 200 mg/kg and 300 mg/kg of root extract of Croton macrostachys, respectively and G5 (GC5) was given Glibenclamide 5 mg/kg. Blood glucose level was measured after 7, 14 and 21 days of treatment.

Ethical consideration: The animals were maintained and cared according to the international guidelines for the use and maintenance of experimental animals [18]. The study protocol was approved by the board of ethics of college of medicine and health sciences of Bahir Dar University (Ref No PGCS-CMHS/1843). Formal letter was written to the concerned bodies from the College of Medicine and Health Sciences post graduate, research and community service coordinator. Diethyl ether was used for euthanasia.

Statistical Analysis: The data from the experiment was processed by using SPSS version 20, statistical software using one way ANOVA followed by Tukey's test. All the results were expressed as mean ± S.E.M of five mice in each group. A P-value <0.05 was considered as significant.

RESULTS

Acute toxicity study: The acute toxicity study revealed that the root extract of Croton macrostachys caused no mortality in both doses (2 and 5 g/kg) within the first 24 h as well as for the following 14 days. Physical and behavioural observations of the experimental mice also indicated no visible signs of overt toxicity like lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhoea and convulsion. This suggests that LD50 of the extract is greater than 5 g/kg.

Phytochemical screening: Phytochemical screening of the hydroalcoholic root extract Croton macrostachyus revealed the presence of alkaloids, phenolic compounds, tannins, terpenoids, saponins, phlobatannins and flavonoids. Anthraquinones, cardiac glycosides and simple sugars were absent in the extract (Table 1).

Effect of the extract on fasting blood glucose level (FBGL): Three different doses of the root extract of Croton macrostachys, CM100, CM200 and CM300 were administered to three different groups of overnight fasted mice to determine oral hypoglycemic potentials of the plant. Glibenclamide was used as a reference drug and another group of mice were provided with distilled water and used as normal controls. The extract has significant hypoglycemic activity in dose dependent manner. Compared to the normal controls, CM100 ($P<0.05$), CM200 ($P<0.001$) and CM300 ($P<0.001$) produced significant reduction in blood glucose level after two hours of administration. The hypoglycemic activity was very high after four hours of administering the

extracts. The highest dose (CM300) and the reference drug produced significant hypoglycemic activity compared to CM100 (P<0.001) and CM200 (P<0.01). There was no significant difference in hypoglycemic activity of the reference drug and the highest dose (Table 2).

Effect of the extract on oral glucose tolerance test (OGTT): The blood glucose levels, after oral administration of 50% (w/v) glucose solution in normal control and treated mice are given in table 3. Both the reference drug and extract showed significant hypoglycemic effect. The hyperglycemia due to glucose load in negative controls returned back near to normal levels after 120 minutes whereas, all the extract doses and reference drug depressed the hyperglycemia level 60 minutes after loading. CM100 (P<0.01), CM200 (P<0.001) and CM300 (P<0.001) produced significant lowering effect compared to normal mice. The highest dose (CM300) and reference drug (GC5) produced significant (P<0.001) decrease of blood glucose level compared to the lower doses after 60, 90 and 120 minutes of glucose loading. There was no significant difference in blood glucose lowering activities of CM300 and GC5 (Table3).

Oral antidiabetic activity: Streptozotocin induced diabetic mice having blood glucose level > 200 mg/dl were used to determine oral antidiabetic activity of the extract. The blood sugar levels measured in normal controls and treated group of mice at the 0, 7, 14 and 21th day of treatment are given in table 4. Oral administration of the hydroalcoholic root extract of Croton macrostachys produced significant (P<0.001) antidiabetic activity with CM100, CM200 and CM300 doses compared to the normal controls after 21 days of treatment. Glibenclamide (GC5) also produced significant antidiabetic activity compared to normal controls (P<0.001), CM100 (P<0.001) and CM200 (P<0.01). CM300 had significant antidiabetic activity compared to CM100 (P<0.01). There is no significant antidiabetic difference between CM300 and GC5 (Table 4).

