doi.org/10.36721/PJPS.2021.34.2.REG.529-535.1

Antioxidant and haematinic effects of methanolic and aqueous methanolic roots extracts of *Rauwolfia serpentina* Benth in type 2 diabetic mice

Muhammad Bilal Azmi[1,2*], Shamim A Qureshi[1], Shakil Ahmed[3], Saleha Sultana[1], Auwais Ahmed Khan[4] and Hina Akram Mudassir[5]

[1]Department of Biochemistry, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan
[2]Department of Biochemistry, University of Karachi, Karachi, Pakistan
[3]Industrial Analytical Center, HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan
[4]Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[5]Department of Biochemistry, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

Abstract: Present investigation was carried out to evaluate the antioxidant and haematinic effects of methanolic (MREt) and aqueous methanolic (AqMREt) root extracts of *R. serpentina* in mice model of type 2 diabetes (T2D). Experimental mice were divided into nine groups (six per group) as: fructose-induced (T2D) diabetic group (distilled water 1ml/kg), negative control (0.05% DMSO 1ml/kg), positive control (pioglitazone 15mg/kg) and six test groups (MREt 10, 30 & 60mg/kg & AqMREt 50, 100 & 150mg/kg). Whereas tenth group was served as normal control (1ml/kg distilled water). All test doses of MREt & AqMREt significantly ($p<0.05$) decreases the percent inhibition of catalase (CAT) and superoxide dismutase (SOD) when compared with diabetic controls. Treatment with both extracts also improved the total hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) counts, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in test groups. Fourier transform infrared (FTIR) spectral analysis revealed the presence of phenols moiety in both extracts. Findings suggested that AqMREt possesses more antioxidant and haematinic potential while the MREt of *R. serpentina* moderately possesses the same activities, which might be due to the high content of phenols present in AqMREt.

Keywords: Antioxidant, haematinic, *Rauwolfia serpentina.*

INTRODUCTION

Oxidation is the characteristic feature of majority of living cells for the generation of energy to fortify the needs (fuel) of biological processes. Biochemically, oxidation represents a set of chemical reaction that involves the transfer of electrons or hydrogen atoms from a substance to an oxidizing agent which ultimately generates free radicals in the cellular environment (Davies, 2000). These radicals can generally be categorized into reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Pham-Huy *et al.,* 2008). Most common sources of these reactive species include electron transport chain (ETC) in mitochondria, NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, myeloperoxidase of neutrophils and xanthine oxidase of endothelial cells (Seifu *et al.,* 2012). Frequent generation of ROS or RNS produce oxidative stress, that have harmful impacts on body by reacting with macromolecules including carbohydrates, lipids, nucleic acids and proteins (Davies, 2000) and reported to induce many problems like aging, atherosclerosis, cancer, cardiovascular diseases, diabetes, non-enzymatic glycosylation, inflammatory consequences, due to the imbalance between radical generating and scavenging systems in body (Seifu *et al.,* 2012). Similarly, the subsequent productions of ROS also reduce the half-life (t½) of erythrocytes by altering their osmotic fragility through engendering superoxide radicals, with increased xanthine oxidase action (Azmi *et al.,* 2013). These radicals involved in oxidation of erythrocytes' membrane proteins and lipids, that make them more prone to hemolysis (Saba *et al.,* 2010), which in turn disturb the entire haematinic potential of the body (Azmi *et al.,* 2013).

Interestingly, nature equipped living machinery with important defense systems to detoxify radicals, which are referred as 'antioxidants' (Pham-Huy *et al.,* 2008; Seifu *et al.,* 2012). These antioxidants prevent the radical-induced oxidative process either by direct scavenging the free radicals or preventing their formation by inhibiting the activity of particular oxidase enzyme which thereafter improves the defense mechanisms of body.

Worldwide recognized antihypertensive phytomedicine *Rauwolfia serpentina* Benth (family *Apocynaceae*) is also well-known for its ethno-medicinal usefulness which include the cure of snake (venom) bite, gastrointestinal tract disorders, breast cancer, skin problems, etc (Qureshi and Udhani, 2009; Dey and De, 2011). In recent years, its

**Corresponding author:* e-mail: azmibilal@gmail.com

short and long-term antidiabetic activities have been reported in alloxan-induced diabetic mice and in fructose-induced type 2 diabetic mice, where it was found to improve the cardioprotective indices (Qureshi *et al.,* 2009; Azmi and Qureshi, 2012; Azmi and Qureshi, 2016). Similarly, in type 1 diabetic mice, the antioxidant and heamatinic potential of the same plant extract was also reported (Azmi and Qureshi, 2013). In relevance to the past concepts, the present study aimed for the exploration of antioxidant and haematinic effects of methanolic (MREt) and aqueous methanolic (AqMREt) root extracts of *R.serpentina* in mice model of type 2 diabetes (T2D).

