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Effect of *Osbeckia Chinensis* root extract on aldose reductase and sorbitol dehydrogenase activity in mice of two different age groups

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ABSTRACT

The present study was performed toevaluate the effect of aqueous-methanolic extract of Osbeckia chinensis roots, on the polyol pathway enzymes i.e., aldose reductase (AR) and sorbitol dehydrogenase (SDH) in normoglycemic and diabetic mice of two different age groups (six- and one-month old). Polyol pathway under hyperglycemic condition has been associated with the pathogenesis of diabetic complications. Varying doses (50-250 mg/kg b.w.) of extract was administered intraperitoneally to six-month normoglycemic mice in alternate days for upto 4 weeks. The optimised dose of 150 mg/kg b.w. was then administered to one-month old normoglycemic and diabetic mice. The activities of AR and SDH was then measured spectrophotometrically in liver, kidney and eye tissues of normoglycemic and diabetic mice of these two age groups treated with extract. Curcumin (20 mg/kg b.w.), a natural product known to inhibit enzymes of the polyol pathway was used as reference.Extract of O. chinensis exhibited the capacity to inhibit AR/SDH activity in selected tissues of both normoglycemic and diabetic mice in both age groups when compared to the control group. However, OCE was more effective in reducing AR activity than SDH. Curcumin showed significant inhibition against both AR and SDH in tissues of normoglycemic and diabetic mice of both age groups.The extract showed differential pattern of AR and SDH inhibition as a function of age, tissue and pathophysiologial condition. These results suggest that O. chinensis extract might contain active components which could represent potential compounds for the prevention and /or treatment of diabetic complications.

Keywords: Aldose reductase, curcumin, hyperglycemia, *Osbeckia chinensis*, sorbitol dehydrogenase

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INTRODUCTION

Diabetes is a hyperglycemic condition that results due to abnormalities in thelevel of insulin secretion and /insulin action which can lead tolong term complications in different organs [1].Tissue damage can be caused by hyperglycemia via different mechanism and among these; thepolyol pathway is generally accepted to be the mechanism of prime importance in the pathogenesis of diabetic complications [2-4].Polyol pathway consists of two enzymes in which glucose is reduced to sorbitol by AR (EC 1.1.1.21)using a cofactor NADPH and then sorbitol is converted to fructose by SDH (EC 1.1.1.14)using NAD^+ as a cofactor [5,6].Accumulation of polyols has been attributed to the pathological changes in various organs including kidney[7,8],eye [9]and liver [10,11].Therefore, inhibition of polyol pathway has been an attractive approach to the prevention and treatment of diabetic complications.Aldose reductase inhibitors (ARIs), which directly inhibit AR, are potential therapeutic drugs of diabetic complications[12].Polyol pathway flux via SDH has been link to changes in cytosolic NADH/NAD^+ ratio[13,14] and these changes have been reported in ischemic and diabetic state[15,16].Therefore, search of sorbitol dehydrogenase inhibitor (SDI) would attenuate the increased NADH/NAD^+ and fructose levels in diabetic tissues.

A number of molecules have been developed to inhibit AR. However, only a limited number of drugs have been made available in the market [17].Commercially, epalrestat is the only available AR inhibitor. Some drugs have been withdrawn due to their safety concerns and others are still in clinical trials[18]. Thus, it is very crucial to develop new ARIs with improved efficacy and safety profile[19]. Similarly, there is a need for developing new SDI

which can be used to inhibit the polyol pathway by attenuating the increased NADH/NAD^+ level during diabetic condition.

Plants are rich sources of active principles and a vast majority of currently available therapeutic drugs were derived directly or indirectly from plants [20]. Moreover, there is an increased interest in recent times to identify natural sources for their therapeutic properties. It is because most of the plants are mostly free from adverse effects and are being used as a source of diet and traditional medicine[21]. Thus, the aim of the current study was to determine the AR and SDH inhibitory potential of aqueous-methanolic extract of *Osbeckiachinensis* L.(Family: Melastomataceae) roots in normoglycemic and diabetic mice of two different age groups (six- and one-month old).These ages were chosen to represent the effect of developmental stages on the enzyme activities of the polyol pathway in the presence of the plant extracts. The roots of *O.chinensis* have been reported to possess hypoglycemic and antihyperglycemic activity[22]. Further, curcumin was also studied as a reference for it is known to inhibit the enzymes of the polyol pathway [23,24].

