

Full Length Research paper

Anti-diabetic and some haematological effects of ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract in alloxan - induced diabetic wistar rats

A. Mohammed^{1*}, A. B. Adelaiye¹, A. G. Bakari² and M. A. Mabrouk³

¹Department of Human Physiology, ABU, Zaria, Nigeria.

²Department of Medicine, ABUTH, Zaria, Nigeria.

³Department of Physiology, Faculty of Medicine, Al-Azhar University, Cairo.

Accepted 16 November, 2019

Diabetes mellitus has become a major public health concern, especially in the developing countries. The current orthodox treatment modalities for diabetes mellitus have many drawbacks including undesirable side-effects and the high cost of long -term treatment. The aim of this study is to determine the anti- diabetic and some haematological effects of Ethylacetate and n-Butanol fractions of *Ganoderma lucidum* (Tuwon biri) aqueous extract in alloxan-induced diabetic. Preliminary phytochemical screening and acute toxicity studies of the two fractions of *G. lucidum* were carried out. A dose of 50 mg/kg of Ethylacetate and n-Butanol fraction of *G. lucidum* aqueous extract was given intraperitoneally daily for two weeks to the alloxan-induced wistar rats. Insulin was also used as a standard anti-diabetic drug and was given intraperitoneally to alloxan-induced diabetic wistar rats. The fasting blood glucose levels were determined weekly for two weeks. At the end of the two weeks, the animals were sacrificed and blood samples were taken from all the groups for the determination of hematological parameters. The preliminary phytochemical screening of the two fractions of *G. lucidum* aqueous extract revealed the presence of alkaloids, flavonoids, and saponins. The LD₅₀ was 1265 and 471 mg/kg for Ethylacetate and n-Butanol fractions of the *G. lucidum* aqueous extract respectively. There was a significant ($p < 0.05$) reduction of 188.8 ± 19.51 mg/dl and 162.8 ± 24.67 mg/dl of the fasting blood glucose of the alloxan-induced diabetic groups after 1 and 2 weeks of treatment with Ethylacetate fraction of *G. lucidum* aqueous extract respectively. Similarly, There was a significant ($p < 0.05$) reduction of 182.2 ± 56.09 mg/dl and 148.8 ± 32.82 mg/dl of the fasting blood glucose of the alloxan-induced diabetic groups after 1 and 2 weeks of treatment with n-Butanol fraction of *G. lucidum* aqueous extract respectively. There was a statistically significant ($p < 0.05$) reduction of $3.68 \pm 0.28 \times 10^{12}$ /L in the red cell count of the n-Butanol fraction treated group. The total leukocytes showed a significant ($p < 0.05$) increase of $16.62 \pm 0.53 \times 10^9$ /L and $16.48 \pm 1.35 \times 10^9$ /L after the two weeks treatment with Ethylacetate and n-Butanol fractions of *G. lucidum* aqueous extract respectively. In conclusion, both Ethylacetate and n-Butanol fractions of *G. lucidum* aqueous extract were found to have potent anti-diabetic effects. The Ethylacetate fraction has no significant erythropoetic effect while the n-Butanol fraction showed a significant decrease in the red cell parameters.

Key words: Blood glucose, diabetes mellitus, *Ganoderma lucidum*, red blood cell, leucocytes.

INTRODUCTION

Diabetes mellitus is a metabolic disorder with different

aetiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and /or action (Balkau et al., 2000). The disease has become a real problem of public health in developing countries,

*Corresponding author. E-mail: amohd177@hotmail.com.