DISCUSSION

Croton macrostachys root extract had produced no observable toxicity signs at doses up to 5 g/kg doses. The LD50 of the plant is higher than 5 g/kg. This finding is in line with previous studies [22]. Phytochemical screening revealed presence of secondary metabolites such as flavonoids, alkaloids, tannins which has been reported to have antidiabetic activity [23, 24]. Hypoglycemic activity of a drug might be produced by both pancreatic and non-pancreatic mechanisms. Glibenclamide which is grouped under sulfonylureas oral antidiabetic drug is known for its hypoglycemic activity [25]. It produces hypoglycemic activity mainly by blocking ATP sensitive K+ channels and thereby increasing insulin secretion from pancreas β-cells. It is also reported that sulfonylureas reduce serum glucagon levels contributing indirectly to their hypoglycemic activity [26, 27]. In our study both the reference drug, Glibenclamide, and the extract particularly with the highest dose (CM300) produced comparable oral hypoglycemic activity when provided to overnight fasted non-diabetic mice. This shows that the extract like Glibenclamide reduced the blood glucose level below the baseline value. Even though the exact mechanism for this hypoglycemic effect of the extract is yet to be determined, the extract might exert its hypoglycemic activity by similar mechanism as Glibenclamide.

Oral glucose tolerance testing (OGTT) is a standard experimental technique used to diagnose diabetes mellitus. Each year, 1-5% of people with impaired glucose tolerance (IGT) actually develop diabetes mellitus. Since impaired oral glucose tolerance is indicative of predisposition to diabetes, agents that exhibit antihyperglycemic activity and capable of reducing blood glucose concentration within normal limits (<140 mg/dl) after two hours of glucose load will help in halting the progression of impaired glucose tolerance to diabetes [28].

Glibenclamide and the root extract improved the glucose tolerating efficiency of the mice compared to the normal controls. This effect is supported partly by the fact that both Glibenclamide and the extract have hypoglycemic activity in non-diabetic mice. The extract might improve the glucose tolerance by different mechanisms. It might increase insulin secretion or improve sensitivity of peripheral tissues to insulin. Both mechanisms have been reported to be mechanisms for improving oral glucose tolerance in the literature [29, 30].

The screening of antidiabetic activity of natural products and synthetic compounds is performed in experimental animal models after induction of diabetes by several methods. To induce non-insulin-dependent diabetes in animals, streptozotocin (STZ) is commonly used which produces moderate hyperglycemia with clinical symptoms similar to type 2 diabetes [31]. STZ causes alkylation of pancreatic deoxyribonucleic acid by entering to the β-cell via glucose transporter 2 and induces activation of poly (ADP ribosylation) that causes depletion of cellular nicotinamide adenine dinucleotide (NAD+) and adenosine triphosphate. As a result, generation of

free radicals causes pancreatic β-cells necrosis [32]. The extract after chronic administration produced a very significant reduction of blood glucose level in streptozotocin induced diabetic mice. All the three doses of the extract showed significant antidiabetic activity after 21 days of oral administration in dose dependent manner. The highest dose lowered the blood glucose level to values comparable with effect of Glibenclamide. Previous studies indicated that many plants have significant antidiabetic activity [30, 33].

Although the active compound is yet to be identified, the antidiabetic activity of Croton macrostachyus could be attributed to a single or a combination of its secondary metabolites such as alkaloids, flavonoids, terpenoids and phenolic compounds. These metabolites have been reported to have different extents of antidiabetic activity in the literature. Reduction in blood glucose by these bioactive compounds from plants might act by one of several mechanisms. Some of them may inhibit endogenous glucose production [34] or interfere with gastrointestinal glucose absorption [35]; some may have insulin-like substances [36]; some may inhibit insulinase activity and some may increase secretion of insulin from the ß cells of the pancreas i.e. pancreatotropic action [37, 38], while others may increase beta cells in pancreas by activating regeneration of these cells [39].

CONCLUSION

The results indicate that hydroalcoholic root extract of Croton macrostachys possess a very significant antidiabetic activity in dose dependent manner. It is generally accepted that streptozotocin causes permanent destruction of β-cells. Therefore, it is evident that the hypoglycemic activity of extract may be due to the insulin mimetic or insulin secretagogues components present in the extract. It is recommended to further investigate the plant with solvent fractions and bioguided fractionations to identify possible lead compounds for drug development as well as elucidating the mechanism of action for the antidiabetic activity.