MATERIALS AND METHODS

Chemicals

Alloxan, DL-glyceraldehyde, curcumin were procured from Sigma-Aldrich (St. Louis, USA), and D-sorbitol, nicotinamide adenine dinucleotide phosphate (NADPH),nicotinamide adenine nucleotide (NAD), bovine serum albumin was purchased from Sisco Research Laboratories (SRL), India. Other chemicals were of analytical grade obtained from E-merck and SRL, India.

Experimental Animals

Healthy, Swiss albino mice of approximately six- and one-month old were used for the study. Mice were kept in animal house under temperature controlled conditions maintained at 22°C on a 12h light/12h dark cycle. Mice were fed with mice feed obtained from Amrut Laboratory, Pune, India. The number of animals used for all the experiments were six.The protocols on animal models were followed as per the guidelines of the Institutional Animal Ethical Committee.

Preparation of extract

The plant material of*O. chinensis*(Voucher No: 71) was collected from North-Eastern Hill University (NEHU), Meghalaya, India. The specimen was identified by Dr. P. B. Gurung, Department of Botany, NEHU, Meghalaya, India. The roots of *O. chinensis*(OC) were washed and dried. These were then powdered and mixed in 10 volume of aqueous-methanol solution (1:4) [25]. The mixture was filtered using Whatmann No. 1 filter paper. The filtrate was lyophilized (Lyolab-3000, HetoLyolab, Switzerland) to dryness which was used as *O. chinensis* extract (OCE).

Toxicity study

Normoglycemic mice of six-month old were administered with OCE (50-450 mg/kg b.w.)for alternative daysvia intraperitoneal (i.p.) routeand kept in observation up to 4 weeks for any signs of distress, convulsion, coma or death [26].

Administration of OCE/curcumin to normoglycemic mice of two age groups

(i) Six-month old mice were administered via i.p. route for alternative days with OCE (50 and 150 mg/kg b.w. dissolved in 2% ethanol)upto 4 weeks period. The most effective dose (150 mg/kg b.w.) determined was administered to one-month old mice. The control group received 2% ethanol. (ii) Curcumin (20 mg/kg b.w.) dissolved in dimethylsulfoxide (DMSO)[27]was administered via i.p. route for alternate days to six- and one-month old miceupto 4 weeks period. The control group received DMSO. Mice groups of (i) and (ii) were sacrificed by cervical dislocation and their individual organs(liver, kidney and the eye balls) were removed and homogenized separately for AR and SDH activity measurement using spectrophotometer (Cary 50 UV-Vis).

Preparation of diabetic mice

Overnight fasted mice (given water ad libitum)were administered with alloxan monohydrate (180 mg/kg b.w. for six-month old and 150 mg/kg b.w. for one-month old) prepared in acetate buffer (0.15 M, pH 4.5) via i.p. route. Diabetic mice (more than 3-4 fold increase in blood glucose) were considered for further experiments.

Administration of OCE/curcumin to diabetic mice of two age groups

Alloxan-induced diabetic mice (six- and one-month old) were administered on alternate days with OCE(150 mg/kg b.w.)/curcumin (20 mg/kg b.w.) via i.p. route for a period of 4 weeks. The control group of OCE and curcumin received 2% ethanol and DMSO respectively. Organs (liver, kidney and eye balls)from respective groups were removed and homogenized separately.

Enzyme assays

Enzyme solution for AR and SDH assays were prepared by homogenizing the selected tissues(liver, kidney and eye balls). The homogenized tissues were centrifuged(9000 xg for 15 min)in 0.225 M sucrose-Tris buffer (2.5 volume, pH 7.4) and supernatant was further centrifuged at 16,000 xg for 30 min. The obtained supernatant was used as enzyme preparation for AR and SDH assays. AR activity was measured according to Wolff and Crabbe[28] with some modifications. The reaction mixture (1 ml) contained Na-phosphate buffer (50 mM,pH 6.5), enzyme preparation (100 µl), dl-glyceraldehyde (100 mM) and NADPH (0.2 mM). Measurement of SDH activity was according to the method of Gerlach [29]. Incubation medium (3 ml) contained Tris-HCl (0.1M, pH 9), enzyme preparation (50µl), D-sorbitol (1.1 mM) and NAD^+ (32mM). Absorbance for both AR and SDH assays were measured at λ340 nm. Enzyme activity was expressed as U/mg protein [30]. Protein was estimated using Bradford's method [31].

Statistical analysis

The data are reported as mean ± S.E.M. The level of significance was determined between the control and treated group using student's't'-tests. A value of P less than 0.05 was considered statistically significant.