where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable (Djrolo et al., 1998). Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed, because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries (WHO, 2002). Well over 400 medicinal plants are available globally for the medication of diabetes mellitus, with a few having been subjected to scientific authentication to ascertain their effectiveness as anti-diabetic agents (Onoagbe and Esekheigbe, 1999). Substances with hypoglycemic properties would be effective in the management of diabetes mellitus (Young and Maciejewski, 1997). The family of *Ganodermataceae* consists of a large group of tree fungi of the class *Polyporaceae*, specifically the genus *Ganoderma* and other related genera. *Ganoderma* fungi are mainly found in tropical and subtropical areas; the typical species is *Ganoderma lucidum*. This mushroom is reported to have various biological activities, such as anti-tumor, antibacterial, and antiviral activities (Wang et al., 1997; El-Mekkawy et al., 1998; Eo et al., 2000; Yoon et al., 2003). Mohammed et al. (2007) has earlier reported the hypoglycemic activity of aqueous extract of *G. lucidum*. Also several plants extracts, for example, *Solanum tuberosum*, *S. lycopersium*, *S. eleagnofolium*, *S. nigrum*, *Mercurialis perennis*, *M. annua* have been reported to destroy RBCs thus leading to anemia (Blood and Radostits, 1989; Adedapo et al., 2004; Adedapo et al., 2007). Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. It can also be used to explain blood relating functions of chemical compounds/plant extract (Yakubu et al., 2007).

The aim of this research work was to determine the effects of Ethylacetate and n-Butanol fractions of *G. lucidum* aqueous extract on the blood glucose levels and some hematological parameters of alloxan-induced diabetic Wistar rats.

MATERIALS AND METHODS

Animals

A total of seventy four albino Wistar rats of both sexes between the ages of 8 - 12 weeks old and weighing 120 - 250 g were used for this study. The animals were housed in the Animal House, Department of Human Physiology, ABU, Zaria. The animals were randomized into experimental and control groups and were kept in polypropylene cages. The animals were maintained on standard animal feeds and drinking water *ad libitum*.

Plant material

The fruiting bodies of wild *G. Lucidum* were collected within Zaria and its environs between July and October, 2006. The plant material

was identified and authenticated by a taxonomist, Mallam M. Mohammed, of Biological Science Department A.B.U., Zaria where a voucher specimen (No. BSTCC 005) has been deposited at the herbarium section.

Chemicals and drugs

All chemicals and drugs used were of analytical grade.

Preparation of plant extracts

The fruiting bodies of *G. Lucidum* were air dried under the shade and grounded into a fine powder using mortar and pestle. Five hundred grams of the powdered material was macerated in 2.5 L of distilled water at room temperature for 24 h. It was then filtered using a filter paper (Whatmann size 1). The filtrate was then partitioned with Ethylacetate to get the Ethylacetate fraction which was evaporated to dryness in an oven at 40°C. A greenish-brown residue weighing 8.5 g (1.7% w/w) was obtained and kept in a sealed container at 4°C in a refrigerator until use. Another five hundred grams of the powdered material was macerated in 2.5 L of distilled water at room temperature for 24 h. It was then filtered using a filter paper (Whatmann size 1). The filtrate was then be partitioned with n-Butanol to get an n- Butanol fraction which was evaporated to dryness in an oven at 40°C. A brownish residue weighing 6.5 g (1.3% w/w) was obtained and kept in a sealed container at 4°C in a refrigerator until use.

Phytochemical screening of plant fraction

Preliminary phytochemical screening of the two extracts were performed for the presence of secondary metabolites using the following reagents and chemicals: alkaloids - with Mayer's and Dragendorff's reagents (Farnsworth, 1966; Harborne, 1998); flavonoids with the use of Mg and HCl (Silva et al., 1993; Houghton and Raman, 1998); tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds (Houghton and Raman, 1998).

Acute toxicity studies (LD₅₀)

The LD₅₀ determination for each of the fractions was conducted separately using modified method of Lorke (1983). For each of the fractions, the evaluation was done in two phases. In phase one, three groups of three rats each, were treated with 10, 100 and 1000 mg extract/kg body weight intraperitoneally (ip) respectively. The control groups (the fourth group for each fraction) received Tween-20. The rats were observed for clinical signs and symptoms of toxicity within 24 h and death within 72 h.

Based on the results of phase one for the Ethylacetate extract, fifteen fresh rats with three per group were each treated with 600, 1000, 1600 and 2900 mg extract/kg (ip) respectively. The control groups (the fifth group for each fraction) received Tween-20. Clinical signs and symptoms of toxic effects and mortality were then observed for seven days. Also based on the results of phase one for the n-Butanol extract, fifteen fresh rats with three per group were each treated with the extract at the doses of 140, 225, 370 and 600 mg/kg (ip) respectively. Clinical signs and symptoms of toxic effects and mortality were then observed for seven days.