ACKNOWLEDGEMENT

The financial and material support of Bahir Dar University is highly acknowledged. It is also our pleasure to thank Daniel Seyfu Melka (PhD) for his invaluable support of chemicals. Ato Maru Desta is also highly acknowledged for his contribution to this work.

Table 1: Phytochemical constituents of hydroalcoholic root extract of *Croton macrostachys*

Secondary metabolites	Root extract
Alkaloids	+
Saponins	+
Phenolic compounds	+
Cardiac glycosides	-
Tannins	+
Flavonoids	+
Terpenoids	+
Anthraquinones	-
Phlobatannins	+
Simple sugars	-

-, absent; +, present

Table 2: Oral hypoglycemic activity of hydroalcoholic root extract of *Croton macrostachys*

Animal Group	T0	T2	T4	T6
CON	128.4±1.94	121.2±3.47	115.8±3.63	103±4.18
CM100	128.8±1.89	109.4±3.21 ^{a*}	101.8±3.54 ^{a**}	100.2±4.12 $^{a£}$
CM200	129.4±1.92	99.0±2.98 ^{a***b*}	92.2±3.46 ^{a***}	90.1±3.97 ^{a*b*}
CM300	130.6±1.88	93.4±3.10 ^{a***b***}	78.7±3.44 ^{a***b***c**}	72.4±3.79 ^{a***b***c**}
GC5	130.6±1.90	84.6±3.33 $^{a***b***c**d£}$	76.4±3.38 $^{a***b***c**d£}$	69.5±3.62 $^{a***b***c**d£}$

Data are expressed as Mean ± SEM; n=5; a= compared to control, b= to CM100 mg/kg, c= to CM200 mg/kg, d= to CM300 mg/kg; *= p< 0.05; **=p<0.01, ***=p<0.001; £= not significant; T0= pre-treatment value, T2= 2 hours post-treatment, T4 4 hours post treatment, T6 6 hours post treatment; CON= control

Table 3: Effect of hydroalcoholic root extract of *Croton macrostachys* on oral glucose tolerance

Animal group	T0	T30	T60	T90	T120
CON	120.8±5.15	169.6±2.59	158.6±3.60	142.5±3.67	131.4±3.30
CM100	119.6±5.23	152.2±2.61 ^{a***}	140.7±3.58 ^{a**}	134.4±3.55 $^{a£}$	123.4±3.31 $^{a£}$
CM200	121.6±4.98	142.4±2.45 $^{a*** b**}$	130.4±3.57 ^{a***}	120.1±3.45 ^{a***b**}	109.1±3.32 ^{a***b**}
CM300	120.8±5.15	132.2±2.57$^{a*** b** c**}$	102.4±3.55^{a***b***c***}	90.8±3.43$^{a***b*** c***}$	79.6±2.99$^{a***b*** c***}$
GC5	123.5±5.31	127.3±2.4$^{a***b***c***d£}$	92.6±3.3 $^{a***b** *c***d£}$	85.8±63 $^{a***b** *c***d£}$	76.6±3.3$^{a***b** *c***d£}$

Data are expressed as Mean ± SEM; n=5; a= compared to control, b= to CM100 mg/kg, c= to CM200 mg/kg, d= to CM300 mg/kg; *= p< 0.05; **=p<0.01, ***=p<0.001; £= not significant; T0= pre-treatment value, T30= 30 minutes post-treatment, T60= 60 minutes post treatment, T90= 90 minutes post treatment, T120= 120 minutes post treatment; CON= control

Table 4: Oral antidiabetic activity of hydroalcoholic root extract of *Croton macrostachys*

Animal group	D0	D7	D14	D21
CON	255.3±14.63	276.0±9.93	284.6±7.61	298.3±7.47
CM100	253.5±13.96	240.6±9.91 ^{a**}	226.2±7.45 ^{a***}	214.2±7.23 ^{a***}
CM200	254.4±14.23	222.4±9.90 $^{a***b£}$	202.3±6.97 ^{a***b*}	188.6±6.93 ^{a***b*}
CM300	250.7±14.55	211.2±9.85 $^{a***b£c£}$	179.8±6.93 $^{a***b***c£}$	168.8±6.92 $^{a***b***c£}$
GC5	252.3±13.98	209.1±9.67 $^{a***b*c£d£}$	170.4±7.31 $^{a***b***c**d£}$	161.4±7.21 $^{a***b***c**d£}$