RESULTS

Toxicity study

Mice treated with 150 mg/kg b.w. dose of OCE did not show any adverse effects during the 4weeks of treatment. However, doses of 250mg/kg b.w. and above doses resulted in severe hypoglycemia followed by death.

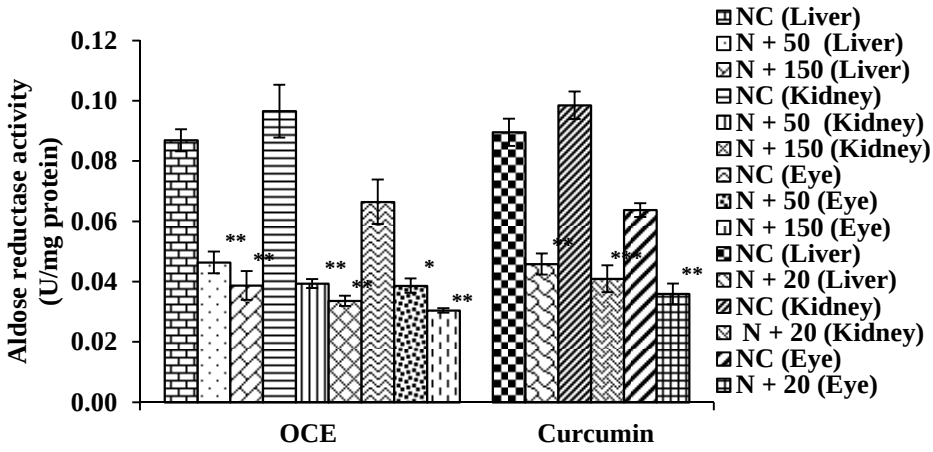

Figure 1: Effect of OCE (50-150 mg/kg b.w.)/curcuminon the AR activity (U/mg protein) in selected tissues of six-month old normoglycemic mice (N) whereNC: normoglycemic control.Values are represented as mean ± SEM with n=3, level of significance at p*<0.05, **<0.01, *<0.001 respectively.**

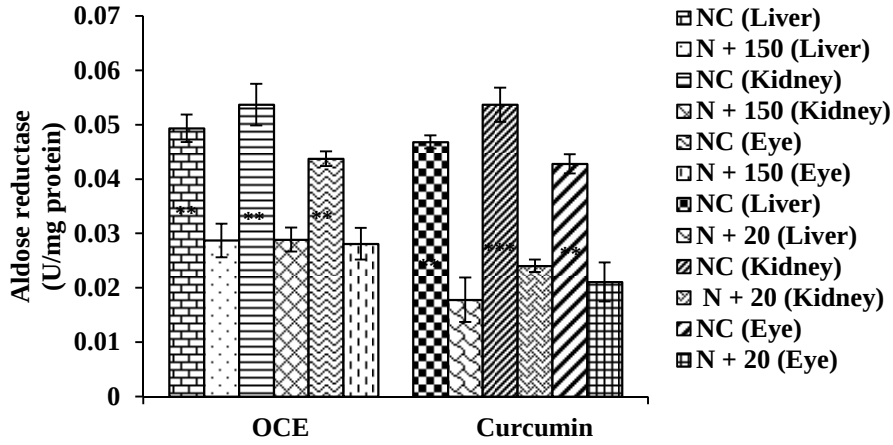

Figure 2: Effect of OCE (150 mg/kg b.w.)/curcuminon the AR activity (U/mg protein) in selected tissues of one-month old normoglycemic mice (N) whereNC: normoglycemic control.Values are represented as mean ± SEM with n=3, level of significance at p*<0.05, **<0.01, *<0.001 respectively.**

Effect of OCE/curcumin on AR activity in normoglycemic and diabetic mice

Among the doses of OCE, 150 mg/kg b.w. inhibited the AR activity most effectively by 55% ($p<0.01$), 65% ($p<0.01$) and 54% ($p<0.01$) in liver, kidney and eye tissue respectively in six-month old normoglycemic mice from that of control group[Figure 1].Reduction in the AR activity of one-month old mice treated with OCE was also observed in liver (42%, $p<0.01$), kidney (46%, $p<0.01$) and eye tissue (36%, $p<0.01$) from that of control group[Figure 2].Curcumin also showed reduction in the AR activity in selected tissues of both six- and one-month old mice[Figure 1 and2].