The LD₅₀ were then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose that is the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

Induction of experimental diabetes mellitus

The animals were fasted for 16 - 18 h with free access to water prior to the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9% v/v cold normal saline solution at a dose of 150 mg/kg body weight (Katsuamat et al., 1999). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani et al., 2002). The diabetes was assessed in alloxan-induced rats by determining the blood glucose concentration 72 h after injection of alloxan. The rats with blood glucose level above 200 mg/dl were then selected for the study.

Experimental design

After the induction of diabetes mellitus in the Wistar rats, the animals were randomly divided into experimental and control groups. All animals were fasted for 16 - 18 h before treatment. Fasting blood glucose levels of each group was determined weekly for the two weeks. All the animals were sacrificed at the end of the two weeks after fasting them for 16 - 18 h. The rats were anaesthetized at the time of sacrifice by being placed in sealed cotton wool soaked chloroform inhalation jar. Blood was collected via cardiac puncture from each animal for determination of haematological parameters. The Wistar rats were subdivided as follows;

- Group 1 (n = 5)-----Diabetic control Wistar rats (Received Tween 20,5 ml/Kg body weight)
- Group 2 (n = 5)-----Diabetic Wistar rats were treated with 50 mg/Kg of Ethylacetate fraction of the aqueous extract of *Ganoderma lucidum*.
- Group 3 (n = 5)-----Diabetic Wistar rats were treated with 50 mg/Kg of n-Butanol fraction of the aqueous extract of *G. lucidum*
- Group 4 (n = 5)-----Diabetic Wistar rats were treated with Insulin [6 I.U/Kg body Weight (Stanley et al., 2001)]

Determination of blood glucose levels

Fasting blood glucose levels were determined by using the glucose oxidase method (Trinder, 1969) with ONE TOUCH BASIC® Glucometer (LIFESCAN, Inc 2001 Milpitas, CA 95035, USA) and results were reported as mg/dl (Rheney and Kirk, 2000).

Determination of haematological parameters

After two weeks treatment with the two extracts, blood samples were obtained through cardiac puncture of the rats for the determination of the blood parameters: Red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), white blood cell count (WBC) and its differential counts using the method of Dacie and Lewis (1991). The red cell indices were also calculated.

Statistical analysis

All the data are expressed as mean \pm SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests (Duncan et al., 1977). The results were considered statistically significant if the p values were 0.05 or

less. The data were analyzed using SigmaStat v2.0 (Jandel Scientific, Palo Aho, CA, USA).

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the two fractions of *G. lucidum* aqueous extracts revealed the presence of alkaloids, flavonoids and saponins. However, there were no tannins detected in both fractions.

Acute toxicity studies

The signs of toxicity were first noticed after 4 - 5 h of extracts administration. There were decreased locomotor activity and sensitivity to touch and pain. Also there was decreased feed intake, tachypnoea and prostration after 8 - 12 h of extracts administration. Early deaths were recorded after 12 hours and late deaths 48 h after extract administration. The LD₅₀ were then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose that is the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

For the Ethylacetate fraction, there was 0% mortality at 1000 mg/Kg and 33.3% mortality was the next highest lethal dose at 1600mg/Kg. The LD₅₀ of the Ethylacetate fraction was thus; $1000 \times 1600 = 1264.9$ mg/Kg.

For the n-Butanol fraction, there was 0% mortality at 370mg/Kg and 33.3% mortality was the next highest lethal dose at 600 mg/Kg. The LD₅₀ of the n-Butanol fraction was thus; The LD₅₀ was thus; $370 \times 600 = 471.2$ mg/Kg.

Effects of daily doses of ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract on blood glucose levels of diabetic wistar rats

There was a significant decrease ($p < 0.05$) in the blood glucose levels of the fractions treated diabetic groups after the 7th and 14th days of treatment period when compared to the control as shown in Table 1.

