**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.com/
**Research Article**

# Glucose lowering potential of hydromethanolic extract of *Rauwolfia*

Muhammad Bilal Azmi and Shamim A. Qureshi*

Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan

**Received: 15-01-2014 / Revised: 08-02-2014 / Accepted: 25-02-2014**

## ABSTRACT

The objective of the present study was to evaluate the phytochemistry, acute toxicity and glucose lowering potential of hydromethanolic roots extract (HMREt) of *Rauwolfia serpentina*. The qualitative analysis of HMREt showed the presence of many important phyto-constituents except anthraquinones, carbohydrates and saponins whereas quantitatively it found rich in total phenols. In acute toxicity study, orally administrated HMREt from 5-250 mg/ kg was observed safe and non-sedative while its doses from 500-2500 mg/kg were found sedative and induced mortalities (17-100%) within 4 hours of administration. The median lethal dose ($LD_{50}$) of same extract was calculated as 1412.54 mg / kg (log $LD_{50}$ = 3.15 mg/ kg) from log doses verses probit graph. The HMREt in doses of 50, 100 and 150 mg/kg induced significant percent decrease in blood glucose level at 30, 60 and 120 minutes in normo-hyperglycemic test mice as compared to control and negative control groups ($p$<0.05). The results concluded that HMREt has glucose lowering potential either by developing glucose tolerance or by pancreatic action in normo-hyperglycemic mice.

**Key words:** acute toxicity, $LD_{50}$, phytochemistry, qualitative, *Rauwolfia serpentina*

## INTRODUCTION

Diabetes is a commonly found endocrine disorder in the world which primarily associated with absolute or relative insulin deficiency that severely disturbs glucose homeostasis, lipid and protein metabolism [1]. The disturbance in glucose homeostasis need immediate attention and life-time treatment otherwise it serves as a beginning of chronic complications that affect important tissues of the body including brain, eye, heart and kidney in old age [2]. Today, the rapidly progressing prevalence of diabetes is becoming a serious health challenge and stands as one of the leading causes of mortalities in the world [3, 4]. Pakistan is also a big sufferer of this health associated risk and international agencies ranked our country as the fourth largest country that shared the burden of morbidities and mortalities of this disorder [5]. Many orally and sub-cutaneously administrated medicines have been practiced for the management of this health dynamite globally, however, literature also witnesses the importance of herbal remedies in this regard [6]. Interestingly, commercially available metformin, a widely used medicine in type-II diabetes, is also a derivative of guanidine compound isolated from *Galega officinalis* (lilac) extract. Hence, plant kingdom is always a potential target for ethanopharmacologists in order to find a cure of many diseases including diabetes. The antidiabetic aspect of *Rauwolfia serpentina* Benth (family *Apocynaceae)* has been recently reported. However, traditionally, the same plant was reported for the treatment of snake bite, gastrointestinal tract disorders, skin problems and hypertension [7-9]. The antihypertensive activity of *R.serpentina* was strongly established after the isolation of many alkaloids including ajmaline, reserpine, etc which are widely used as hypotensive agents in herbal medicines [9, 10]. The methanolic root extract (MREt) of *R.serpentina* has been investigated so far for its antioxidant, antiatherogenic and cardioprotective activities in alloxan-induced diabetic mice [11-13]. Therefore, in the present study, hydromethanolic roots extract (HMREt) of *R.serpentina* is made and used to investigate its glucose lowering potential in normo-hyperglycemic mice after determining its total phenol content and median lethal dose ($LD_{50}$). This study will provides a good comparison between glucose lowering effect of HMREt and previously described effect of MREt on same aspect.

***Corresponding Author Address: Dr. Shamim A. Qureshi, Assistant Professor,** Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan; E-mail: qureshi29@live.com*

## MATERIALS AND METHODS

***Roots of Rauwolfia serpentina:*** These were purchased from Hamdard Dawakhana, Saddar, Karachi (Voucher specimen: KU/BCH/SAQ/02) and identified by an expert in Botany Department, University of Karachi (UoK), Karachi-75270, Pakistan.

***Preparation of Hydromethanolic Roots Extract (HMREt):*** It was prepared by extracting 10 grams of ground roots powder of *R.serpentina* with 100 ml of 80% aqueous methanol repeatedly at room temperature. The extract was filtered through *Whatman* filter paper No.42. Then filtrate was transferred into a crucible and evaporated over a water bath to obtain brown residue that referred as HMREt, this process continues until a constant weight is achieved [11]. The quality of extract was maintained by keeping in an airtight container and stored in refrigerator below 10°C until used.