Data are expressed as Mean ± SEM; n=5; a= compared to control, b= to CM100 mg/kg, c= to CM200 mg/kg, d= to CM300 mg/kg; *= p< 0.05; **=p<0.01, ***=p<0.001; £= not significant; D0= pre-treatment value, D7= 7day post-treatment, D14= 14 days post treatment, D21= 21 days post treatment; CON= control

References

1. Edwin J, Siddaheswar BJ, Dharam CJ: Diabetes and Herbal Medicines. *Iran J Pharmacol & Therapeut* 2008, 7: 97-106.
2. Feleke Y, Enquselassie F: An assessment of the health care system for diabetes in Addis Ababa, Ethiopia. *Ethio J health dev* 2005, 19: 203-210.
3. WHO. Diabetes program: Facts and figures about diabetes: WWW.who.int/diabetes/facts/world-figure/en/index1.html. accessed on 22/10/2014
4. Power AC: Diabetes Mellitus. In Harrison's principles of internal medicine. 16th edition. Edited by Kasper DL, Braunwald E, Hauser SL, Fauci AS, Longo DL, Jameson JL. New York, McGrow-Hill medical publishing division; 2005: 2152-2180.
5. Fowler MJ. Microvascular and macrovascular complications of diabetes. *Clin diabetes* 2008, 26(2):77-82.
6. International diabetes federation clinical guidelines task force. Global guideline for type 2 diabetes. Brussels: International diabetes federation, 2005
7. Al-Awdi FM, Gumaa KA: Studies on the activity of individual plants of an antidiabetic plant mixture. *Acta diabetologica Latina* 1987, 24:37-41.
8. Rai, MK: A review on some antidiabetic plants of India. *Ancien scien life* 1995, 14: 42-54
9. Mohamed B, Abderrahim Z, Hassane M, Abdelhafid T, Abdelkhaleq L: Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). *Int J Diabetes Metab* 2006, 14:1-25.
10. Upendra RM, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM: 2010. Herbal Medicines for Diabetes Mellitus: A Review. *Int J Pharm Tech* 2010, 2(3):1883-1892.
11. Tala MF, Tan N-H, Ndonsta BL, Tane P: Triterpinoids and phenolic compounds from *Croton macrostchyus*. *Biochem Syst Ecolo* 2013, 51:138–141.
12. Kapingu CM, Guillaume D, Mbwambo HZ, Moshi JM, Uliso CF, Mahunnah ALR: Diterpenoids from the roots of *Croton macrostachys*. *Phytochem* 2000, 54:767–770.
13. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A (2009). Agroforestree Database http://www.worldagroforestry.org/af/treedb accessed on April 2nd, 2014.
14. Gidey M, Teklehaymanot T, Animut A, Mekonnen Y: Medicinal plants of Shinasha, Agew-awi and Amhara peoples in North West Ethiopia. *J Ethnopharmacol* 2007, 110:516–525.
15. Mazzanti G, Bolle P, Martinoliu L, Piccineliu D, Grugurina I, Animate F, Mugne Y: *Croton macrostach*ys a plant used in traditional medicine: purgative and anti-inflammatory activity. *J Ethnopharmacol* 1987, 19(2): 213–217.
16. Yirga G: Use of traditional medicinal plants by indigenous people in Mekele town, capital city of Tigray regional state of Ethiopia. *J Med Plants Res* 2010, 4(17):1799–1804
17. Mesfin F, Demissew S, Teklehaymanot T: An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *J Ethnobio Ethnomed* 2009 doi: 10.1186/1746-4269-5-28.
18. OECD guideline for testing of chemicals 423: Acute Oral Toxicity: Acute Toxic Class Method 2001.
19. Trease GE, Evans W: Pharmacognosy. 13th ed. Bailliere Tindall, London 1989, 176–180.