Effects of daily doses of ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract on erythrocytes indices of diabetic wistar rats

There was a significant decrease ($p < 0.05$) and a significant increase ($p < 0.05$) in the red cell count and the red cell indices (MCV and MCH) respectively in the n-Butanol treated group of the diabetic Wistar rats as shown in Table 2. There was a significant increase ($p < 0.05$) in the red cell count, Hb and PCV in the Insulin

Table 1. Effects of daily doses of ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract on blood glucose levels of diabetic Wistar rats.

Group (n = 5)	Treatment given	Fasting blood glucose levels(mg/dl)		
		1 st Day	7 th Day	14 th Day
1	Control (Tween-20)	379.6 ± 62.18	397.2 ± 55.22	364.0 ± 60.12
2	Ethylacetate (50 mg/Kg body weight)	395.4 ± 53.20	188.8 ± 19.51 ^a	162.8 ± 24.67 ^a
3	n-Butanol (50 mg/Kg body weight)	350.0 ± 63.29	182.2 ± 56.09 ^a	148.8 ± 32.82 ^a
4	Insulin (6 IU/Kg body weight)	322.2 ± 43.89	197.6 ± 31.56 ^a	210.6 ± 58.28 ^{ns}

Values are mean ± SEM.

Values are statistically significant compared to control group at: ^aP < 0.05, ns = not significant.

Mean fasting blood glucose levels at 1st Day: 322.2 - 395.4 mg/dl.

Table 2. Effects of daily doses of ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract on erythrocytes indices of diabetic wistar rats.

Group (n=5)	Treatment	RBC × 10 ¹² / L	Hb (gm/dl)	PCV (%)	MCV(Cubic μ)	MCH(pg)	MCHC (%)
1	Control (Tween 20)	4.24 ± 0.17	12.9 ± 0.28	38.8 ± 0.86	91.8 ± 2.08	30.5 ± 0.71	33.2 ± 0.04
2	Ethylacetate(50mg/Kg)	4.72 ± 0.24 ^{ns}	13.5 ± 0.34 ^{ns}	40.6 ± 1.03 ^{ns}	86.6 ± 2.73 ^{ns}	28.8 ± 0.91 ^{ns}	33.2 ± 0.03 ^{ns}
3	n-Butanol(50 mg/Kg)	3.68 ± 0.28 ^a	12.5 ± 0.54 ^{ns}	37.6 ± 1.60 ^{ns}	103.2 ± 3.58 ^a	34.3 ± 1.17 ^a	33.2 ± 0.03 ^{ns}
4	Insulin(6 IU/Kg)	5.02 ± 0.12 ^a	14.7 ± 0.27 ^a	44.0 ± 0.83 ^a	87.8 ± 2.11 ^{ns}	29.3 ± 0.69 ^{ns}	33.3 ± 0.01 ^{ns}

Values are mean ± SEM.

Values are statistically significant compared to control group at: ^aP < 0.05, ns = not significant.

Table 3. Effects of daily doses of ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract on leucocytes of diabetic wistar rats.

Group (n=5)	Treatment given	WBC×10 ⁹ /L	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Lymphocyte (%)	Monocytes (%)
1	Control(Tween20)	10.86± 0.57	29.6 ± 1.50	3.2 ± 0.37	1.0 ± 0.0	61.2 ± 1.07	4.6 ± 0.51
2	Ethylacetate(50mg/Kg)	16.62±0.53 ^a	39.8 ± 1.16 ^a	3.2 ± 0.58 ^{ns}	1.0 ± 0.0 ^{ns}	51.4 ± 1.08 ^a	4.8 ± 0.58 ^{ns}
3	n-Butanol(50mg/Kg)	16.48±1.35 ^a	38.8 ± 1.24 ^a	3.4 ± 0.6 ^{ns}	0.6 ± 0.24 ^{ns}	52.0 ± 2.17 ^a	5.2 ± 0.97 ^{ns}
4	Insulin (6 IU/Kg)	8.5 ± 1.15 ^{ns}	34.6 ± 1.66 ^a	3.6 ± 0.51 ^{ns}	0.8 ± 0.2 ^{ns}	56.6 ± 1.69 ^{ns}	4.6 ± 0.75 ^{ns}

Values are mean ± SEM.

