International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 233-244, Jan-Mar 2012

Antihyperglycemic effect of Aqueous and Ethanol extract of Aerial part of *Osbeckia nepalensis* Hook in Alloxan induced Diabetic rats

M. Neshwari Devi[1]*, Kh. Biren Singh[2], S.R. Singh[3], C.B. Singh[4],Deb Lukesh[5], Dey Amitabh[6].

[1]Scholar J.N.U., [2]Modern College, Imphal, [3]HRDRI, Canchipur, Manipur, India. [4]Dept. of Natural Product Chemistry, IBSD, [5,6]Dept. of Pharmacology, IBSD, Takyelpat, Manipur, India.

Corres.author: hrdri 2008@ gmail.com

Abstract: Aqueous and ethanolic extract of *Osbeckia nepalensis* Hook was evaluated for the antihyperglycemic activity in alloxan induced diabetic rats. Hyperglycemic was confirmed to Albino rats (150- 200 g) by treated with alloxan upto 320.6 ± 3.59 at 1 hr, 445.6 ± 4.23 at 5 days from non treated rats of 316 ± 3.56± mg/ dL. A significant hypoglycemic activity of aqueous and ethanolic extract was confirmed in alloxan induced diabetic rats ($P<0.001$). The serum glucose level of alloxan induced diabetic rats reduced upto 312.2 ± 3.65 and 232 ± 3.05 mg/dL at 1 hr and 120 hrs from 359±3.8 mg/ dL in aqueous extract and 320.6 ± 3.59, 445.6 ±4.24 mg /dL at 1 hr and 120 hrs. after treatment from 330 ± 3.64 mg/ dL before treatment in ethanolic extract of the test herbal. The aqueous and ethanolic extract of *Osbeckia nepalensis* enhance the checking of reduction in weight in comparision to normal and diabetic control animals. Further in the present work, phytochemicals of antioxidant (IC_{50} 23 µg/ml); saponin (20.0 ± 0.89 mg/g); flavonoid (38.28 mg/g) and minerals of K, (6.02 ± 0.51); N(22.1± 0.94); P(0.53±0.14) and Co (N.D) have screened out from the methanolic extract of *Osbeckia nepalensis* suggesting a unique supportive to the health care ingredients. The extracts both in aqueous and ethanolic substantiates for pharmaceutical formulation upto 4000 mg/kg oral toxicity.

Key words: *Osbeckia nepalensis* Hook, Antihyperglycemic activity, Phytochemical constituents, Minerals.

INTRODUCTION

Due to the change in lifestyle, the number of the people in the world with diabetes has increased dramatically over recent years. It is reported that the diabetic population will increased continuously around the world [1]. Diabetes mellitus is one of the most serious, chronic diseases i.e. developing with an increase in obesity and ageing in the general population.

Furthermore, diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism[12]. The non-insulin dependent diabetes mellitus (NIDDM) conditions, which has assumed epidemic proportion today[18], is characterized by reduced circulating

concentration of insulin, poor insulin sensitivity or insulin resistant, poor glucose tolerance resulting in high sugar in plasma[2]. Again, prolonged hyperglycemia leads to other micro vascular and macro vascular complication[34]. Current drugs used for treatment of diabetes including few groups of chemical compounds such as the drug biguanide (metformin), which although anti hyperglycemic does not affect hypoglycemia in the normal subject[6] and involves extra-pancreatic mechanisms[19]. Further, it is apparent that different mechanisms are involved in bringing down blood sugar level in normal or hyperglycemic conditions. However, some, such as biguanides, reportedly have undesirable side effects, and hence there is need for effective, safe and better oral hypoglycemic agents[25].

Osbeckia nepalensis Hook, a shrub growing to 1 m or more, with 4-angled branches covered with addressed hairs. Lance like leaves with entire margins have 3-5 parallel veins. Handsome flowers are mauve-purple or white, 3-5 cm across, in rather dense branched clusters at the end of branches. Sepals are covered with large flat scales, fringed with bristles. Stamens are straight, and all similar. The anthers also do not have apical beaks. It is found at altitudes of 600-2300 m. flowering in July-November. Traditionally the plant is used in health care system since immemorial time in management of diabetes in Manipur. There were scanty reports of *Osbeckia nepalensis* on diabetes. Hence, the present work was undertaken to investigate on its antihyperglycemic activity of therapeutic compounds from aqueous and ethanolic extract of *Osbeckia nepalensis* in alloxan induced diabetic rats.

MATERIALS AND METHODS

Chemicals and instruments used

The following chemicals used in the study: Alloxan (Labo. Chemic Bombay, India).

Plant material

For detecting the organic phytochemicals of therapeutic compounds, all the aerial parts of the plant of the fresh *Osbeckia nepalensis* Hook was collected freshly from Manipur near Kakching, Thoubal District during the month of July - August 2009 and its authentication has been confirmed by BSI, Shillong and deposited the plant in the Post Graduate Study centre, HRDRI, Canchipur. HRDRI/NES-10 for future reference.

Preparation of plant extracts

The dried plant material (1.5 kg) was ground in a warning blender and shifted through a wire screen. The powdered material (500 g) was extracted exhaustively first in soxhlet apparatus with ethanol and water for 48 hrs. The extracts was filtered and concentrated on a rotary vacuum evaporator. These are stored in room temperature and then refrigerated. The ethanol extract was used for acute toxicity and antidiabetic investigation was made under standardized condition.