***Phytochemical Screening:*** HMREt was subjected to qualitative analysis in order to determine important groups of phyto-constituents by using standardized methods as described earlier by Azmi and Qureshi [12] and then same extract was analyzed for total phenols through spectrophotometric method [14].

***Dimethyl Sulphoxide (DMSO) and Positive Control:*** DMSO of Fisher Scientific (UK) was used (0.05%) as vehicle for administering the doses of HMREt in experimental test mice and glibenclamide (commercial name: *Daonil*) of Sanofi-Aventis Pakistan Ltd. in a dose of 5mg/kg used as positive control [15].

***Animals and Experimental Protocol****:* The protocol of present study was approved by Board of Advance Study and Research (BASR) of UoK (*BASR No./0532/Sc*). The *Wister* albino male mice (20 - 30g) were purchased from the animal house of Dow University of Health Sciences (DUHS), Ohja campus Karachi, Pakistan and kept according to the international guidelines of animal handling by placing them individually in cages under temperature (23±02 $^0$C) with free access to water *ad libitum* and fed standard diet.

***Determination of Acute Toxicity and $LD_{50}$:*** Overnight fasted total sixty six (66) mice were randomly divided into eleven groups (6 /group). HMREt in a dose of 5, 10, 50, 100, 250, 500, 1000, 1500, 2000 and 2500 mg/kg was separately administrated orally to mice of its respective test group, whereas six mice took as a control group and treated with distilled water (1 ml/kg) orally. The mice in both test and control groups were then allowed free access to food and water but their activity was carefully monitored over a period of 12 hours for observing behavior change (sedative or not) and mortality rate [16]. Finally, $LD_{50}$ of HMREt was determined by plotting a graph between log doses of extract verses probit [17].

***Glucose Lowering Effect of HMREt in Normo-hyperglycemic Mice:*** Nearly 12-14 hours overnight fasted mice were divided into different groups (6 /group) on the basis of oral treatments such as control & negative control groups treated with distilled water and 0.05% DMSO respectively, each in dose of 1 ml/kg and positive control group treated with glibenclamide (5 mg/kg). The test group was further sub-divided into four groups and treated with HMREt (10, 50, 100 and 150 mg/kg respectively). Each group after receiving its allotted treatment was immediately administered with glucose load (2 g/kg) orally. Blood glucose was monitored by pricking the tail vein of mice at 0, 30, 60, and 120 min in each group via a glucometer (*Optium Xceed*, Diabetes Monitoring system by Abbott). Percent change (decrease/increase) in blood glucose level between control and test groups on each time interval was calculated by using the following formula [15]

**Percent change in blood glucose level (%) =**
**$[(G_x - G_o) / G_o] \times 100$**

Where Go = blood glucose level of control group on specified time interval and Gx = blood glucose level of positive control and all test groups on time interval respective to control.

***Statistical Analysis****:* The results are expressed as mean ± SEM (Standard Error of Mean) and analyzed by *one way* ANOVA and LSD (least significance difference) tests (Statistical Package Software for Social Sciences version 18). The differences of test group were considered significant at $p<0.05$ when compared with respective control.

## RESULTS AND DISCUSSION

Medicinal plants are traditionally used in the treatment of diseases, of which many plants are successfully available for the treatment of diabetes by herbal practitioners in developing countries like Pakistan. Similarly, the alcoholic extract of *R. serpentina* is still in use as a hypotensive agent in homeopathic medicines. On the contrary, the antidiabetic activity of methanolic root extract (MREt) of same plant has recently been introduced in scientific literature [11-13, 15]. In order to give more strength to antidiabetic potential of *R.serpentina*, the present study used hydromethanolic roots extract (HMREt) of same

plant which showed the presence of alkaloids, flavonoids, glycosides, cardiac glycosides, phalobatanins, resins, steroids, tannins, triterpenoids, total phenols but no anthraquinones, carbohydrate (reducing sugars) and saponins (Table 1) whereas carbohydrate and saponins were present in previously reported MREt [12]. In addition, the HMREt was found to have more total phenol content (321.6 mg/g of starting material) quantitatively (Table 1) than MREt that contained 233.33 mg/g [12]. Interestingly, in acute toxicity study, the doses of HMREt from 5 to 250 mg/kg were found non-sedative and non-lethal by observing normal behavior and no mortality in mice of their respective test groups.