Values are statistically significant compared to control group at: ^aP < 0.05, ns = not significant.

treated group. There was no significant change in the red cell indices in the Ethylacetate treated group when compared to control group.

Effects of daily doses of ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract on leucocytes of diabetic wistar rats

There was a statistically significant increase (p < 0.05) in the total white cell count and Neutrophils and a significant (p < 0.05) decrease in the Lymphocytes counts of both the Ethylacetate and n-Butanol treated diabetic groups when compared to control as shown in Table 3. There was statistically significant increase (p < 0.05) in the Neutrophils count, but no significant change in the total white cell count in the group treated with Insulin when compared to control as shown in Table 3.

DISCUSSION

Medicinal plant extracts have been valuable anti-diabetic agents and may involve one or more active components responsible for blood glucose reduction (Marles and Farnsworth, 1995; Grover et al., 2002). Flavonoids of different plant origin showed a promising anti-diabetic activity, as demonstrated in diabetic animal models (Zarzuelo et al., 1996; Nojima et al., 1998; Kim et al., 2004). Saponins are glycosides of triterpenes, steroids or alkaloids. Previous researchers have demonstrated the hypoglycemic activity of triterpenoid glycosides (Reher et al., 1991; Kako et al., 1997). There was a significant reduction (p < 0.05) in the fasting blood glucose levels of diabetic Wistar rats treated for two weeks with both Ethylacetate and n-Butanol fractions of *G. lucidum* aqueous extract when compared to control group. However, the diabetic group that received Insulin treatment had a sig-

nificant reduction ($p < 0.05$) in the fasting blood glucose levels after one week of treatment only. The preliminary phytochemical screening of these fractions of *G. lucidum* aqueous extract revealed the presence of flavonoids and saponins which may be responsible for the observed anti-diabetic effects of these fractions by possibly stimulating insulin release from pancreatic beta cells. In consonant with this study, some researchers reported that alcoholic extract of leaves of *Cinnamomum tamala* (Bayberry) produced hypoglycemic activity in alloxan induced diabetic rats when administered orally for two weeks at a dose of 250 mg/kg (Kar et al., 2003). Also, aqueous and ethanolic extracts of the fruit-pulp of *Eugenia jambolana* has been reported to produce antihyperglycemic effect in alloxan diabetic rats, and 24.4% raise in plasma insulin level in mild diabetic and 26.3% in severely diabetic rabbits (Sharma et al., 2006). Kumar et al. (2007) also reported a significant reduction ($p < 0.05$) of the fasting blood glucose levels of alloxan-induced diabetic rats when given ethanolic extract of *Ficus microcarpa* leaves orally for two weeks.

Reactive oxygen species has also been implicated in the mechanism of red cells damage (Rao et al., 2003). During diabetes the excess glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin. So the total haemoglobin level is decreased in alloxan diabetic rats (Sheela and Augusti, 1992). The results of the red cell indices of this study revealed a statistically significant ($p < 0.05$) decrease in the red cell count and a significant ($p < 0.05$) increase in MCV and MCH of the diabetic groups that received n-Butanol fraction of *G. lucidum* aqueous extract. There was no significant change in the red cell indices of the diabetic group that received Ethylacetate fraction of *G. lucidum* aqueous extract. This indicates that saponins found in the n-Butanol fraction have deleterious effects on red blood cells by possibly causing haemolytic effects on red cell membranes. In Insulin treated diabetic group, there was a statistically significant ($p < 0.05$) increase in the red cell count, Hb and PCV. These results are consistent with the findings of some researchers on the effects of plant extracts on red cell indices of experimental animals (Maphosa et al., 2008; Ashafa et al., 2009).

The increase in total leucocytes and the neutrophils by Ethylacetate and n- Butanol fractions of *G. lucidum* aqueous extract may indicate an anti- infective effect, but not a boost in the immune system as exhibited by some other plant extracts reported earlier by some researchers (Yakubu et al., 2007). However, the results of this study are consistent with the results of some researchers who reported that triterpenoids such as ganoderiol F, ganodermanondiol, and ganodermanontriol from *G. lucidum* had a potent anticomplement activity against the classical pathway of complement system (Min et al., 2001).