MATERIALS AND METHODS

Animals

In present study, adult male *Wister* albino mice (25±5 g) were used. Mice were acclimatized for one week prior starting experiments. Each mice was retained in a cage of stainless steel at controlled temperature (23±2^{o}C), humidity (55±10%), with consecutive light/dark (12/12 hr) cycle. Mice had free access to food and water. Animal handling and care were in relevance to the worldwide established standard procedures for animal handling.

Institutional ethical permission

Experimental design and animal handling procedures was approved by the Institutional Ethical Review Board (IERB) of Dow University of Health Sciences (DUHS), Karachi, Pakistan (Reference Letter Number: IRB-186/DUHS-10).

Medicines and reagents

Pioglitazone (*Zolid* 15mg/kg) as positive control (antidiabetic medicine) was obtained from Getz Pharma, Pakistan Ltd. Analytical grade dimethyl sulphoxide (DMSO) was procured from Fisher Scientific (UK), and used as vehicle (0.05%) for administering the doses of MREt & AqMREt in experimental mice. White crystalline powder of D (-) Fructose with molecular weight 180.16 and chemical formula $C_6H_{12}O_6$ was purchased from AnalaR, BDH Laboratories Supplies, England.

Plant and preparation of experimental extracts

Roots of *R.serpentina* were used as an experimental material and purchased from the Hamdard Dawakhana, Saddar, Karachi, Pakistan. The plant specimen was verified and authenticated from Department of Botany, University of Karachi, Pakistan, before use. Methanolic roots extract (MREt) and aqueous methanolic roots extract were prepared in accordance to the methods described earlier in Azmi and Qureshi, 2012 and Azmi and Qureshi, 2014, respectively.

Induction of type 2 diabetes

It was induced in fasted (12-14 hrs) mice by daily oral administration of 10% solution of D (-) fructose (1ml/kg) 30 min before providing standard laboratory diet (Azmi and Qureshi, 2016).

Experimental grouping of animals

Overnight fasted mice (12-14 hrs) were randomly divided into ten groups (6/group), according to the treatments (fig. 1). Each treatment was given orally once per day, for consecutively 14 days.

Each treatment was given orally once per day for consecutively 14 days. After the completion of animal trial, mice were sacrificed. Whole blood, serum and livers were collected to analyze hematological, antioxidant and biochemical parameters (Neeharika *et al.,* 2012).

Determination of fasting blood glucose

Blood glucose fasting levels were examined by pricking the tail vein of each mice of each group by using glucometer at initial (0) and final (14th) day of trial and recorded as milligram per deciliter (mg/dl).

Determination of antioxidant enzymes

Antioxidant enzymes viz., catalase (CAT) and superoxide dismutase (SOD) were measured in liver homogenate by manual methods (Azmi and Qureshi, 2013).

Blood sampling and assessment of blood profile

At the end of trial, mice were sacrificed a portion of whole blood was collected in a commercially available ethylenediamine tetra acetic acid (EDTA) coated tubes (purple-topped tube) for analyzing complete blood profile (CBP) including total hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), packed cell volume or hematocrit (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration by Sysmex (XS-1000i) automated hematology analyzer.

Preparation of samples for fourier transform infra-red (FTIR) spectroscopic analysis

FTIR analysis of root extracts (MREt and AqMREt) were performed on Nicolet 6700 Thermo Fischer Scientific Infrared spectrophotometer. In conjugation with infrared spectroscopy Attenuated Total Reflectance (ATR) of smart orbit of Thermo Scientific (Diamond 30,000-200 cm^{-1}) was used. The spectrum was recorded in a region of 4000-500cm^{-1}. Sample preparation of MREt of *R. serpentina* was done by soaking the 0.4 g of ground root powder in 10ml of methanol (95%) for overnight, sonicated for 10-15min then 7-8ml of methanol was added, vortexes for 5-10minutes and centrifuged for 10 minutes at 4000 rpm to separate the supernatant. It was transferred to 50ml volumetric flask. The whole procedure was repeated till colorless extract was appeared. However, 5-10mg of AqMREt extract of *R. serpentina* was dissolved in 10ml of 80% aqueous

Table 1: Effect of root extracts on blood glucose levels of fructose-induced T2D mice

Groups	Blood glucose level (mg/dl)	
	Initial day	Final day
Group I	104.92 ± 14.33	103.43 ± 8.85
Group II	103.33 ± 5.44	185.83 ± 4.23
Group III	99 ± 7.26	176 ± 39.66
Group IV	95.67 ± 20.01	116.33 ± 13.10^{***ab}
Group V	97.33 ± 9.29	122 ± 15.94^{***ab}
Group VI	99 ± 6.68	116.75 ± 6.55^{***ab}
Group VII	95 ± 10.68	110.33 ± 3.40^{***ab}
Group VIII	86.25 ± 15.92	128 ± 25.15^{**ab}
Group IX	111.33 ± 9.57	115.67 ± 10.66^{***ab}
Group X	104.33 ± 19.14	109.50 ± 9.18^{***ab}

Values are expressed as mean ± SD (n = 6). **p <0.01, and ***p <0.0001, when compared with group II (a) and III (b).