Determination of free radial scavenging of plant extracts by the use of DPPH radical[11],Estimation of total flavonoid content[10]. Saponin[30].

Mineral element determination of Potassium is determined using flame photometer, phosphorous by vanado-molybdate yellow method and nitrogen by Kjelhdal method.

Experimental animals

Albino rats of both sexes (150- 200 g) were kept in approved standard animal house conditions, fed standard pellet diet (Hindustan Lever Ltd., Bombay). Fasted animal were deprived of food for at least 16 hr. but were allowed free access water. The study was carried out with prior approval obtained from the institutional animal ethical committee of Institute of Bioresource and Sustanable Development (IBSD) Takyelpat, Imphal.

Acute toxicity and selection of doses

The acute toxicity studies was carried out in adult female Wister albino mice weighing about 200g by up and down method as per OECD 425 guidelines (OECD 425 guidelines). Overnight fasted animals received test drug at a dose of 2000 mg/kg body weight orally. Then the animals were observed continuously once in half an hour for next 4hrs and then after 24hrs from the time of administration of extract for general behavioral, neurological, autonomic profiles and find out mortality. The extracts found safe up to a dose of 200 mg/kg body weight.

Biochemical parameters test in Alloxan induced diabetic rats

For analysis of haematological and biochemical changes, blood samples were centrifuged at 4000rpm for 15minutes, then collected serum was determined according to the ERBA diagnostic kit instruction manual. The fasting glucose level was determined by Accu chek Narmoglycemic study Goglucan etc. some plasma used for biochemical parameters test in Alloxan induced diabetic rat estimation of plasma cholesterol triglycerides creatinine and urea by spectrophotometric assays

according to the method presented in the Erba diagnostic kit instruction manual.

For hormoglycemic study rats were divided into three groups (n=3) and were administered 2% gum acacia solution, since alloxan is comparable of producing fatal hypoglycemic as a result of massive pancreatic insulin release rats were treated with 20% glucose solution (15-20 ml).

Induction of experimental diabetes

Diabetes has induced in overnight fasted rats by single intraperitoneal injection of 150mg/kg alloxan monohydration saline (2%IP). Hyperglycemia was confirmed by the elevated blood glucose level determined at 48 hrs after the dose. Animal that exhibited glycosuria after 48 hrs tested by urine test strips (Uristix, Bayer diagnostic Ltd., India) were considered as diabetic.

Experimental and design

The animals were divided into four groups of five animals in each group.

Group I : Normal healthy control.

Group II:Diabetic control (alloxan monohydrate 150 mg/kg in saline 2% IP).

GroupIII:Diabetic + Aqueous extract 200 mg/kg BW/day orally.

Group IV: Diabetic + Ethanol extract 200 mg/kg BW/ day dose orally.

The extract treatments were carried out for a period of 7 days. During the period, animals for all groups had free access to standard diet and water. Body weight and blood glucose level were estimates on first day before and after treatment and 7th days of extract treatment. On the 7th day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for hematological and biochemical analysis.

Statistical analysis

The result were expressed as mean ± SEM. Statistical analysis were carried out using paired t-test and one way ANOVA following Bonferroni Multiple Comparisons test using graph pad install version 3 (Graph Pad software). All graphs prepared were made by graph and prism software. Differences below p <0.05 implied statistically significance.

Table A.1. The phytochemical constituents of saponin, flavonoid in mg/g and antioxidant in µg/ml of *Osbeckia nepalensis Hook.*

Plant species	Antioxidant IC_{50} (µg/ml)	Saponin	Flavonoid
Osbeckia nepalensis	**23**	**20.00**±0.89	38.28±1.24

