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Research Article
Antidiabetic and Oxidative Stress Ameliorative Potential of Ethanolic Extract of *Pedicularis longiflora* Rudolph

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Abstract

This study demonstrated the antidiabetic and antioxidant potential of *Pedicularis longiflora* Rudolph, a Himalayan wild herb, in alloxan induced diabetic rats. Seven groups of wistar rats (n = 6) were divided into healthy control (I) and diabetic control (II) groups, both without any treatment, test groups (III, IV and V) treated with varying concentration of ethanolic extract of *Pedicularis longiflora* and standard groups treated with N-acetyl cysteine as standard antioxidant (VI) and glibenclamide as standard antidiabetic drug (VII). Diabetic biomarkers and oxidative stress indices were evaluated on day 0, 7 and 14 of treatment. Glucose, glycated haemoglobin (GHb) and fructosamine showed a decrease of 23.08, 33.85 and 43.46%, respectively in group IV than group III (15.06, 46.13 and 23.95%) and group V (15.99, 27.77 and 24.84%) whereas, insulin showed significant ($p<0.05$) increase in group IV (15.43%) and group V (5.12%), but not than group III (16.82%) on day 14 of treatment. Concentration of reduced glutathione (GSH) and activity of superoxide dismutase (SOD) was increased ($p<0.05$) whereas, levels of lipid peroxide (LPO) and Nitric Oxide (NO) were decreased ($p<0.05$) in test groups on day 14 of treatment compared to diabetic control group. Liver homogenate showed lower levels ($p<0.05$) of LPO and NO, whereas, pancreatic homogenate showed higher levels ($p<0.05$) of GSH and lower levels ($p<0.05$) of LPO in test groups. These findings can be attributed to antidiabetic and antioxidant potential of *P. longiflora* Rudolph, however, group IV (500 mg kg^{-1} b.wt.) showing better response indicating its safe use as natural medicine.

Key words: Antioxidants, diabetes, oxidative stress, *Pedicularis longiflora*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a multisystem disease and involves oxidative stress in both etiology and pathogenesis (Yang *et al*., 2011; Fiorentino *et al*., 2013). Oxidative stress induced injury to pancreatic tissue especially beta cells of islets of Langerhans, is considered as one of the modern cause of diabetes mellitus (Shah and Khan, 2014; Yang *et al*., 2011; Khodaie *et al*., 2012a). Free radicals act as oxidants and produce oxidative stress by damaging cell organelles; plasma membrane, mitochondria and genetic material (Opara, 2002; Maritim *et al*., 2003). Antioxidants by scavenging these oxidants can protect sensitive cellular organelles thus preventing development of diabetes (Osawa and Kato, 2005). Various antioxidants especially of plant origin, have been found essential for ameliorating diabetes mellitus (Kaneto *et al*., 1999; Johansen *et al*., 2005).

Pedicularis longiflora Rudolph commonly known as Luguruk serpo, is used in traditional medicine Sowa-rigpa by Amchis in Ladakh (India) and Tibet (China) for curing hepatic and pancreatic diseases (Angmo *et al*., 2012). It is one of the important medicinal plant of genus Pedicularis rich in flavonoids, iridoids and polyphenols (Jia and Liu, 1992; Fujii *et al*., 1995; Wang *et al*., 1996; Chu *et al*., 2009; Khodaie *et al*., 2012a; Zhao *et al*., 2013). These phytochemicals have antioxidant properties (Chu *et al*., 2009; Shi *et al*., 2011; Khodaie *et al*., 2012a). Extracts from different species of Pedicularis have been found beneficial in diabetes (Gao *et al*., 2011a; Khodaie *et al*., 2012a), but *in-vivo* studies on many such related aspects are lacking. Also antioxidant and antidiabetic potential of *Pedicularis longiflora* Rudolph, a native wild herb of high altitude Himalayas is yet to be explored. Present study was undertaken with the same objectives.

MATERIALS AND METHODS

Plant material: Aerial plant parts (stem, leaves and flowers) of wild herb *Pedicularis longiflora* Rudolph collected from trans Himalayan Changthang region of Ladakh (India) were cleaned and air dried (1500 g) without exposure to sunlight. The plant was identified by Dr. Kunzes Angmo (Angmo *et al*., 2012), scientist at High Mountain Arid Agricultural Research Institute, Ladakh (India). Extraction of coarsely powdered whole aerial part mixture (1000 g) was done in 80% ethanol in Soxhlet extraction apparatus three times at room temperature. The ethanolic extract was filtered using Whatmann filter paper no. 1 and evaporated to dryness over rotary evaporator under room temperature. The ethanolic extract yielded a dark brown sticky semisolid, weighing 246.42 g. The extract was preserved in a refrigerator for further use.

Animals: Seven groups of male wistar rats of about 2 months age and weighing 200-250 g, were selected, each group having six animals (n = 6). The animals were acclimatized to standard laboratory conditions (temperature 24±1°C, relative humidity 55±5%) and a 12 h photoperiod in wire meshed galvanized cages (6 rats cage^{-1}) for one week before the commencement of the experiment. During the entire period of study, the rats were supplied with a semi-purified basal diet and water *ad libitum*. All animals were maintained according to the criteria of Saha *et al*. (2001).

Induction of diabetes: Diabetes was induced by alloxan (Sigma Aldrich) and treatments were started after stabilization of diabetes and continued till end of experiment (Misra and Aiman, 2012). The rats were given single intraperitoneal injection of alloxan 150 mg kg^{-1} freshly prepared in normal saline.