The decrease in the activity of AR was also observed in diabetic mice treated with OCE (150 mg/kg b.w.) which was comparable to curcumin (Figure 3 and 4). In six-month old diabetic mice treated with OCE, the reduction in AR activity was found to be 36% ($p<0.01$) in liver, 34% ($p<0.01$) in kidney and 28% ($p<0.01$) in eye while in one-month old diabetic mice, the reduction was 35% ($p<0.01$), 35% ($p<0.01$) and 26% ($p<0.05$) in liver, kidney and eye respectively from that of the diabetic control.

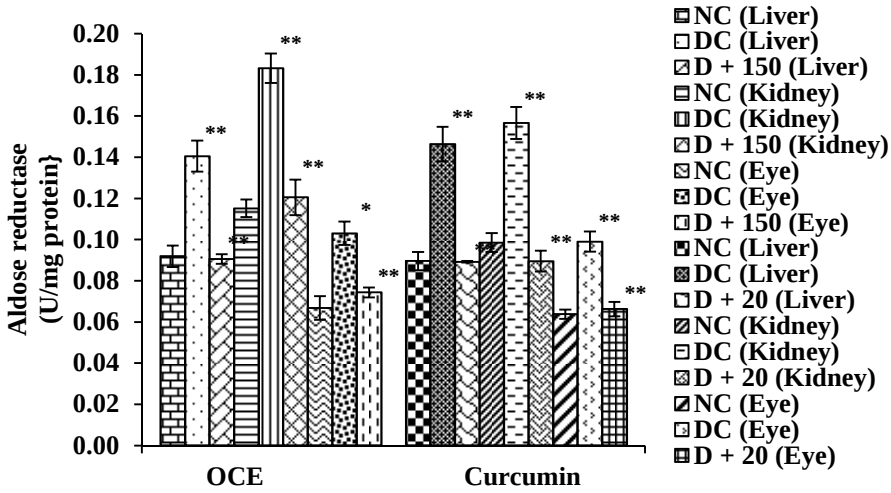

Figure 3:Effect of OCE (150 mg/kg b.w.)/curcuminon the AR activity (U/mg protein) in selected tissues of six-month old diabetic mice (D) whereNC: normoglycemic control, DC: diabetic control.Values are represented as mean ± SEM with n=3, level of significance at p*<0.05, **<0.01, *<0.001 respectively.**

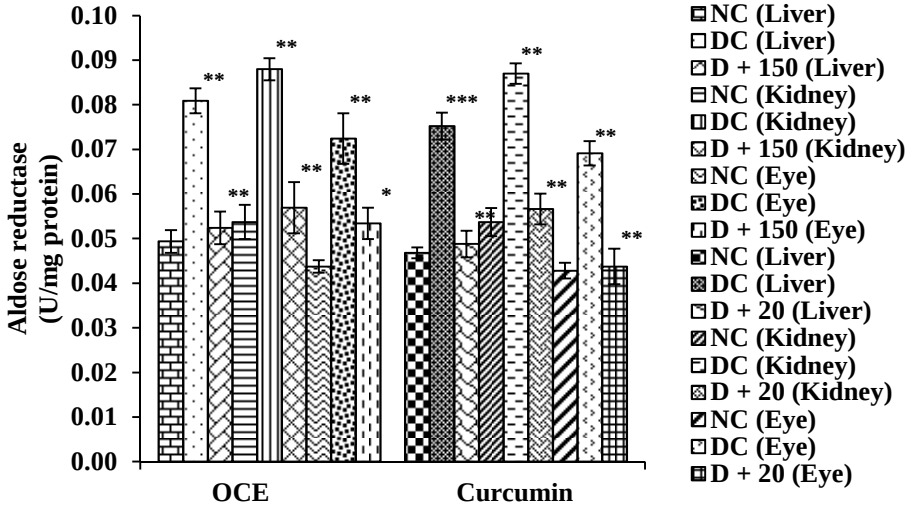

Figure 4:Effect of OCE (150 mg/kg b.w.)/curcuminon the AR activity (U/mg protein) in selected tissues ofone-month old diabetic mice (D) whereNC: normoglycemic control, DC: diabetic control. Values are represented as mean ± SEM with n=3, level of significance at p*<0.05, **<0.01, *<0.001 respectively. NC:- normoglycemic control.**

Effect of OCE/curcumin on SDH activity in normoglycemic and diabetic mice

OCE resulted in significant reduction of SDH activity at 150 mg/kg b.w. by 15% ($p<0.05$) in liver, 24% ($p<0.01$) in kidney from that of the normoglycemic (six-month) control group. SDH activity did not alter in a significant manner in eye tissue. However, in curcumin treated normoglycemicmice (six-month),SDH activity was significantly

inhibited in all three tissues by 36% (p<0.01)in liver, 42% (p<0.01) in kidney and 41% (p<0.01) in eye from that of the control group [Figure 5].