Value are expressed as mean ± SEM; n = 3 in triplicate for each data

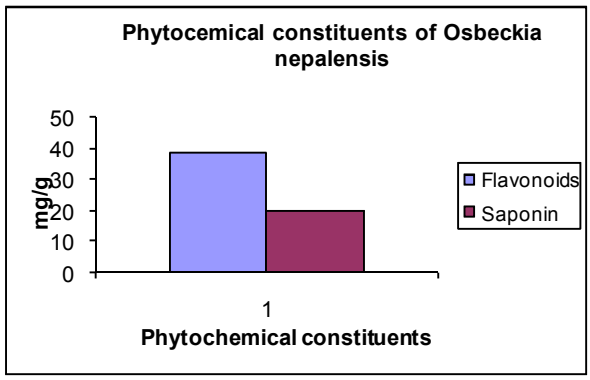

Fig.A.1. Phytochemical composition of *Osbeckia nepalensis*

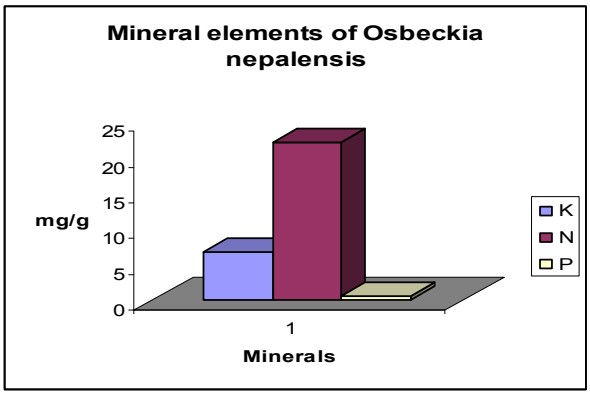

Fig.A.2. Mineral composition of *Osbeckia nepalensis.*

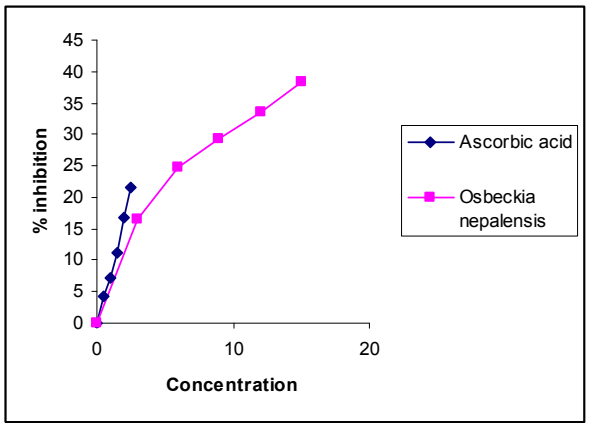

Fig.A.3. DPPH free radical scavenging activity of methanolic extract *Osbeckia nepalensis* added to methanolic solution of DPPH as compared to standard Ascorbic acid

Table A.2. Composition of Mineral elements of *Osbeckia nepalensis.* (Average of three replications).

Plant species	K	N	P	Co
Osbeckia nepalensis	6.72±0.51	22.1±0.94	0.53±0.14	ND

Value are expressed as mean ± SEM; n = 3 in triplicate for each data

Table. B.1. Effect of aqueous and ethanol extract from from *Osbeckia nepalensis* on serum glucose level (mg/dL) in alloxan diabetic rats.

Group	Treatment	Blood glucose level (mg/dL)			
		Before treatment	After 1 hr of treatment	After 5 days of treatment	Remarks
I	Diabetic control (Aloxan only)	316± 3.56	320.6 ± 3.59	445.6 ±4.23	
II	Aqueous extract + Alloxan	359.4±3.8	312.2±3.65	232.4±3.05	
III	Ethanol extract + Alloxan	330.0±3.64	261.0±3.23	110.8±2.11	

Value are expressed as mean ± SEM; n = 5 in duplicate for each treatment.

Table. B.2. Effect of Alloxan on experimental rats

Group	Treatment	Blood glucose level (mg/dL)			
		Before treatment	After 1 hr of treatment	After 5 days of treatment	Remarks
I	Normal control (1ml distilled water P.O./day + 2 ml/kg saline I.P.)	89.20 ± 1.89	89.40±1.89	89.40± 1.89	89.20± 1.89
II	Diabetic control (1ml distilled water P.O./day + 150 ml/kg saline I.P.)	89.20±1.89	316.00 ±3.56	320.6±3.59	445.6± 4.23

Value are expressed as mean ± SEM; n = 5 in duplicate for each treatment.

Table. B.3. Effect of aqueous and ethanol extract from from *Osbeckia nepalensis* on serum glucose level (mg/dL) in normal fasted rats.

Groups	Treatment	Glucose level (mg/dL)					
		O_1	O_2	O_3	O_4	O_5	Mean
I	Normal control /Diabetic control	85	103	78	87	93	89.20 ±1.89
II	Sample aqueous extract	85	101	78	87	90	88.20±1.88
III	Sample ethanol extract	85	100	77	86	89	87.40±1.87

Value are expressed as mean ± SEM (Standard error Mean)

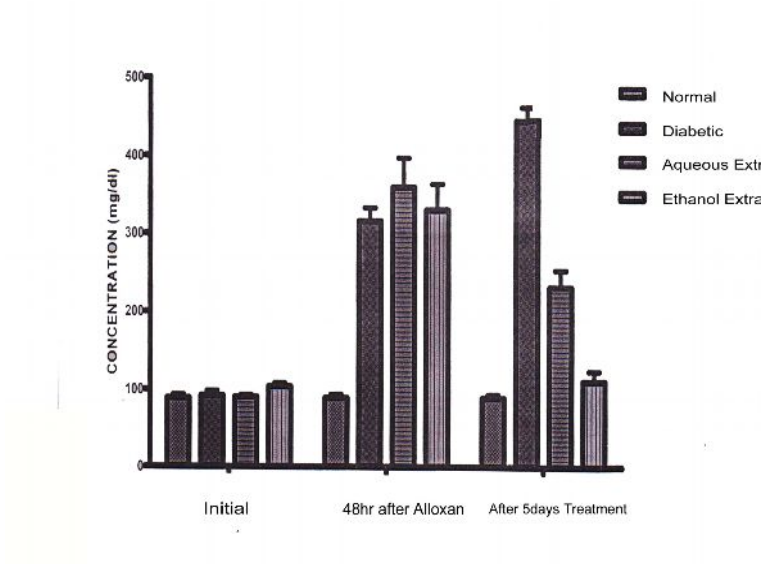

Fig: B.1. Effects of aqueous and Ethanol Extract from *Osbeckia nepalensis* on blood glucose level in Alloxan monohydrate induced diabetic rats