However, in previous study MREt of *R. serpentina* in a dose of 250 mg/kg appeared highly toxic and produced 100% mortality in mice [15]. On the other hand, the presently used HMREt in doses of 500 and 1000 mg/kg found mild sedative by showing 17-33% mortalities while doses of 1500 and 2500 mg/kg of same extract were found moderate to extremely sedative in mice of their respective test groups by inducing 50-100% mortalities within 4 hours of their administration (Table 2). Therefore, the $LD_{50}$ of HMREt was determined as 1412.54 mg/kg (log $LD_{50}$ = 3.15 mg/kg) by plotted a graph between log doses verses probit (Figure 1) and it is approximately ten (10) times higher than $LD_{50}$ of MREt found in our previous study [15]. This marked increased in $LD_{50}$ may be due to the presence of high total phenol content in HMREt.

In the present study, for determining glucose lowering effect of HMREt, its doses of 10, 50, 100 and 150 mg/kg were selected (Table 3). Of which, last three doses (50, 100 and 150 mg/kg) were found to produce significant percent reduction in blood glucose levels in normo-hyperglycemic mice at 0, 30, 60 and 120 min immediately after glucose load (2 g/kg) as compared to control (normo-hyperglycemic) and negative control groups ($p<0.05$). Interestingly, the magnitude of decreasing blood glucose level at 30, 60 and 120 min increases exponentially with the increase in dose concentration (10-150 mg/kg) of extract as -14.09 to -32.39 %, -26.44 to -33 % and -6.15 to -28.75% respectively. However, the same extract at 10 mg/kg was not found as effective as its other three high doses. Whereas, a significant percent decrease in glucose level at each time interval was observed by glibenclamide in positive control group ($p<0.05$). Therefore, the glucose lowering effect of HMREt may be either by inhibiting glucose absorption in gastrointestinal tract as extract of plants rich in phenols was reported to inhibit glucose absorption in rabbit intestinal epithelial cells [18] or by inducing glucose tolerance in mice via enhancing the glucose uptake in tissues such as muscles, liver and thereby stimulating the processes of glycolysis & glycogenesis and/or by improving the insulin release from β-cells of pancreas as did by MREt of *R.serpentina* in previous study [12]. The last two possibilities were also approved by observing the glucose lowering effect of glibenclamide which is an efficacious oral hypoglycemic agent belongs to a class of sulfonylureas (SU), primarily responsible to enhance the secretion of insulin from functional β-cells of pancreas in non-insulin dependent diabetes [19]. However, the extra-pancreatic effect of SU has also been reported [20]. There are many medicinal plants reported to have insulin releasing effect from β-cells of pancreas such as *Gymnema sylvestre*, *Ocimum sanctum*, *Phyllanthus species* [21-24].

## CONCLUSION

The $LD_{50}$ of HMREt of *R. serpentina* found much higher than MREt of same plant. Similarly, the same extract in doses of 50, 100 and 150 mg/kg found effective in lowering the blood glucose levels at 0, 30, 60 and 120 min in normo-hyperglycemic mice.

**Conflict of Interest:** The authors declare that there is no conflict of interests regarding the publication of this article.