Table 2: Effect of root extracts on blood profile of fructose-induced diabetic mice

Groups	RBC (10^6/µl)	WBC (10^3/µl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group I	4.10 ± 0.42	3.18 ± 0.50	24.13 ± 7.50	41.41 ± 7.75	18.78 ± 2.90	37.41 ± 6.19
Group II	3.16 ± 0.18	2.08 ± 0.30	13.83 ± 2.80	37.53 ± 5.04	22.89 ± 8.48	64.70 ± 8.09
Group III	3.41 ± 0.65	3.60 ± 0.86	14.85 ± 8.07	36.30 ± 8.76	20.76 ± 7.79	50.75 ± 16.58
Group IV	3.01 ± 0.48	2.51 ± 0.31^{*b}	17.46 ± 1.38	36.17 ± 15.88	19.49 ± 7.80	39.94 ± 10.05
Group V	2.71 ± 0.77	1.30 ± 0.67^{***b}	14.07 ± 3.81	52.34 ± 4.89^{*ab}	22.50 ± 3.02	43.67 ± 9.78
Group VI	5.01±0.46^{***a**b}	1.85±0.82^{**b}	25.83 ± 3.74^{*ab}	54.78 ± 4.62^{**ab}	24.30± 0.71	46.47 ± 1.47
Group VII	3.34 ± 0.72	0.95 ± 0.53^{*a***b}	20.86 ± 7.39	55.28 ± 4.78^{**ab}	24.65± 0.86	48.70 ± 5.47
Group VIII	3.12 ± 1.60	3.17 ± 0.91	14.27 ± 7.07	46.33 ± 1.70	28.07 ± 6.68	60.60 ± 14.09
Group IX	4.39 ± 0.80^{*a}	1.83 ± 0.93	18.40 ± 6.02	41 ± 6.16	30.30 ± 3.83	64.73 ± 17.78
Group X	5.29 ± 1.02^{**ab}	1.27 ± 0.63^{*ab}	27.83 ± 5.93^{**ab}	52.33 ± 1.70^{*a}	32.27 ± 1.32	65.83 ± 3.76

Values are expressed as mean ± SD (n = 6). *p <0.05, **p <0.01, and ***p <0.0001, when compared with group II (a) and III (b)

methanol and finally made up volume till 50ml with the same 80% aqueous methanol. The root samples were kept separately on water bath at 40-50^{o}C until the dried or solid state of both extracts was obtained which then subjected to FTIR spectroscopic analysis.

STATISTICAL ANALYSIS

Results of the present study are expressed as Mean ± SD (Standard Deviation). The data were analyzed by using *one-way* ANOVA followed by LSD (least significant difference) test at p<0.05 available in statistical package for social science (SPSS *version* 18). The differences of means of test groups were considered significant at p< 0.05, p<0.01, p<0.001 and p<0.0001 when compared with means of diabetic control groups.

RESULTS

Effect of root extracts on percent glycemic change

At 14th day all the three doses of MREt (10, 30 & 60 mg/kg) showed significant (p<0.01 & p<0.0001) reduction in fasting blood glucose level with similar pattern also observed in positive control group (pioglitazone 15mg/kg) as compared to diabetic and negative control groups which showed marked increase in same parameter in fasting state. In case of AqMREt, gradual significant (p<0.0001 & p<0.0001) decrement was also observed in fasting blood glucose level of test groups in comparison with same diabetic and negative control groups (table 1).

Effect of root extracts on antioxidant enzymes

The significant (p<0.0001, p<0.001 & p<0.05) decreased in percent inhibition of CAT from 40-55% and SOD from 38-63% was observed in positive control and test groups treated with pioglitazone and doses (10-60mg/kg) of MREt as compared to diabetic and negative control groups (group II & III) that showed high percent inhibition of these enzymes up 78 and 83% respectively (fig. 2). Similarly, all three doses (50-150 mg/kg) of AqMREt significantly (p<0.0001, p<0.001 & p<0.05) decreased the percent inhibition of CAT from 42-58% and SOD from 33-67% in their respective test groups (fig. 2).