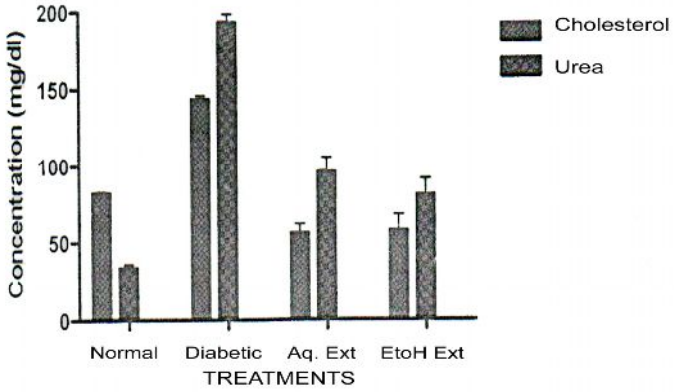

Fig: B.2. Effects of aqueous and Ethanol Extract from *Osbeckia nepalensis* on Cholesterol & urea in Alloxan monohydrate induced diabetic rats

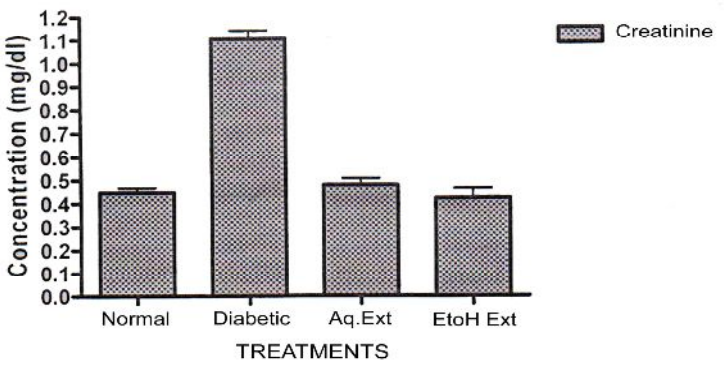

Fig: B.3. Effects of aqueous and Ethanol Extract from *Osbeckia nepalensis* on Creatinine in Alloxan monohydrate induced diabetic rats

Table. B.4. Effect of unknown sample on body weight (gm) of rats

Group	Treatment	Initial (0) day (g)		Final 7 Days (g)		Remark Changes ±increases ±decreases
I	Normal control (Vehicle)	200	202 ± 2.85	208	210 ± 2.9	+8
		250		260		+10
		170		150		+10
		190		195		+5
		200		207		+7
II	Diabetic control	200	200 ± 2.83	185	183 ± 2.71	- 15
		210		200		- 10
		210		190		-20
		200		190		- 10
		180		150		- 30
III	Sample aqueous extract 200mg/kg BW/day dose	190	198 ± 2.82	190	191 ± 2.77	- 00
		210		200		- 10
		210		200		- 10
		200		195		- 5
		180		170		- 10
IV	Ethanol extract 200mg/kg BW/day dose	200	196 ± 2.86	195	187 ± 2.74	-5
		200		190		- 10
		180		170		-10
		190		180		- 10
		210		200		-10

Value are expressed as mean ± SEM; n = 5 in duplicate for each treatment.

RESULTS AND DISCUSSION

The phytochemical investigation of *Osbeckia nepalensis*, revealed the presence of with constituents of saponin and flavonoid (Table A.1.). Investigation on quantitative phytochemical constituents of *Osbeckia nepalensis* yields a number of organic compounds inclusive of antioxidant (Table A.1.). Graphically it is represented in fig. A.1. and fig. A.3. The potential of concentration of antioxidant ranged to 23 µg /ml, saponin 20m/g and flavonoid 38.28 mg/g.

The present phytochemical test of plant extract estimated for antioxidants accord IC_{50} 23 µg/ml, under free radical scavenging activity technique and confirmed the unique presence of

antioxidants and patented in the test herbal *Osbeckia nepalensis*. It is shown in table A.1. and fig. A.3.

Blood glucose levels of alloxan induced diabetic rats accord 316 ± 3.56, 320.6 ± 3.59, 445.6± 4.23 mg/dL while the control accord 89.2±1.89, 89.40 ±1.89, 89.4±1.89, 89.2±1.89 mg/ dL respectively at 48 hrs, 49 hrs and 5 days after injection(Table B.1.,B.2. and Fig. B.1.).

Blood glucose level of animal treated with aqueous extract of *Osbeckia nepalensis* accord 88.20 ± 1.88mg/dL and 87.40±1.87mg/dL with ethanol extract of the same test plant while the control (without any extract) accord 89.20 ± 1.89. (Table B. 3. and Fig. B.3.).

In the experimentation on the effect of *Osbeckia nepalensis* extracts in aqueous and ethanol, on blood glucose level of alloxan induced diabetic rats accord 359.4 ± 3.8, 332.2 ±3.65, 232.4 ±3.65mg/dL in aqueous extract and 330.0 ±3.64, 261.0 ±3.23, 110.8 ±2.11mg/dL in ethanol extract at 0hrs and 120 hrs after treatments respectively(Table B.1. and Fig. B.1.).

Experimentation on effect of *Osbeckia nepalensis* extract in aqueous and ethanol for body weight (gm) of alloxan induced diabetic rats strike with 198±2.82gm and 191±2.77gm in aqueous extracts and 196±2.86 gm, 187±2.74 gm in ethanol extract at 0 and 7th day respectively after treatment. While diabetic control accord 200±2.83gm and 183±2.7gm and normal control accord 202±2.85gm and 210±2.9gm at 0 and 7th day after treatment. (Table B.4.).