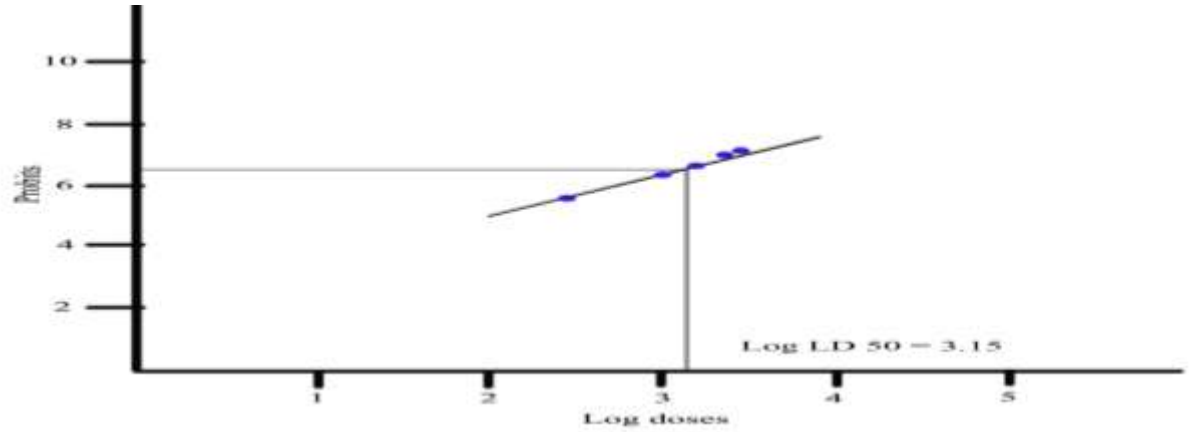


Figure 1: **$LD_{50}$ of HMREt**

**Table 1: Phytochemical analysis of HMREt**

| Constituents | Analysis | |
|---|---|---|
| | **Qualitative** | **Quantitative** |
| Alkaloids | Positive | - |
| Anthraquinones | Negative | - |
| Carbohydrates (reducing sugars) | Negative | - |
| Flavonoids | Positive | - |
| Glycosides | Positive | - |
| Cardiac glycosides | Positive | - |
| Phlobatannins | Positive | - |
| Resins | Positive | - |
| Saponins | Negative | - |
| Steroids | Positive | - |
| Tanins | Positive | - |
| Triterpenoids | Positive | - |
| Total Phenols | - | 321.6 mg/g |

**Table 2: Acute Toxicity of HMREt**

| Treatments | Behavioral change | | |
|---|---|---|---|
| | **Normal** | **Sedative** | **Mortality rate (%)** |
| Control (DW 1ml/kg) | √ | - | 0 |
| HMREt (5mg/kg) | √ | - | 0 |
| HMREt (10mg/kg) | √ | - | 0 |
| HMREt (50mg/kg) | √ | - | 0 |
| HMREt (100mg/kg) | √ | - | 0 |
| HMREt (250mg/kg) | √ | - | 0 |
| HMREt (500mg/kg) | - | + | 17(1/6) |
| HMREt (1000mg/kg) | - | + | 33(2/6) |
| HMREt (1500mg/kg) | - | ++ | 50(3/6) |
| HMREt (2000mg/kg) | - | +++ | 83(5/6) |
| HMREt (2500mg/kg) | - | +++ | 100(6/6) |

DW = distilled water; √ = normal behavior; - = sedation or normal behavior was not observed
+ = slight sedation and 17- 33% mortality were observed within 4 hours of administration of dose
++ = moderate sedation and 50% mortality were observed within 4 hours of administration of dose
+++ = extreme sedation with 83-100 % mortality was observed

**Table 3: Glucose Lowering Effect of HMREt**

| Groups | Treatments | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 120 min |
| Control | distilled water(1ml/kg) + glucose load (2g/kg) | 113± 3.51 | 198.67± 25.44 | 179 ± 12.70 | 135.67 ± 3.18 |
| Negative control | 0.05% DMSO (1ml/kg) + glucose load (2g/kg) | 141± 4.58 | 260.33 ± 9.77 | 184± 10.41 | 128.33± 11.67 |
| Positive control | *Glibenclamide* (5 mg/kg) + glucose load (2g/kg) | 106± 7.26*[b] (-6.19%) | 117± 14.80*[ab] (-41.11%) | 96.67± 6.96*[ab] (-45.99%) | 82.67 ± 4.41*[ab] (-39.07%) |
| Test | HMREt (10 mg/kg) + glucose load (2g/kg) | 111.67± 4.26*[b] (-1.18%) | 203.33± 31.02 (+2.35%) | 175.67± 29.76 (-1.86%) | 130 ± 14.42 (-4.18%) |
| | HMREt (50 mg/kg) + glucose load (2g/kg) | 78.33 ± 6.96*[ab] (-30.68%) | 170.67 ± 17.89*[b] (-14.09%) | 131.67± 8.41*[ab] (-26.44%) | 127.33 ± 6.17 (-6.15%) |
| | HMREt (100 mg/kg) + glucose load (2g/kg) | 87.67± 11.35*[ab] (-22.42%) | 138 ± 23.86*[b] (-30.54%) | 118.67± 6.49*[ab] (-33.70%) | 114.33 ± 5.24 (-15.73%) |
| | HMREt (150 mg/kg) + glucose load (2g/kg) | 97.67± 12.24*[b] (-13.57%) | 134.33 ± 12.44*[ab] (-32.39%) | 119.67 ± 8.35*[ab] (-33.15%) | 96.67 ± 3.84*[ab] (-28.75%) |

Values are expressed as mean ± SEM (n=6). Values in parenthesis represent percent decrease (-) / increase (+) in blood glucose level compared with respective control group. * = $p < 0.05$ when compared with respective control (a) and negative control (b) groups.