Effect of root extracts on haematological parameters

Blood profile parameters including RBC, WBC, PCV, MCV and MCHC were significantly improved (p<0.05) in test groups treated with 30 and 60mg/kg of MREt when compared with diabetic and negative control groups (table 2). Beside this, all three doses of AqMREt of *R. serpentina* have more significant impact on RBC count (p<0.05 & p<0.001) and showed gradual improvement in same count like 3.12, 4.39 and 5.29 x 10^6 /µl in their

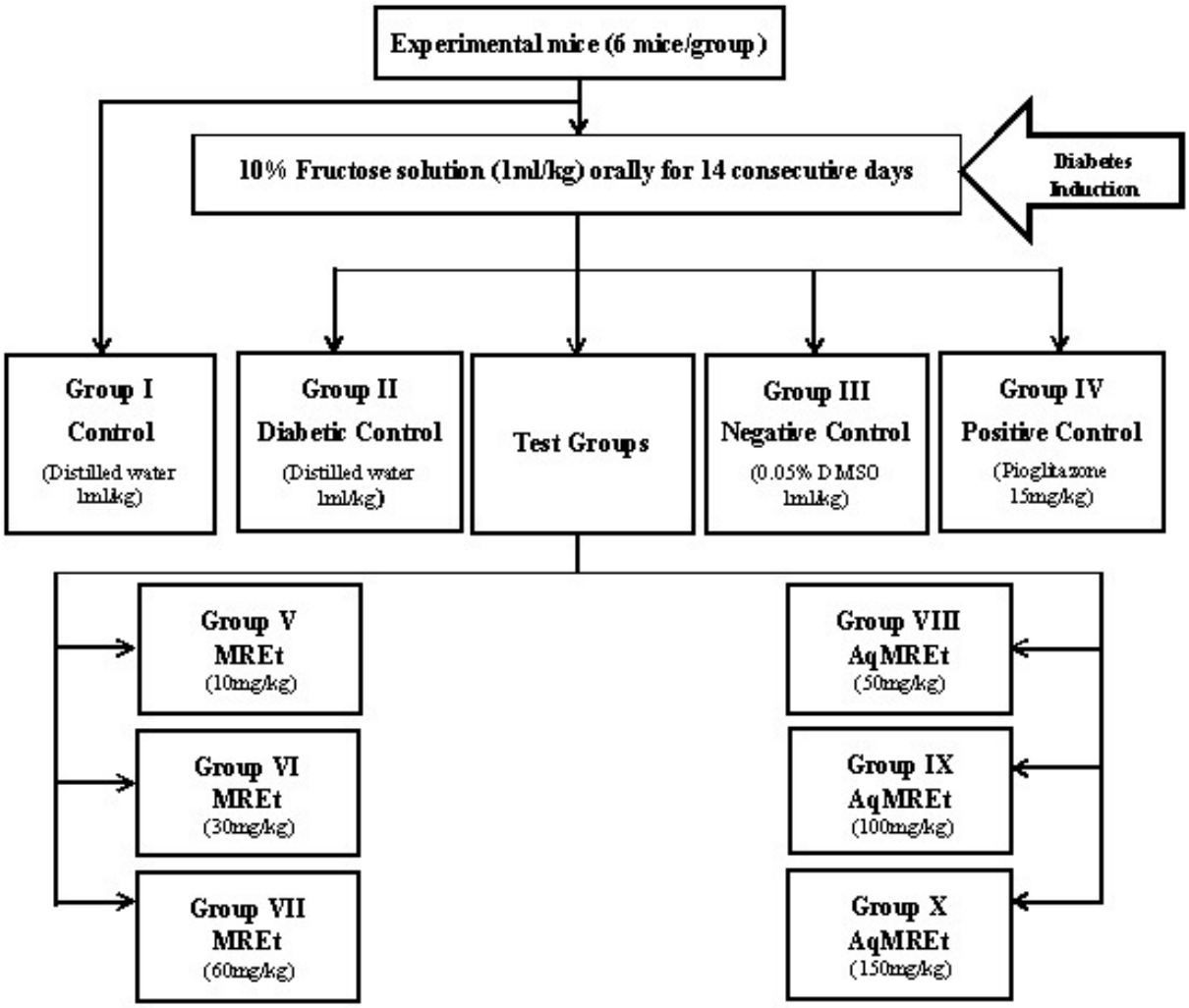


Fig. 1: Animal grouping

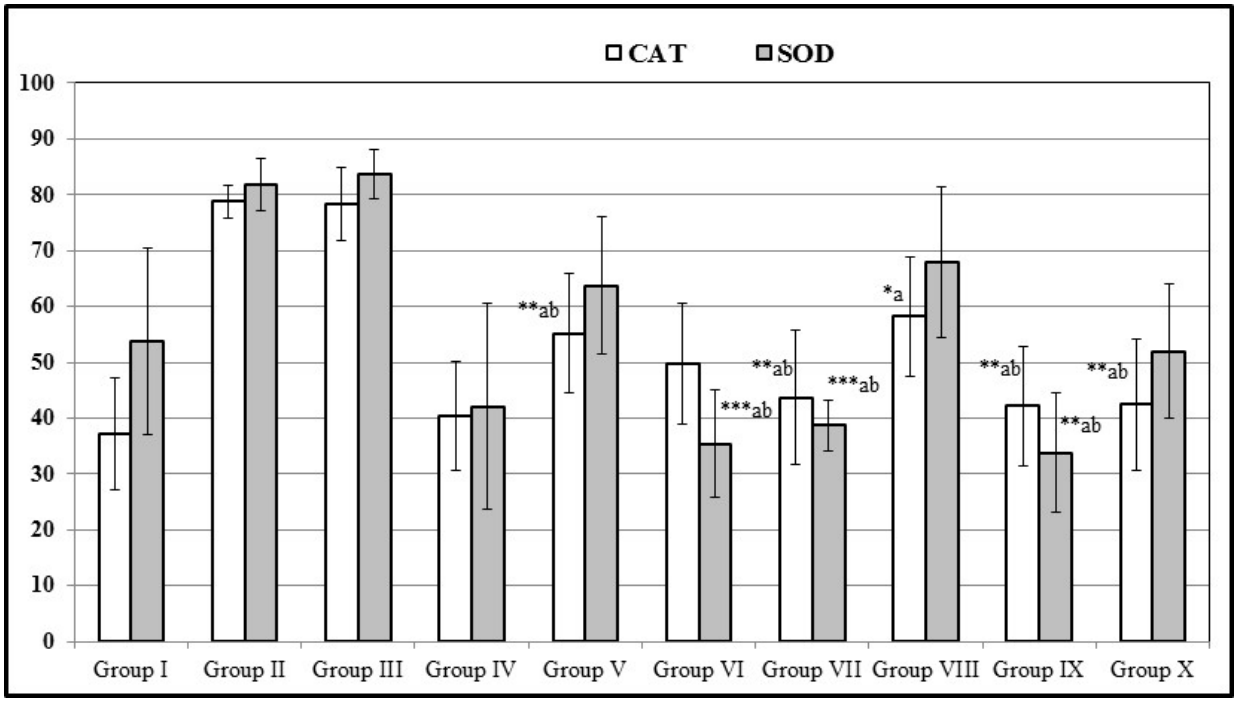
respective test groups when compared with diabetic and negative groups. In contrast, WBC count was decreased significantly ($p<0.05$) with respect to increase in dose magnitude as 3.17, 1.83 and 1.27 x 10^3/µl while their elevated levels were observed in diabetic and negative controls. Similarly, PCV ($p<0.01$), MCV ($p<0.0001$), MCH and MCHC ($p<0.05$) levels were significantly and gradually improved in all AqMREt treated test groups (table 2).

FTIR analysis of root extracts of R. serpentina