In one-month normoglycemic group, OCE reduced the activity of SDH by 18% (p<0.05), 34% (p<0.05) and 17% (p<0.05)in liver, kidney and eye respectively while in curcumin treated normoglycemic (one-month)mice, the activity of SDH was reduced by 32% (p<0.01) in liver, 48% (p<0.01) in kidney and 46% (p<0.01) in eye from that of the control group [Figure 5 and 6].

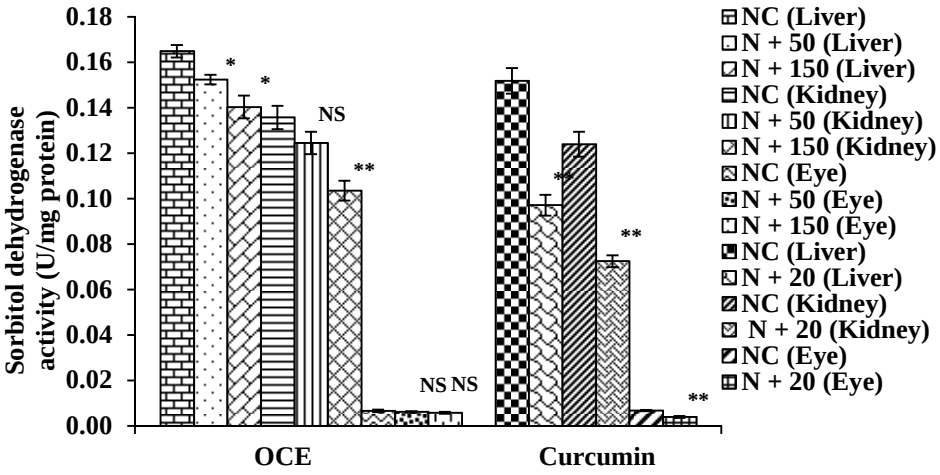

Figure 5: Effect of (50-150 mg/kg b.w.)/curcuminon the SDH activity (U/mg protein) in selected tissues of of six-month old normoglycemic mice (N)whereNC: normoglycemic control, NS: non-significant.Values are represented as mean ± SEM with n=3, level of significance at p*<0.05, **<0.01, *<0.001 respectively. NC:- normoglycemic control.**

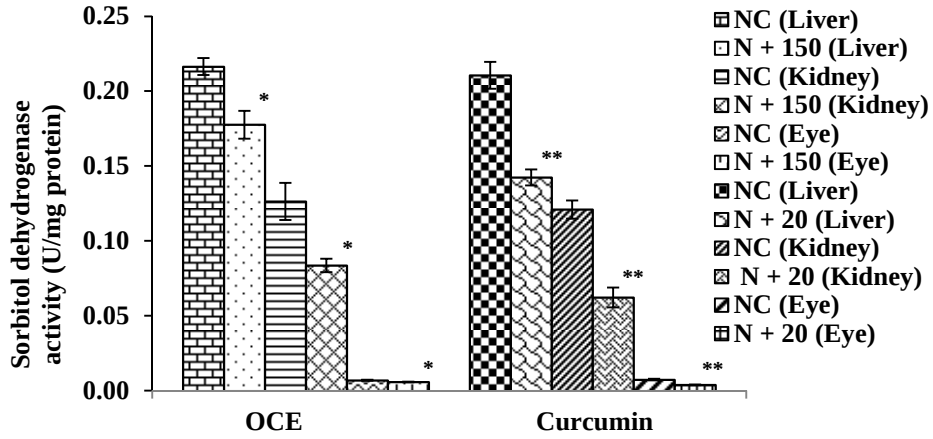

Figure 6: Effect of OCE (150 mg/kg b.w.)/curcuminon on the SDH activity (U/mg protein) in selected tissues ofone-month old normoglycemic mice (N)whereNC: normoglycemic control. Values are represented as mean ± SEM with n=3, level of significance at p*<0.05, **<0.01, *<0.001 respectively. NC:- normoglycemic control.**

Figure 7 shows effect of OCE/curcumin on the SDH activity in selected tissues of diabetic (six-month) mice. The activity of SDH was inhibited by 16% (p<0.05) and 12% (p<0.05) in liver and kidney respectively while in eye tissue the reduction was found to be insignificant from that of the diabetic control group. However, curcumin also reduced the SDH activity in liver (30%, p<0.01), kidney (32%, p<0.01) and eye (23%, p<0.01) in diabetic mice when compared from that of diabetic control group. Similar reduction in the activity of SDH was also observed in one-month old diabetic mice treated with OCE/curcumin from that of the diabetic control group [Figure 8].