**REFERENCES**


1. Shrivastava SR et al. Role of self-care in management of diabetes mellitus. J Diabetes & Metab Dis 2013; 12: 14.
2. Hussein AA et al. Myocardial and metabolic dysfunction in type 2 diabetic rats: impact of ghrelin. Canadian J Physiol & Pharmacol 2012; 90: 99-111.
3. Aggarwal N, Shishu. A review of recent investigations on medicinal herbs possessing anti- diabetic properties. J. Nutrit Dis & Theraphy 2011; 1: 102.
4. Wild S et al. Global prevalence of diabetes; estimates for the year 2000 and projection for 2030. Diabetes Care 2004; 27: 1047-1053.
5. Shaikh ZA et al. Diabetic patients, awareness about life style modifications. Prof Med J 2011; 18**:** 265-268.
6. Tiwari S. Plant: A rich source of herbal medicine. J. Nat Prod 2008; 1: 27-35.
7. Dey A, De JN. Ethnobotanical aspects of *Rauwolfia serpentina* (L). Benth. ex Kurz. in India, Nepal and Bangladesh. J Medicinal Plant Res 2011; 5: 144–150.
8. Itoh A et al. Indole alkaloids and other constituents of *Rauwolfia serpentina*. J Nat Prod 2005; 68: 848–852.
9. Stanford JL et al. Rauwolfia use and breast cancer: a case-control study. J National Cancer Instit 1986; 76: 817– 822.
10. Von Poser G et al. Genotoxic, mutagenic and recombinogenic effects of *rauwolfia* alkaloids. Mutat Res 1990; 232: 37-43.
11. Qureshi SA et al. Hypoglycaemic and hypolipidemic activities of *Rauwolfia serpentina* in alloxan - induced diabetic rats. Int. J Pharmacol 2009; 5: 323-326.
12. Azmi MB, Qureshi SA. Methanolic root extract of *Rauwolfia serpentina* Benth improves the glycemic, antiatherogenic and cardioprotective indices alloxan induced diabetic mice. Adv Pharmacol Sci 2012; 2012, Article ID 376429: 11pages.
13. Azmi MB, Qureshi SA. *Rauwolfia serpentina* ameliorates hyperglycemic, haematininc and antioxidant status in alloxan-induced diabetic mice. J Appl Pharm Sci 2013; 3: 136-141.
14. Sengul M et al. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci 2009; 22: 102–106.
15. Azmi MB, Qureshi SA. Methanolic root extract of *Rauwolfia serpentine* Benth improves the glucose tolerance in Wister mice. J Food Drug Anal 2012; 20: 54-58.
16. Bhatia A, Mishra T. Hypoglycemic activity of *Ziziphus mauritiana* aqueous ethanol seed extract in alloxan- induced diabetic mice. Pharm Biol 2010; 48: 604 – 610.
17. Miller LC, Tainter ML. Estimation of $LD_{50}$ and its error by means of log-probit graph paper. Proceeding of the Society for Experiment Biol & Medicine 1944; **57**: 261-264.
18. Shimizu M et al. Regulation of intestinal glucose transport by tea catechins. Biofactor 2000; 13**:** 61-65.
19. Davis SN. Insulin, oral hypoglycemic agents, and the pharmacology of the Endocrine Pancreas. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th ed, Brunton LL, Lazo JS, Parker KL, Eds; McGraw-Hill Co. Incorp, USA, 2006; pp. 1613-1645.
20. Kaku K, Inoue Y, Kaneko T. Extrapancreatic effects of sulfonylurea drugs. Diabetes Res Clini Pract 1995; 28: S105-S108.
21. Al-Romaiyan A et al. A novel *Gymnema sylvestre* extract stimulates insulin secretion from human islets *in vivo* and *in vitro*. Phytotherap Res 2010; 24**:** 1370-1376.
22. Daisy P, Averal HI, Modilal RD. Curative properties of *Phyllanthus* extract in alloxan induced diabetic rats. J Tropical and Medicinal Plants 2004; 5: 21-27.
23. Hannan JM et al. *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic beta-cells. The J Endocrinol 2006; 189**:** 127-136.
24. Qureshi SA, Asad W, Sultana V. The effect of *Phyllanthus emblica* Linn on type II diabetes, triglycerides and liver-specific enzymes. Pak J Nutri. 2009; 8: 125-128.