MREt and AqMREt samples showed almost similar pattern of spectra while to some extent few divergence in peak order was also observed. The first broad peak was observed in MREt at 3277.24cm^{-1} and in AqMREt at 3264.67cm^{-1}. Interestingly, both peaks showed the presence of phenol functional group with O-H bend. Secondly, two consecutive peaks of MREt at 2922.13 cm^{-1}, 2852.06cm^{-1} and AqMREt peak at 2927.61 cm^{-1} showed CH saturated stretching. Third, MREt peak at 1618.34 cm^{-1} and AqMREt peak at 1623.75 cm^{-1} showed the C=C aromatic stretching. Fourth, MREt peak at 1411.93 cm^{-1} and AqMREt peak at 1405.96cm^{-1} showed the presence of C-C stretch in aromatic ring. Another single peak of MREt appeared at 1253.49cm^{-1} that showed the C-N stretch of alkaloids. MREt peak at 1040.75cm^{-1} and AqMREt peak at 1045.77 cm^{-1} showed C-O stretch of phenols. Similarly, MREt peak of 829.67 cm^{-1} showed the presence of N-H wagging of secondary amines in alkaloids while this was not observed in AqMREt spectrum. MREt peak at 520.45 cm^{-1} and AqMREt peak at 539.71 cm^{-1} showed the C-Cl stretch of alkyl halide (fig. 3).