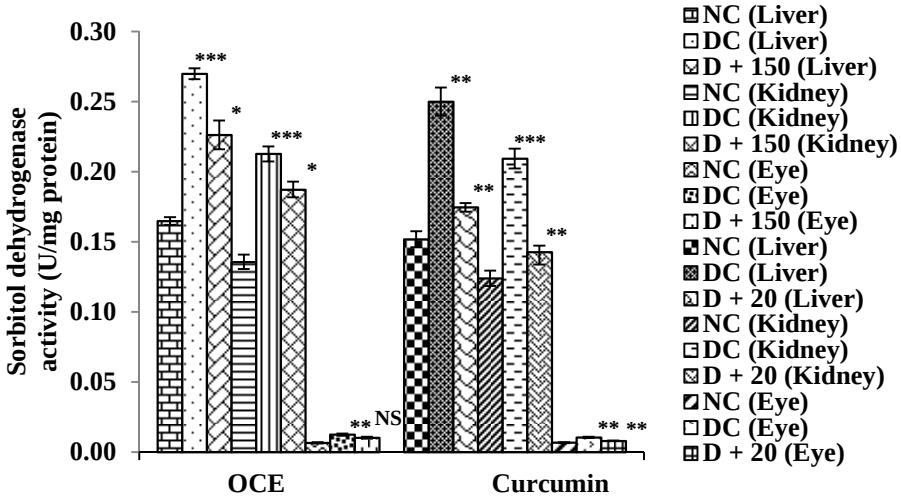

Figure7:Effect of OCE (150 mg/kg b.w.)/curcuminon on the SDH activity (U/mg protein) in selected tissues ofsix-month old diabetic mice (D) whereNC: normoglycemic control, DC: diabetic control, NS: non-significant. Values are represented as mean ± SEM with n=3, level of significance at p*<0.05, **<0.01, *<0.001 respectively. NC:- normoglycemic control.**

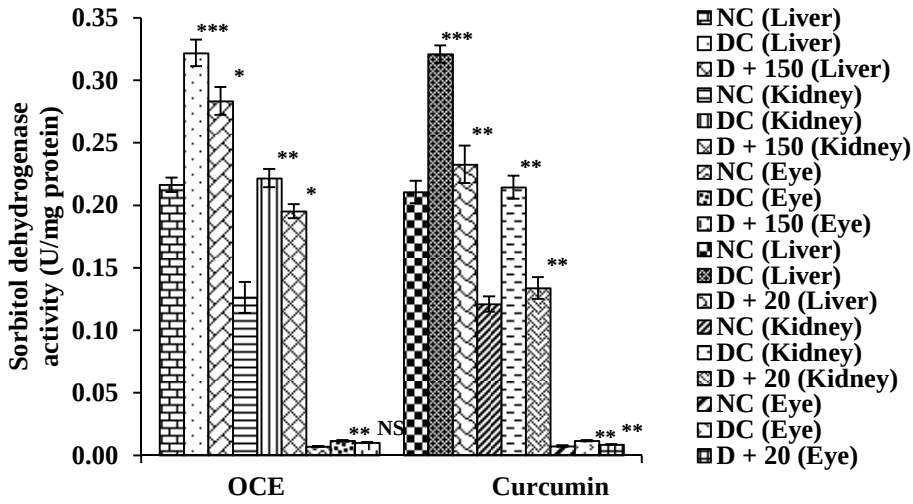

Figure8: Effect of OCE (150 mg/kg b.w.)/curcuminon on the SDH activity (U/mg protein) in selected tissues ofone-month old diabetic mice (D) where NC: normoglycemic control, DC: diabetic control; NS: non-significant. Values are represented as mean ± SEM with n=3, level of significance at p *<0.05, **<0.01, *<0.001 respectively. NC:- normoglycemic control.**

DISCUSSION

The present study was performed to determine the effect of OCE on AR and SDH activity in both normoglycemic and diabetic mice of two different age groups. As reported in previous studies[32],AR activity in our result was observed to exhibit an age- and tissue-specific pattern of distribution with the highest activity in kidney, followed by the liver and eye in mice. The activity of SDH was highest in liver followed by kidney with lowest activity in eye tissue which is consistent with previous report [33, 34]suggesting the complexity of its regulation and function. Toxicity studies performed in six-month old mice showed that at 250 mg/kg b.w., administration of the OCEresulted in fatality/mortality. Therefore, the i.p. route at 150 mg/kg b.w. was selected for further studies.