20. Jones P, Kinghorn D: Extraction of Plant Secondary Metabolites. In Methods in Biotechnology Natural Products Isolation, 2nd edition. Edited by Sarker D, Latif Z, Gray A. Totowa, New Jersey Human Press; 2005: 323–351.
21. Center for Drug Evaluation and Research: Guidance for industry single dose acute toxicity testing for chemicals 1996.
22. Bantie L, Assefa S, Engidawork E, Teklehaimanot T: *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of Croton macrostachyus Hocsht. (Euphorbiaceae) against Plasmodium berghei in mice. BMC Complem Altern M 2014, 14:79
23. Chandrika UG, Wedage WS, Wickramasinghe SMDN, Fernando WS: Hypoglycemic actions of the flavonoids fraction of *Artocaprusheterophyllus* leaf. *Afr J Trad CAM* 2006, 3(2): 42-50
24. Saidu AN, Mann A, Onuegbu CD: Phytochemical screening and hypoglycemic effects of aqueous *Blighia sapida* root bark extract on normoglycemic albino rats. Brit J Pharmaceut Res 2012, 2(2):89-97.
25. Rydberg T, Jonsson A, Roder M, Melander A: Hypoglycemic activity of Glyburide (Glibenclamide) metabolites in humans. *Diabetes care* 1994, 17(a):1026-1030.
26. Lebovitz HE, Feinglos MN: Sulfonylurea drugs: mechanism of antidiabetic action and therapeutic usefulness. *Diabetes care* 1978, 1(3):189-198.
27. Sarkar A, Tiwari A, Bhasin PS and Mitra M: Pharmacological and pharmaceutical profile of Gliclazide: A review. *J appl pharmaceut sci* 2011, 1(9):11-19
28. American diabetes association: Diabetes management guidelines. WWW.ndei.org/ADA-2014-guidlines-diabetes-diagnosis-A1c.aspx. retrieved on 23/10/2014
29. Bhoyar PK, Tripathi AK, Baheti JR, Biyani Dm, Khaliqu M, Kothmire MS, Amgaonkar YM, Bhanarkar AB: Herbal antidiabetics: A review. *Int J Res Pharm Sci* 2011, 2(1):30-37
30. Hassan Z, Yam MF, Ahmad M, Yusof APM: Antidiabetic properties and mechanism of action *Gynura procumbens* water extract in streptozotocin induced diabetic rats. *Molecul* 2010, 15:9008-9023
31. Srinivasan K, Ramarao K: Animal models in type 2 diabetes research: an overview. *Ind J Med Res* 2007, 125(3): 451–472.
32. Szkudelski T: The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res* 2001, 50(6): 537–546.
33. Ramachandran S, Rajasekaran A, Manisenchilkumar KT: Investigation of hypoglycemic, hypolipidemics and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. *Asian Pacif J Tropi Biomed* 2012, 2(4):262-268
34. Eddouks M, Jouad H, Maghrani M, Lemhadri A, Burcelin R: Inhibition of endogenous glucose production accounts for hypoglycemic effect of *Spergularia purpurea* in streptozocin mice. *Phytomedicine: Int J Phytother Phytomacol* 2003, 10(6-7):594-599.
35. Musbayane CT, Bwititi PT, Ojewole JAO: Effect of oral Administration of some herbal extracts on food consumption and blood glucose levels in normal and streptozotocin treated diabetic rats. *Methods and Findings in Exper Clin Pharmacol* 2006, 28(4): 223-228.
36. Gray AA, Flat PR: Insulin releasing like activity of the traditional Antidiabetic plant *Coriander sativum* (coriander). *J Nutr* 1999, 81:203-208.
37. Trivedi NA, Mazumder B, Bhatt JD, Hemavathi KG: Effect of Shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. *Indian J Pharmacol* 2004, 36: 373-376.
38. Yadav JP, Kalia AN, Dangi AS: Hypoglycemic activity of extract of *Salvadora oleoides* in normal and alloxan induced diabetes rats. *Indian Pharmacol* 2008, 40: 23-27
39. Jelodar G, Mohesn M, Sharam S: Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreases Alloxan induced diabetic rats. Afr J Trad Compl & Altern Med 2007, 4(3): 299-305.