DISCUSSION

Increase intake of fructose not only induced hyperglycemia, hyperinsulinemia but also accelerate the formation of ROS to create oxidative stress (Johnson *et al.,* 2009; Bray, 2007). Hyperglycemia may lead to the activation of polyol pathway (an alternate pathway of glucose metabolism in diabetic condition) that involve in reduction of sweet substance (glucose) to sorbitol (alcohol) in the presence of aldose reductase, the key



Values are expressed as mean ± SD (n = 6). *p <0.05, **p <0.01 and ***p <0.0001, when compared with group II (a) and III (b)

Fig. 2: Effect of root extract on antioxidant enzymes of fructose-induced T2D mice

enzyme of this pathway (Pathania *et al.,* 2013). This increased sorbitol production in turn leads to the formation of ROS (super oxide anions, hydroxyl radicals, etc) and advanced glycation end products (AGE) which accelerate the production of micro-vascular complications of diabetes by inducing lipid peroxidation and altering membrane structure and function (Barnett, 1986). On the other hand, this oxidative stress suppresses the activity of antioxidant enzymes system in body (Pham-Huy, 2008). The same was observed in present investigation where high percent inhibition of CAT and SOD activities were found in fructose-induced diabetic groups whereas the percent inhibition of CAT and SOD was effectively (p< 0.05 & p<0.01, p<0.0001) lowered in both root extracts (MREt and AqMREt) treated test groups. Interestingly, a current computational study based on our reported antidiabetic activity of *R. serpentina* described that few alkaloids present in roots of *R. serpentina* are potent inhibitors of aldose reductase enzyme thereby inhibiting formation of ROS and reducing oxidative stress (Pathania *et al.,* 2013).

In diabetes linked production of ROS, it was reported that the half-life ($t_{1/2}$) of RBC become shortened due to oxidative stress and non-enzymatic glycation of RBC membrane proteins (Saba *et al.,* 2010). In addition, increased amount of HbA_{1c} and decreased amount of total Hb also affects RBC count (Azmi and Qureshi, 2013). Hb improving effect of root extracts of *R.serpentina* that observed in present study also improves (p<0.01 & p< 0.0001) the RBC count in their respective test groups especially AqMREt found more effective in this aspect and produced dose-dependent prominent improvement (p<0.05 & p<0.01) in RBC count. This finding correlates with the hematopoietic effect of MREt and AqMREt found in alloxan-induced type 1 diabetic mice model and of course secondary to the antiglycation and antioxidant activities of both root extracts of *R. serpentina* thereby stabilizing the osmotic fragility of RBC membrane. On the other hand, the significantly (p<0.05 & p<0.01, p<0.0001) lowered and better leukocyte count was found in test groups as compared to elevated level of same cells count observed in diabetic control groups which reflected the inflammatory response occurred in these control groups that might be either due to hyperglycemic or hyperlipidemic conditions induced by high-fructose intake (Johnson *et al.,* 2009; Tanko *et al.,* 2011). Ameliorative effect of MREt and AqMERt on total Hb level and RBC count also induced improvement in further hematological parameters (including PCV, MCV, MCH and MCHC) in test groups (p<0.05). Therefore, root extracts of *R. serpentina* may possibly minimize the chances of mild anemia which normally observed in diabetes especially after prolong use of thiazolidinediones (Gale, 2001).

FTIR is a time-saving analytical technique which evaluates the functional groups possess by compounds present in extracts of natural sources (Ashokkumar and Ramaswamy, 2014; Jagdish and Irudayaraj, 2004; Sasidharan *et al.,* 2011). In the present study, this technique is used to reveal the functional groups present

in both MREt and AqMREt that could give an idea of possible active compounds present in both of these root extracts responsible for significant antidiabetic and antioxidant activities. The spectrum of each root extract showed peaks at different wave numbers from 4000 to 500 nm. Of which, the FTIR analysis of both MREt and AqMREt showed the presence of phenols by displaying peaks for their characteristic functional groups (O-H & C-O) and aromatic ring at 3277.24 cm, 3264.67 cm and 1040.74 cm, 1045.77 cm respectively. This finding verified the initial quantitative analyses of phenols in both root extracts (MREt & AqMREt) of *R. serpentina* and reported that AqMREt contained more total phenols as compared to MREt (Azmi and Qureshi, 2016). Literature proved that medicinal plants rich in phenolic compounds are significant antidiabetic agents and also ameliorate hyperglycemia-induced oxidative stress in experimental diabetic models (Yang *et al.,* 2014). It has also been well-reported that phenolic compounds inhibited the aldose reductase, NADPH oxidase and xanthine oxidase enzymes, thereby diminishing the production of ROS in cells and enhancing the antioxidant capacity of biological system (Seifu *et al.,* 2012).