The extract of OC exerted inhibitory effects on the activities of AR and SDH in selected tissues of normoglycemic mice in both the age groups. However, the magnitude of reduction in AR and SDH activity varied in adose-dependent manner. Various ARI are known to show different effectiveness among animal species and tissues [35]. Similarly, the SDH activity in different tissues of both the age groups of normoglycemic mice was also found to show variation in the pattern of SDH inhibition. It was observed that on OCE treatment, AR activity in selected tissues was reduced maximally in six-month old than in one-month normoglycemic mice. The more marked effect observed with the six-month old group may be due to maturity of metabolic response compared to the one-month old mice which is just past the weaning period and thus, the differential response. However, in the case of SDH

activity in normoglycemic mice, there were no significant differences with respect to the degree of inhibition by the extract between the age groups. Results showed that OCE was more effective in reducing the AR activity than compared to SDH activity in both the age groups. There are previous reports where compounds isolated from medicinal plants have been found to inhibit AR more effectively than SDH eg., umbelliferone inhibited recombinant AR with an IC_{50} of 85.7µM and SDH with $IC_{50}$of 120µM;esculetin has been reported to inhibit the activity of the enzymes AR (IC_{50}: 36.5µM) and SDH (IC_{50}: 82.9µM) with different IC_{50} value[36].

Administration of OCE to diabetic mice reduced the activity of both AR and SDH in selected tissues to varying extent in an age- and tissue-specific manner as observed in normoglycemic mice. However, our results showed that the inhibitory effect of OCE on enzymes was lesser in diabetic mice than observed in normoglycemic mice. Our findings are in agreement with previous reports where AR isolated from diabetic or hyperglycemic tissues exhibited lesser susceptibility to inhibition [37]. Further, it has been reported in cell culture studies that hyperglycemia induces progressive resistance of the enzyme inhibition [38]. Likewise, reduced effect of inhibition of SDH by extracts observed under diabetic conditions may be due to prolonged hyperglycemic condition which is known to result in glycation of enzymes and proteins. SDH is also reported to be glycated at Lys-210, Lys-319 and Lys-369 [39]. This may in part be responsible for the lower response in diabetic mice compared to normoglycemic mice. From our results, it was found that the percentage of AR inhibition shown by OCE was comparable to curcumin but in terms of SDH inhibition, curcumin was found to be more effective than OCE. Therefore, *O. chinensis* root extract could be a potential source of inhibitor for aldose reductase. ARI from plant origin have been reported previously [40].These results suggest that *O. chinensis* extract might contain active components which could have potential role in the prevention and /or treatment of diabetic complications.

CONCLUSION

In conclusion, the results imply that the methanolic extract of *O. chinensis* contains principle(s) that inhibit the enzymes of polyol pathway. The magnitude of inhibition being dependent on age and pathophysiological conditions of the animals. Further studies will be needed to isolate active components present in the extract and to understand the mechanism of inhibition of the enzymes of the polyol pathway.