Effect of test herbal *Osbeckia nepalensis* extract in aqueous and ethanol on the biochemical parameters (mg/dL) of cholesterol, creatinine and urea of alloxan induced diabetic rats has detected. The aqueous extract hits 56.00 ± 1.15, 0.4780±0.13 96.21±1.96 and ethanol extract strike 57.79 ± 1.52, 0.4220± 0.13, 81.27±1.8mg/dL, for cholesterol, creatinine and urea respectively as against the record of the diabetic control rats with 143.5±2.4, 1.106±0.21, 193.3±2.78 mg/dL for cholesterol, creatinine and urea respectively. While the normal control rats accord 82.68 ± 1.82, 0.448 ±0.13, 33.86±1.16 mg/dL for cholesterol creatinine and urea respectively. (Table B.5. and Fig. B.2. ; B.3.).

Table. B.5. Effect of aqueous and ethanol extract from *Osbeckia nepalensis* on biochemical parameter in alloxane induced diabetes rats.

Treatment	Biochemical Parameters (mg/dl)		
	Cholesterol	Creatinine	Urea
Normal control (1ml distilled water P.O./day + 2 ml/kg saline I.P.)	84.68	0.45	40.22
	86.73	0.40	37.31
	79.49	0.51	35.30
	82.92	0.42	30.21
	81.23	0.46	26.27
Mean ± SEM	82.68±1.82	0.448±0.13	33.86±1.16
Diabetic control (1ml distilled water P.O. + 150 mg/kg alloxane monohydrate in saline(2%, I.P.)	139.42	1.02	190.7
	145.23	1.14	200.2
	152.32	1.23	187.2
	142.31	1.09	179.3
	138.24	1.05	209.0
Mean ± SEM	143.5±2.4	1.106±0.21	193.3±2.78
Sample aqueous extract 200mg/kg BW/day dose	44.75	0.44	87.16
	69.06	0.52	132.49
	40.66	0.40	85.43
	68.29	0.47	97.35
	57.23	0.56	78.62
Mean ± SEM	56.00±1.5	0.478±0.13	96.21±1.96
extract 200mg/kg BW/day dose	86.80	0.31	54.63
	37.72	0.44	80.40
	31.50	0.35	72.51
	63.40	0.49	119.50
	69.58	0.52	79.32
Mean ± SEM	57.79±1.52	0.422±0.13	81.27±1.8

ONE –WAY ANALYSIS OF VARIANCE (ANOVA)
GLUCOSE COMPARISION DATA (7TH DAY)
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

Comparision	Mean difference	t	P value
Diabetic vs Aq. Extract	213.20	9.718	***P<0.001
Diabetic vs Ethanol extract	334.80	15.261	***P<0.001

Differences	Mean difference	95% confidence Interval From To
Diabetic –Aq. Extract	213.20	158.95- 267.45
Diabetic -Ethanol extract	334.80	280.55- 389.95

ONE –WAY ANALYSIS OF VARIANCE (ANOVA)
CHOLESTROL DATA
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

Comparision	Mean difference	t	P value
Diabetic vs Aq. Extract	87.500	10.202	***P<0.001
Diabetic vs Ethanol extract	85.710	9.993	***P<0.001

Differences	Mean difference	95% confidence Interval From To
Diabetic –Aq. Extract	87.500	66.291- 108.71
Diabetic -Ethanol extract	85.710	64.501- 106.92

ONE –WAY ANALYSIS OF VARIANCE (ANOVA)
UREA DATA
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

Comparision	Mean difference	t	P value
Diabetic vs Aq. Extract	97.090	8.923	***P<0.001
Diabetic vs Ethanol extract	112.03	10.296	***P<0.001

Differences	Mean difference	95% confidence Interval From To
Diabetic –Aq. Extract	97.090	70.182- 124.00
Diabetic -Ethanol extract	112.03	85.122- 138.94

ONE –WAY ANALYSIS OF VARIANCE (ANOVA)
CREATININE DATA
The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Bonferroni Multiple Comparision Test
If the value of t is greater than 2.473 then the P value is less than 0.05.

Comparision	Mean difference	t	P value
Diabetic vs Aq. Extract	0.6280	14.021	***P<0.001
Diabetic vs Ethanol extract	0.6840	15.272	***P<0.001

Differences	Mean difference	95% confidence Interval From To
Diabetic –Aq. Extract	0.6280	0.5172- 0.7388
Diabetic -Ethanol extract	0.6840	0.5732- 0.7948

For present bio-assaying, LD_{50} was determined by the fixed dose (OECD/OCDE) method and LD_{50} cut off of the different extracts were 1000 mg/kg body weight. All extracts were administered as $1/5^{th}$ the dose of their respective LD_{50} values in vivo experiments. During the present investigation both the aqueous and ethanol extracts of *Osbeckia nepalensis* leaves were subjected for antidiabetic activity in rats where alloxan monohydrate (150 mg/kg body weight) used as the diabetogenic agent. The findings accord a marked rise in blood glucose level upto 445±4.23 mg/dL after 5 days in diabetic control group of rats, which also became hyperlipidine, restless and irritable. Severe thirst and lack of appetite were observed. Among test rats aqueous and ethanol treated groups of rats showed progressively reduction in blood glucose levels upto 232.4±3.05 mg/dL after 5 days and 110.8±2.11 mg/dL after 5 days respectively. All the extracts have produce distinct anti -diabetic activity on 5^{th} day as compared to 1^{st} day of treatment.