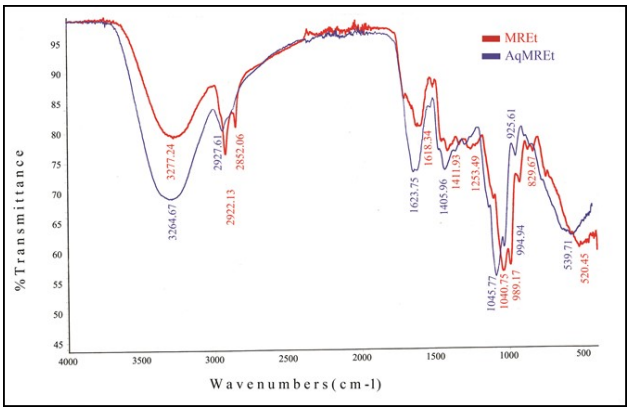


Fig. 3: FTIR profile of root extracts of *R. serpentina*

It has been reported that the roots of *R. serpentina* are rich in indole alkaloids (that contains indole ring like ajmaline, ajmalicine, yuhimbine, etc) having hypotensive and sedative properties (Itoh *et al.,* 2005; Azmi and Qureshi, 2012). The possible presence of these indole alkaloids in MREt of *R. serpentina* was also proved by observing characteristic peaks of C-N and N-H (wagging of secondary amines) stretches of alkaloids in FTIR spectrum at 1253.49 and 829.67 nm. Whereas these peaks are completely absent in FTIR spectrum of AqMREt. This finding also validates our published results of acute toxicity of both extracts which described that –MREt of *R.serpentina* was found to induce sedation from 100 mg/kg to onward whereas AqMREt was found safe till 250mg/kg as a result of which 10 times high LD_{50} of AqMREt was found as compared to MREt through log-probit graphical method (Azmi and Qureshi, 2012: Azmi and Qureshi, 2014). Alkaloids are nitrogen containing compounds of low molecular weights and various studies described their therapeutic potential in the management of clinical disease like Alzheimer, myasthenia gravis, myopathy and dyslipidemia (Seifu *et al.,* 2012). In addition, different biological activities of alkaloids including antiinflammatory, analgesic, gout suppressing, antineoplastic, antiviral, anticholinergic, antioxidant, etc are reported (Mukhopadhyay *et al.,* 2012; Pelletier, 1991). It was also reported that alkaloids can inhibit lipid peroxidation thereby protecting the cells *via* inhibiting lipoxygenase and xanthine oxidase enzymes which considered as ROS generating sources or accelerating the SOD enzyme activity which ultimately provide protection against oxidative stress (Rackova *et al.,* 2007; Ahmad *et al.,* 2010; Seifu *et al.,* 2012; Facchini, 2001).

CONCLUSION

The results conclude that MREt and AqMREt of *R. serpentina* prevent the generation of ROS and maintaining the haematinic potential in fructose-induced type 2 diabetic mice. In addition, the same root extracts exhibited the presence of good quantity of phenols.

ACKNOWLEDGMENT

The authors are highly thankful to Dr. Shamsa Kanwal, Assistant Professor HEJ Research Institute of Chemistry, University of Karachi for interpreting the FTIR spectra of both the extracts used in this study.

REFERENCES

Ahmad I, Ijaz F and Fatima I *et al*., (2010). Xanthine oxidase/tyrosinase inhibiting, antioxidant, and antifungal oxindole alkaloids from Isatis costata. *Pharm. Biol.,* **48**(6): 716-21.

Ashokkumar R and Ramaswamy M (2014). Phyto-chemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants, *Int. J. Curr. Microbiol. App. Sci.,* **3**(1): 395-406.

Azmi MB and Qureshi SA (2012). Methanolic root extract of *Rauwolfia serpentina* Benth improves the glycemic, antiatherogenic, and cardioprotective indices in alloxan-induced diabetic mice. *Adv. Pharmacol. Sci*., article ID 376429(2012): 11 pages.

Azmi MB and Qureshi SA (2013). *Rauwolfia serpentina* ameliorates hyperglycemic, haematinic and antioxidant status in alloxan- induced diabetic mice. *J. App. Pharm. Sci.,* **3**(7): 136-141.

Azmi MB and Qureshi SA (2014). Glucose lowering potential of hydromethanolic extract of *Rauwolfia. World J. Pharmaceut. Sci*., **2**(3): 219-223.

Azmi MB and Qureshi SA (2016). *Rauwolfia serpentina* improves altered glucose and lipid homeostasis in fructose-induced type 2 diabetic mice. *Pak. J. Pharm. Sci.,* **29**(5): 1619-1624.