REFERENCES

[1] American Diabetes Association.*Diabetes Care*,**2014**, 37(1 Suppl), S81-S90.
[2] NECameron;MACotter.*DiabetesMetab Rev*, **1994**, 10, 189-224.
[3] MJStevens;ELFeldman;DAGreene.*Diabetic Med*,**1995**, 12, 566-579.
[4] FGiacco;M Brownlee.*Circ Res*,**2010**, 107(9), 1058-1070.
[5] CYabe-Nishimura.*Pharmacol Rev*,**1998**, 50, 21-33.
[6] OEl-Kabbani;FRuiz;CDarmanin;RP Chung.*Cell Mol Life Sci*,**2004**, 61, 750-762.
[7] ZSong;DTFu;YSChan;SLeung;SSChung;SK Chung.*Mol Cell Neurosci*,**2003**, 23, 638-647.
[8] WOTang;KAMartin;JHwa.*Front Pharmacol*, **2012**, 3, 87.
[9] SDMathebula.*African Vision and Eye Health*,**2015**, 74(1), doi10.4102/aveh.v74i1.13.
[10]KWLee;BCKo; ZJiang;D Cao;SSChung.*Anticancer Drugs*, **2001**, 12, 129-132.
[11]KEBrown;KABroadhurst;MMMathahs;RDKladney;CJFimmel;SKSrivastava;EMBrunt. *Histol Histopathol*, **2005**, 20, 429-436.
[12] CZhu.Aldose reductase inhibitors as potential therapeutic drugs of diabetic complications.In: OOguntibeju (Ed.),Diabetes mellitus-insights and perspectives, Intech Open, Croatia,**2013**; pp. 17-46.
[13] RG Tilton; LDBaier; JE Harlow; SR Smith; E Ostrow; JR Williamson.*Kidney Int*, **1992**, 41, 778-788.
[14] IObrosova;AFaller;JBurgan;EOstrow;JRWilliamson.*Curr Eye Res*, **1997**, 16, 34-43.
[15] NATrueblood;RRamasamy.*AmJPhysiol*, **1998**, 275, H75-H83.
[16] YCHwang;MKaneko;SBakr;HLiao;YLu;ERLewis;SDYan;SIi;MItakura;LRui;HSkopicki;SHomma; AMSchmidt;PJOates; MSzabolcs;RRamasamy.*FASEB J*, **2004**, 18, 1192-1199.
[17] LWang;QGu;XZheng;JYe;ZLiu;JLi;AHagler;JXu.*JChemInf Model*,**2013**, 53(9), 2409-2422.
[18] MARamirez;NLBorja.*Pharmacotherapy*,**2008**, 28(5), 646-655.
[19] PAntony, R Vijayan.*PLoS1*,**2015**, 10(9), e0138186. doi:10.1371/journal.p1.0138186
[20] MJBalunas, ADKinghorn.*Life Sci*,**2005**, 78(5), 431-441.
[21] RKumar, DKPatel, DLaloo, SKrishnamurthy, SHemalatha.*Asian Pac J Trop Med*,**2011**, 4(9), 694-697.
[22] DSyiem;PZKhup.*PharmBiol*,**2006**, 44(8), 613-618.
[23] PSuryanarayana;MSaraswat;TMrudula;TPKrishna;KKrishnaswamy, GBReddy.*InvestOphthalmol VisSci*,**2005**, 46(6), 2092-2099.
[24] B Nagilla;PReddy.*IJBPR*,**2014**, 5(2), 131-139.
[25] JBHarborne.Phytochemical methods, 2nd ed., Chapman & Hall, New York, **1984**; pp.4-7.

[26] MNGhosh.Toxicity studies. In: MNGhosh (Ed.),Fundamentals of experimental pharmacology,(Scientific Book Agency, Kolkata, India, **1984**) 153-158.
[27] SRDasgupta;MSinha;CCSinha;BPMukherjee.*Indian JPharmacol*,**1969**, 1, 49-54.
[28] SPWolff;MJCCrabbe.*Biochem J*, **1985**, 226, 625-630.
[29] U Gerlach.Methodsof Enzymatic Analysis, Vol. 3, 3rd ed., VerlagChemie, Weinheim, Germany, **1984**; pp. 112-117.
[30] D Syiem, SMajaw. *Pharmacologyonline*, **2011**, 3, 63-72.
[31] MM Bradford.*AnalBiochem*,**1976**, 72, 248-254.
[32] Y Wen; IBekhor.*Curr Eye Res*,**1993**, 12, 323-332.
[33] J Jeffery; HJoernvall.*ProcNatlAcad Sci*.**1983**, 80, 901-907.
[34] D Syiem; S Majaw.*Pharmacologyonline*, **2010**, 2, 671-680.
[35] JHKinoshita;SFukushi;PKador;LOMerola.*Metabolism*,**1979**, 28, 462-469.
[36] HMPPoumale;RHamm; YZang;YShiono;V Kuete. Coumarins and related compounds from the Medicinal plants of Africa,1st ed. In: V Kuete (Ed.),Medicinal Plant Research in Africa, Pharmacology and Chemistry, Elsevier Inc., Waltham, MA, USA, **2013;** pp. 285.
[37] MAMansour.*CurrEnzInhib*,**2007**, 3(1), 49.
[38] M Lorenzi;SToledo; GRBoss; MJ Lane;DFMontisano.*Diabetologia*, **1987**, 30,222-227.
[39] H Eklund; E Horjales; HJornvall; CI Branden; J Jeffery.*Biochemistry*, **1985**, 24, 8005-8012.
[40] C Veeresham; K Asres; AR Rao.*Phytother Res*,**2014**, 28(3), 317-33.