Perusal on table A.1. revealed that the fully matured plant of *Osbeckia nepalensis* were free sources of therapeutic organic compounds of antioxidant, saponin, flavonoid. The presence of these bioactive compounds has empathetically emphasized the medicinal potentials of the test herbal. Similar result has reported from different plants [29- 27].

The present phytochemical test of plant extract depict an antioxidant content upto 23 µg /ml under free radical scavenging activity technique confirmed the unique presence of the compound in *Osbeckia nepalensis*. Antioxidant and its ability to balance or trap highly reactive free radicals and oxygen species are very essential in biological systems from a wide variety of sources for longer life of cells. These free radicals may oxidised nucleic acids, protein, lipids or DNA and can initiate degenerative diseases. Further antioxidant compounds like phenolic acid, polyphenols and flavonoid scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism which leads to degenerative disease. There are number of clinical studies suggesting that the antioxidant in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancer [24].

Table A.1. delineated the flavonoid compounds content upto 38.28mg/g in the leaves of *Osbeckia nepalensis*. The value is also represented by fig A.1. showing the status among the 5 phytochemical compounds of the plant. Flavonoids have attracted a great deal of attention in relation to their potential for beneficial effect on health. Over the past few years, several experimental studies have demonstrated biological and pharmacological properties of many flavonoids especially their antimicrobial activity[28], anti-inflammatory[26], antioxidant[31] and anti-tumor[8] effects, which are associated with free radical scavenging action.

Flavonoids have also been reported to posses hypoglycaemic and anti-diabetic effect[4] .

The present phytochemical test determined the presence of saponin with 20 mg/g (Table A.1.). Saponins are useful in medicine and pharmaceutical industries due to its foaming ability that produces for the effects in the food industries[15]. Saponins is also used in the manufacture of sampoos, insecticides, various drug preparation and synthesis of steroidal hormones [32]. Saponins have also been reported to have hypoglycaemic and antidiabetic effects which are very useful in the management of diabetes mellitus[3, 35] .

Table A.2. revealed that the *Osbeckia nepalensis* accounts distinct mineral content with remarkable amounts viz. potassium 6.72 mg/g, nitrogen 22.1mg/g, phosphorous 0.53 mg/g, cobalt not detected. Minerals are naturally occurring chemical elements found throughout the human body in the bones, muscles, teeth, blood and nerve cells. Minerals help to maintain a normal water balance within the body, Phosphorus is needed for energy production, metabolism, and healthy bone development. Potassium is needed for muscle contractions and nerve function[33].

Preliminary phytochemical screening of the methanolic extract of *Osbeckia nepalensis* reveals the presence of flavonoids, saponin and antioxidant. Different doses of herbal plant extract in aqueous and ethanol was screened for their oral toxicity. No mortality was recorded upto 4000mg/kg with both of the extracts, hence the extract were confirmed to be safe up to the dose levels of 4000mg/kg, consequently the antidiabetic effect of the test herbal extracts in aqueous and ethanol was further experimentally investigated.

Table B.1. reveals the blood glucose levels of the alloxan induced diabetic rat's with 316 ± 3.56mg/dL, 320.6 ± 3.59; 445.6± 4.32 at 48, 49 and 120hrs as against the record of 89.40 ± 1.89mg/dL at 48, 49 and 120 hrs after injection in normal control rats. The finding clearly shows that the alloxan induced diabetic rats have remarkably higher level of blood glucose level ranging from 316±3.56 to 445.6 ± 4.23at 48 hrs. Thus, confirmed the induction of diabetic to the test rats for the present experimentation.

Table B.3. depicts the blood glucose level of animal treated with aqueous extract and ethanol extract with 88.20 ±1.88 and 87.40 ± 1.87mg/dL against the 89.20 ± 1.89mg/dL of control. The finding vividly clarify the extract of the test herbal have significant reducing capability of blood glucose level. The finding was in agreement with that of the other workers from other herbal extracts of different parts of the world [21-,16,36] .

Table B.1. displayed the effect of *Osbeckia nepalensis* in aqueous extract, the blood glucose level of alloxan induced diabetic rats, which accord 332.2 ± 3.65 and 232.4 ± 3.05 mg/dL at 1 hr and 120 hrs. However, in ethanol extract, its accord struck 261.0 ± 3.23, 110.8 ± 2.11 mg/dL at 1 hr and 120hrs after treatment as against the corresponding control values of 320.6 ± 3.59, 445.6 ± 4.24 mg/dL at 1 and 120 hrs after treatment. It is evident from the present finding that a significant hypoglycemic activity of aqueous extract has confirmed in alloxan induced diabetic rats. After oral administration of 200 mg/kg, BW/day of aqueous extract of *Osbeckia nephalensis*, a significant reduction has observed in blood glucose level after 1hr of treatment and then the hypoglycemic become more effective after day 5.