In conclusion, both Ethylacetate and n-Butanol fractions of *G. lucidum* aqueous extract were found to have

potent anti-diabetic effects. Both fractions may also have an anti- infective effect as they were found to have significantly increased the total leucocytes and neutrophils counts. The Ethylacetate fraction has no significant effect on the red cell indices while the n- Butanol fraction showed a significant decrease in the red cell indices after the treatment period.

REFERENCES

- Adedapo AA, Abatan MO, Olorunsogo OO (2004). Toxic effects of some plants in the genus *Euphorbia* on haematological and biochemical parameters of rats. *Vet. Arhiv.* 74: 53–62.
- Adedapo AA, Abatan MO, Olorunsogo OO (2007). Effects of some plants of the spurge family on the haematological and biochemical parameters of rats. *Vet. Arhiv.* 77: 29–38.
- Ashafa AOT, Yakubu MT, Grierson DS, Afolayan AJ (2009). Toxicological evaluation of the aqueous extract of *Felicia muricata* Thunb. leaves in Wistar rats. *Afr. J. Biotechnol.* 8 (6): 949-954.
- Balkau B, Charles MA, Eschwège E (2000). Discussion épidémiologique des nouveaux critères du diabète. *Mt Endocrinologie* 2: 229-34.
- Blood DC, Radostits OM (1989). *Veterinary medicine*. London: Balliere Tindall pp. 127–130.
- Dacie, J.V., Lewis, S. (1991). *Practical Hematology*, 7th edition, Churchill Livingstone, New York pp.50-56.
- Dhandapani, S, Ramasamy SV, Rajagopal S, Namasivayam N (2002). Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol. Res.* 46 (3): 251-255.
- Djrolo F, Houngbe H, Avode G, Addra B, Kodjoh N, Avinadje M, Monterio B (1998). Le diabète lié à la mal nutrition (Diabète tropical). *Médecine Africaine Noire*, 45 (8/9): 538-542.
- Duncan RC, Knapp RG, Miller MC (1977). Test of hypothesis in population Means. In: *Introductory Biostatistics for the health sciences*. John Wiley and Sons Inc. NY: pp.71-96.
- El-Mekawy S, Meselhy MR, Nakamura N, Tezuka Y, Hattori M, Kakiuchi N, Shimotohno K, Kawahata T, Otake T (1998). Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. *Phytochemistry* 49: 1651-1657.
- Eo SK, Kim YS, Lee CK, Han SS (2000). Possible mode of antiviral activity of acidic protein bound polysaccharide isolated from *Ganoderma lucidum* on herpes simplex viruses. *J. Ethnopharmacol.* 72: 475-481.
- Farnsworth RN (1966). Review on Biological and Phytochemical screening of plants. *J. Pharm. Sci.* 55: 225-276.
- Grover JK, Yadav S, Vats V (2002). Medicinal plants of India with hypoglycemic potentials. *J. Ethnopharmacol.* 81: 81-100.
- Harborne JB (1998). *Phytochemical Methods: A guide to modern techniques of plant analysis*; 3rd Edition. Chapman and Hall, London: 235.
- Houghton PJ, Raman A (1998). *Laboratory handbook for fractionation of natural extracts*. Chapman and Hall, London: 199.
- Kako M, Miura T, Nishiyama Y, Ichimaru M, Mariyasu M, Kato A (1997). Hypoglycemic activity of some Triterpenoid Glycosides. *J. Nat. Prod.* 60: 604-605.
- Kar A, Choudhary BK, Bandyopadhyay NG. (2003). Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *J. Ethnopharmacol.* 84: 105-108.
- Katsumata KY, Katsumata TO, Katsumata K (1999). Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan-induced diabetes in rats. *Horm. Metab. Res.* 25: 125-126.
- Kim HY, Moon BH, Lee HJ, Choi DH (2004). Flavonoids glycosides from the leaves of *Eucommia ulmoides* with glycation inhibitory activity. *J. Ethnopharmacol.* 93: 227-230.
- Kumar KA, Maheswari MU, Sivashanmugam AT (2007). Hypoglycemic effects of *Ficus microcarpa* leaves (Chinese Baryan) on alloxan-induced diabetic rats. *J. Biol. Sci.* 7(2): 321-326.
- Lorke D (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol.* 54: 275-287.
- Maphosa V, Masika PJ, Adedapo AA (2008). Safety evaluation of the.