Barnett PA, Gonz´alez RG and Chylack Jr LT *et al.,* (1986). The effect of oxidation on sorbitol pathway kinetics. *Diabetes*, **35**(4): 426-432.

Bray GA (2007). How bad is fructose? *Am. J. Clin. Nutr.,* **86**(4): 895-896.

Davies KJ (2000). Oxidative stress, antioxidant defenses, and damage removal, repair and replacement systems. *IUBMB Life*, **50**(4-5): 279-289.

Dey A and De JN (2011). Ethnobotanical aspects of *Rauvolfia serpentina* (L). Benth. ex Kurz. in India, Nepal and Bangladesh. *J. Med. Plants Res*., **5**(2): 144-150.

Facchini PJ (2001). Alkaloid biosynthesis in plants: Biochemistry, Cell Biology, Molecular Regulation, and Metabolic Engineering Applications. *Ann. Rev. Plant Physiol. Plant Mol. Biol*., **52**(1): 29-66.

Gale EA (2001). Lessons from the glitazones: A story of drug development. *Lancet*, **357**(9271):1870-1875.

Itoh A, Kumashiro T, Yamaguchi M, *et al.* (2005). Indole alkaloids and other constituents of *Rauwolfia serpentina*. *J. Nat. Prod.,* **68**(6): 848-852.

Jagdish T and Irudayaraj J (2004). Quantification of saccharides in multiple floral honeys using fourier transform infrared micro attenuated total reflectance spectroscopy. *J. Agric. Food Chem.*, **52**(11): 3237-3243.

Johnson RJ, Perez-Pozo SE and Sautin YY, Manitius J, Sanchez-Lozada LG, Feig DI, Shafiu M, Segal M, Glassock RJ, Shimada M, Roncal C, and Nakagawa T (2009). Hypothesis: Could excessive fructose intake and uric acid cause type 2 diabetes? *Endocrine Rev.,* **30**(1): 96-116.

Mukhopadhyay MK, Banerjee P and Nath D (2012). Phytochemicals biomolecules for prevention and treatment of human diseases-a review. *Int. J. Scienti. Eng. Res*., **3**(7): 1-32.

Neeharika V, Vamsi KR and Madhava RB (2012). Effect of Madhuriktha on dexamathasone and fructose induced insulin resistance in rats. *J. Nat. Prod. Plant Resources,* **2**(2): 288-294.

Pathania S, Randhawa V and Bagler G (2013). Prospecting for novel plant-derived molecules of *Rauvolfia serpentina* as inhibitors of aldose reductase, a potent drug target for diabetes and its complications. *PLoS ONE,* **8**(4): e61327.

Pelletier SW (1991). Alkaloids: Chemical and biological perspectives. Volume 7, Springer. p.591.

Pham-Huy LA, He H and Pham-Huy C (2008). Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci*., **4**(2): 89-96.

Qureshi SA and Udani SK (2009). Hypolipidaemic activity of *Rauwolfia serpentina* Benth. *Pak. J. Nutr.*, **8**(7): 1103-1106.

Qureshi SA, Nawaz A, Udani SK and Azmi B (2009). Hypoglycemic and hypolipidemic activities of *Rauwolfia serpentina* in alloxan–induced diabetic rats. *Int. J. Pharmacol*. **5**(5): 323-326.

Rackova L, Oblozinsky M, Kostalova D *and* Bezakova L (2007). Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *J. Inflamm.,* **4**(1): 1-7.

Saba AB, Oyagbemi AA and Azeez OI (2010). Antidiabetic and haematinic effects of *Parquetina nigrescens* on alloxan induced type-1 diabetes and normocytic normochromic anaemia in Wistar rats. *Afr. Health Sci*., **10**(3): 276-282.

Sasidharan S, Chen Y, Saravanan D, Sundram KM and Latha LY (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr. J. Tradit. Complement Altern. Med.,* **8**(1): 1-10.

Seifu D, Assefa F and Abay SM (2012). Medicinal plants as antioxidant agents: Understanding their mechanism of action and therapeutic efficacy (Capasso A, ed). pp.97-145.

Tanko Y, Mabrouk MA, Adelaiye AB and Fatihu MY (2011). Antidiabetic and some haemotological effects of ethylacetate and nbutanol fractions of *Indigofera pulchra* extract on alloxan-induced diabetic *Wistar* rats. *J. Diabet. Endocri.,* **2**(1): 1-7.

Yang W (2014). Botanical, pharmacological, phytochemical and toxicological aspects of the antidiabetic plant *Bidens pilosa* L. *Evidence-Based Compl. Alt. Med.,* **2014**, Article ID 698617.