Similarly observation of hypoglycemic effect of ethanol extract of *Osbeckia nepalensis* connote in the blood glucose level of alloxan induce diabetic rats, the effect become more conspicuous after day 5 of treatment. Obviously, in conclusion it patented that both the ethanolic and aqueous extract of *Osbeckia nepalensis* produced significant antihyperglycemic activity against the alloxan induced diabetic rats. The finding was in corroborative with the results of other antidiabetic herbal extracts from other plants[13- 7]. The chronic treatment with methanol extract of *A.occidentale* stem-bark at 200mg/kg may be a safe agent that has a protective role against the diabetogenic and atherogenic effects of high fructose diet by reducing the hyperglycemic and hyperlipidemia as well as lipid peroxidation[22]. Further the α- glucosidase type I recognizes the glucosyl structure of the substrate, while the α- glucosidase type II recognizes the maltosyl structure[20]. *Terminalia belerica* found to be most active to reduce serum glucose level followed by *E. officinalis* and *T. chebula*, *Triphala* that is a combination of all the three produced a significant action in reducing the alloxan induce diabetic. The result is slightly different with the result reportedearlier[5] where, *T. chebula* has a higher α- glucosidase inhibitor activity compared to glucose tolerance method with amylum, maltose and sucrose loading as acarbose mechanism is to competitively inhibiting an enzyme (α- amylase) located at the brush border of the small intestine that is responsible for terminal carbohydrate digestion. This inhibition decrease glucose absorption, thereby reducing alimentary hyperglycemia and hyper-insulinemia[17] . Choudhary and Bandyopadhyay[9] stated that hypokalemia or potassium depletion could cause glucose intolerance. Ca, K, and traces of chromium play an important role in insulin release from the β cells of the Langerhans islet, which finally helps to lower the blood glucose level. The content of the

metals and antioxidant in the extract of *Osbeckia nepalensis* herb may add the information on additional role in the anti-diabetes activity of the *Osbeckia nepalensis* herb.

Table B.4. exhibits the effect on the body weight (gm) of alloxan induced diabetic rats of *Osbeckia nepalensis* hits (198 ± 2.82gm), (191 ± 2.77gm) in aqueous extract and (196 ± 2.86 gm), (187 ± 2.74gm) in ethanol extract at 0 and 7 day. After treatment (200 ± 2.83 gm) and (183 ± 2.71 gm) in diabetic control rats and 202 ± 2.85 gm and 210 ± 2.9 gm in normal control after 0 and 7 days of treatment. The finding revealed that the ethanol extract of *Osbeckia nepalensis* have lesser degree of reduction in weight in comparision to normal and diabetic control animals. In this connection Fisman and Tenenbawn,(2008) rightfully claimed that five types of oral antihyperglycemic drugs currently approved for the treatment of diabetes i.e biguanides, sulfruglureas, meglitinides, glitazones and alpha-glucosidase inhibitors have limitations to some extend especially in patients with Coronary Artery Disease (CAD). Further, current data indicates that combined glibenclamide/metformin therapy seems to present a special risk and should be avoid in the long-term management of type 2 diabetes with proven CAD.

CONCLUSION

The alloxan induced diabetic rats have remarkably higher level of blood glucose ranges from 316 ± 3.56 to 445.6 ± 4.23 at 48hrs. The extract of the test herbal have significant reduction capability of blood glucose levels. A significant hypoglycemic activity on aqueous extract comply with alloxan induced diabetic rats. After oral administration of 200mg/kg BW/day of aqueous extract of *Osbeckia nephalensis*, a significant reduction was accommodated in blood glucose level after one hours of treatment and then the hypoglycemic effect become more pronounce and adopted after day 5.

Further, it patented that both the ethanolic and aqueous extract of *Osbeckia nepalensis* produced significant antihyperglycemic activity against the alloxan induced diabetic rats. Obviously, the present finding incorporated and getting support with antidiabetic activity of the test *Osbeckia nepalensis* due to presence of saponin, flavonoid, antioxidant, N, K, P etc.

Acknowledgement

The authors wish to thank UGC, New Delhi for financial assistance**.**

REFERENCES

1. Aslam M.; Orhan D.D.; Sezizik, E. and Yesiada, E., In vivo antidiabetic and antioxidant potential of Helichrysum plicatum sp. plicatum capitulums in streptozotocin-induced-diabetic rats. J. Ethnopharmacol. 2007, 109, 54-59.
2. Arky R.A., Clinical correlates of metabolic derangements of diabetes mellitus. In: Kazak GP, ed., Complication of diabetes mellitus, Philadelphia, 1982 , 16-20.
3. Anila L.; Vijayalakshmi, N.R. and Tian, C., Beneficial effect of flavonoids from Sesamum indicum, Emblica officinalis and Momordica charantia. Phytother. Res. 2002, 14(8): 592 –595.
4. Ahmad, I.; Mehmood, Z.; Mehmood, P. and Ahmed, S., Antimicrobial potency and synergistic activity of five traditionally used Indian medicinal plants. J. Med. aromatic plant Sc.2000, 22,173-176.
5. Anam, K.; Widharma, R. M. and Kusrini, D., α-glucosidase inhibitor activity of Terminalia species. Int. J. Pharmacol. 2009, 5, 277-280.
6. Bailey, C. I., Biguanides and non-insulin dependent diabetes mellitus. Diabetic care. 1992, 12: 663-564.
7. Bhushan, B.; Prakash, I.and Abhay, F., Evaluation of antimicrobial activity of medicinal plants Jatropha podagrica (Hook). Romanian Society of Bio. Sc. 2008, 13(5): 3873-77.
8. Castillo, M. H.; Perkins E., Campbell J.H.; Doerr, R.; Hassett, J.M.; Kandaswami, C. and Middleton, E., The effects of bioflavonoid quercetin on Sqamous Cell Carcinoma of head and neck region. Am. J. Surgery, 1989,158: 351 – 355.
9. Choudhary, K. A.and Bandyopadhyay, N. G. Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test, J. Ethnopharmacol. 1999, 64, 179-184.
10. Chang, C.; Yang, M.; Wen, H. and Cherr, J., Estimation of total flavonoid content in protein by two complementary colorimetric methods. *J. Food Drug Analy. 2002,*10,178-182.
11. Dudonne, S.; Vitrax, X.; Coutricre, P.; Woilez, M. and Merillon, J., Comparative study of antioxidant properties and total phenolic content at

30 plants extract at industrial Interest using DPPH, ABTs, FRAP, DOS and ORAC Assay *J. Agrc. Chem*. 2009, 57, 1768-1774.