- aqueous extract of *Leonotis leonurus* shoots in rats. *Hum. Exp Toxicol.* 27: 837-843.
- Marles R, Farnsworth N (1995). Antidiabetic plants and their active constituents. *Phytomedicine* 2: 137-165.
- Min BS, Gao JJ, Hattori M, Lee HK, Kim YH (2001). Anticomplement activity of terpenoids from the spores of *Ganoderma lucidum*. *Planta Med.* 67(9): 811-814.
- Mohammed A, Adelaiye AB, Abubakar MS, Abdurahman, E.M. (2007). Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of normoglycemic and Alloxan-induced diabetic Wistar rats. *J. Med. Plant Res.* 1(2): 34-37.
- Nojima H, Kimura I, Chen FJ (1998). Anti-hyperglycemic effects of N-containing sugars from *Xanthocercis zambesiaca*, *Morus bombycis*, *Aglaonema trubii* and *Castanospermin australe* in STZ-diabetic mice. *J. Nat. Prod.* 61: 397-400.
- Onoagbe IO, Esekheigbe A (1999). Studies on the anti-diabetic properties of *Uvaria Chamae* in streptozotocin-induced diabetic rabbits. *Biokemistri* 9: 79-84.
- Rao GU, Kamath C, Raghobhama KSP, Rao P (2003). Maternal and fetal indicators of oxidative stress in various obstetric complications. *Ind. J. Clin. Biochem.* 18: 80-86.
- Reher G, Slijepcevic M, Krans L (1991). Hypoglycemic activity of triterpenes and tannins from *Sarcopoterium spinosum* and two *Sanguisorba* Species. *Planta Med.* 57: A57-A58.
- Rheny CC, Kirk KK (2000). Performance of three blood glucose meters. *Ann Pharmacother.* 34(3): 317-21.
- Sharma SB, Nasir A, Prabhu KM, Murthy PS (2006). Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus. *J. Ethnopharmacol.* 104: 367-373.
- Sheela CG, Augusti KT (1992). Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. *Indian Journal of Experimental Biology*, 30: 523-526.
- Silva LG, Lee IS, Kinghorn DA (1993). Special problems with the extraction of plants. In: *Methods in Biotechnology Natural product isolation*. Cannell JPR (ed) Humana, Press Inc., Totowa, New Jersey, USA. 4: 329-363.
- Stanley, P mainzen P, Venugopal MP (2001). Anti-oxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytother Res.* 15: 213-218.
- Trinder P (1969). Determination of Glucose in blood using glucose-oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.* 6: 24-27.
- Wang SY, Hsu ML, Hsu HC, Tzeng CH, Lee SS, Shiao MS, Ho CK (1997). The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. *Int. J. Cancer* 70: 699-705.
- WHO.(2002). WHO launches the first global strategy on traditional medicine: Press release WHO p.38.
- Yakubu MT, Akanji MA, Oladiji AT (2007). Haematological evaluation in male albino. Rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. *Pharmacol. Manage.* 3: 34-38.
- Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim HS, Lee IK, Bonner-Weir S (2003). Selective -cell loss and -cell expansion in patients with type 2 diabetes mellitus in Korea. *J. Clin. Endocrinol. Metab.* 88: 2300-2308.
- Young NS, Maciejewski J (1997). The pathophysiology of Acquired Aplastic anemia. *N. Eng. J. Med.* 336: 1365.
- Zarzuolo A, Jimenez I, Gamez MJ (1996). Effects of luteolin 5-O-beta-rutenoside in STZ-induced diabetic rats. *Life Sci.* 58: 2311-2316.