12. Davis, S.N. and Granner, D.K., Insulin oral hypoglycemic and the pharmacology of the endocrine pancreas. The pharmacological basis of therapeutics, 9th Ed. New York, McGraw-Hill., 1996, 1487-1517.
13. Day,C.; Cartwright, T. and Provost, J., Hypoglycemic effect of Momordica charantia extracts. Planta Med. 1990, 56, 426-29.
14. Fisman, E.Z. and Tenenbaum, A., Cardiaovascular diabetology : Clinical metabolic and inflammatory facets. Adv Cardiol, 2008, 45,11-13.
15. George, A.G., Legal status and toxicity of saponins in food and cosmetics. Toxicol. 1965, 3:85-91.
16. Grover, J.K. and Gupta, S.R., Hypoglycemic activity of seeds of Momordica charantia . Eur. J.Pharmacol. 1990, 183, 1026-1027.
17. Hasegawa, G.; Kajiyama, S.; Tanaka, T.; Imai, S. and Kozai, H., The α- glucosidase inhibitor acarbose reduces the net electronegative charge of low density lipoprotein in patients with newly diagnosed type 2 diabetes. Clinica Chimica Acta, 2008, 390,110-114.
18. Kilbertis, P. and Robert, L., The puzzle of complex disease. Science. 296:685-689.
19. Karam, J.H. (1982). Pancreatic hormone and anti-diabetic drugs. In: Basic and clinical pharmacology. 1st ed. California 2001, 464-466.
20. Kim, Y. M.; Wang, M. H.; and Rhee, H. I. (2004). A novel α- glucosidase inhibitor from pine bark. Car-Res., 339: 715-717.
21. Leung, L.; Richard, B.; Jyoti, K. and Sharon, C., Antidiabetic and hypoglycemic effects of Momordica charantia (bitter melon): a mini review. British Journal of Nutrition. 2009, 1-5.
22. Lawrence, A.; Olatunji, J.; I. Okwusidi and Aydele, O., Antidiabetic effects of Anacardium occidentale stem bark in fructose - diabetic rat. Pharmaceutical Biology. 2005, 43, 7, 589-593.
23. Marwah, R.G.; Fatop, M.O.; Varma, G.B. and Abai, H.A., Antioxidant capacity of some edible and wound healing plants in Oman, Food Chem., 2007, 101, 465-470.
24. Miller, H. E.; Rigelhof, F.; Marquart, L.; Prakash, A. and Kanter, M Cereal foods world. 2000, 45(2), 59-63.
25. Murthy, P. S., Medicinal plants in diabetes treatment. Indian J. Vlin. Biochem. 1995, 10, 52-53.
26. Middleton, E.J.R.; Kandaswami, C. and Theoharides, T.C.,The effects of plants flavonoids on mammalian cells. Implications on inflammation, heart disease and cancer. Pharmacol. Rev. 2000,52: 673 – 751.
27. Naznin, A. and Hasan, N. (2009)., In vitro antioxidant activity of methanolic leaves and flower extracts of Lippia Alba. Research J. of Medicine and Medical science. 2009, 4(1): 107-110.
28. Narayana, K.R.; Reddy, M.R. and Chaluvad, Krishna, D.R., Bioflavonoid classification, pharmacology, biochemical effects and therapeutic potential. Ind. J.Pharm.,2001,33:2 -16.
29. Okwu, D. E., Phytochemicals, vitamins and mineral contents of two Nigerian Medicinal plants. International journal of molecular medicine and advance science. 2005, 1(4): 375-381.
30. Obadoni, B.O. and Ochuko, P.O., Phytochemical studies and comparative efficacy of crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. Global J. Pure and Applied Science. 2001,8, 203-208.
31. Parker, L.; Rimbach, G. and Virgili, F., Antioxidant activity and biologic properties of aprocyanidin-rich extract from pine (Pinus martima) bark pycnogenol free rad. Biol. Med.1999, 27, 704 – 724.
32. Sodipo, O.A. and Akiniyi, J.A., Studies on certain characteristics of extracts from bark of Pansinystalia macruceras. Pierre Exbeille Global J. Pure and Applied Sc. 2000, 6: 83-87.
33. Siberry, G. K. and Robert, I., The Harriett Lane Handbook, 2000, 15th ed.
34. Steppan, C.M.; Bailey, S.T.; Bhatt, S. and Bannerjee, R.R., The hormone resisting links obesity to diabetes. Nature. 2001, 409, 307-312.
35. Sui, D.Y; Luz Z; Li SH and Cai, Y., Hypoglycaemic effect of saponin isolated from leaves of Acanthopanax senticosus. Int. J., Diabetes and Metabolism, 1994, 19, 683- 685.
36. Syiem, D. and Khup, P. Z., Study of the traditional used medicinal plant Osbeckia chinesis for hypoglycemic and anti-hyperglycemic effects in mice. Pharmaceutical Biology. 2006, 4, 613-618.

*****