Experimental design: The animals with glucose levels >200 mg dL^{-1} were considered diabetic (Maharlooei *et al*., 2011). Healthy control (I) and diabetic control (II) groups were given same amount of plane normal saline. Test groups III, IV and V received ethanolic extract of *Pedicularis longiflora* Rudolph @50, 500 and 5000 mg kg^{-1}, respectively orally daily upto day 14 post diabetes induction. Standard antioxidant group (VI) received N-acetyl cysteine @500 mg kg^{-1} day^{-1} as standard antioxidant (Fiordaliso *et al*., 2004) upto day 14 post diabetes induction. Standard antidiabetic drug group (VII) received glibenclamide (600 µg kg^{-1} day^{-1}) as standard antidiabetic drug (Kumarappan *et al*., 2012) after induction of diabetes. The study is approved by Institutional Animal Ethics Committee (IAEC) under Institute project IVRI/MED/12-15/008.

Blood sample collection and tissue harvesting: Blood samples were collected on 0, 7 and 14 day by capillary tube via inner canthus of eye, in heparinized sterile vials (0.2 mg mL^{-1}) for estimation of diabetic biomarkers and oxidative stress indices. For glucose estimation sodium fluoride (2 mg mL^{-1}) was used as anticoagulant. The heparinized blood samples were preserved at normal refrigeration for estimation of oxidative stress indices within 12-48 h.

After centrifugation at 3000 rpm for 10 min, the plasma and buffy coat were removed to harvest the Red Blood Cells (RBC). Part of RBC pellet was diluted with chilled distilled water in 1:10 for the preparation of 10% stock haemolysate, which was used for the estimation of SOD activity and LPO level and

rest of the RBC pellet was diluted with chilled normal saline in 1:1 to get 50% RBC suspension for GSH estimation.

After 14 day trial animals were sacrificed, liver and pancreas were removed and immediately frozen in liquid nitrogen and kept at -80°C for further study. The liver and pancreas tissues were homogenized in 50 mM phosphate buffer solution (pH 7.4) using a tissue homogenizer at 4°C. The supernatant was collected after centrifuged at 1000 rpm for 5 min and stored at -80°C for further analyses. Part of liver and pancreas tissues were also collected and fixed in 10% buffered formalin for histopathology.

Haemoglobin and blood glucose: Haemoglobin estimation was carried out by standard Drabkin's cyanmethemoglobin method (Balasubramaniam and Malathi, 1992). In brief, 2 µL of whole blood was added into 500 µL of Drabkin's solution (Monozyme Ltd., India) and kept in room temperature for five minutes. Optical density of test was measured against Drabkin's solution at 540 nm on a spectrophotometer. Blood glucose was tested using a portable glucometer (Accu-Chek, Roche Diagnostics). In brief, blood was obtained by pricking the tail of rat on glucometer strips and glucose level was analyzed by glucometer (Singhal *et al*., 2011).

Estimation of diabetic biomarkers: Glycated haemoglobin (GHb) was estimated by using kit (Recombigen laboratories, India) based on the principle of ion exchange chromatography method of Trivelli *et al.* (1971). Initially hemolysate was prepared by mixing 100 µL of blood sample to 500 µL of 10 mm potassium cyanide surfactant (lysing reagent) solution. Glycohemoglobin was prepared by adding 100 µL of hemolysate to 3 mL of cation exchange resin buffered at pH 6.9. Glycohemoglobin fraction was determined by measuring the absorbance of supernatant at 415 nm. Total haemoglobin fraction was determined by adding 20 µL of the hemolysate to 5 mL of deionized water and absorbance was measured at 415 nm. The result was expressed as percentage of glycosylated haemoglobin of the total haemoglobin fraction.

Serum fructosamine was estimated by Nitro Blue Tetrazolium (NBT) reduction method (Sahu and Sarkar, 2008). About 200 µL of serum was added to 1 mL of 9 gm L^{-1} sodium chloride and incubated at 37°C for 10 min. About 1 mL of pre warmed NBT reagent prepared in carbonate buffer (0.2 moL L^{-1}, pH 10.8) was added and absorbance was measured at 530 nm at intervals of 5 min (A1) and 10 min (A2). The difference was calculated and expressed as ΔAA min^{-1}. Stock solution (40 mmoL L^{-1}) of 1 deoxy-1 mopholino-D fructose (DMF) was prepared in bovine serum albumin solution (40 g L^{-1} in 155 mmoL L^{-1} saline). This stock solution was diluted with bovine serum albumin solution to prepare DMF standard containing 4 mmoL L^{-1}. Absorbance was measured at 530 nm at intervals of 5 min and 10 min. The result was expressed in mmoL L^{-1}.

Concentration of insulin was measured in serum using a commercially available radioimmunoassay (RIA) kit (Germany). The intra-day precision of the assay was estimated at 5.4-6.0% (Herbert *et al*., 1965). Glucose was also estimated by glucose oxidase method (Trinder, 1969).

Estimation of oxidative stress indices: The concentration of glutathione (GSH) in sample was estimated by 5, 5-dithiobis-(2-nitro-benzoic acid) (DTNB) method as per the procedure of Prins and Loos (1969). The GSH concentration in the test samples was calculated by employing the molar extinction coefficient of DTNB-GSH conjugate (ηmoL mg^{-1} protein or haemoglobin for haemolysate), 13600/M/cm.

Superoxide dismutase activity was measured using nitro blue tetrazolium as substrate as per the method of Marklund and Marklund (1974) with certain modifications suggested by Masayasu and Hiroshi (1979). Briefly, the assay mixture in a total volume of 3 mL consisted of 50 mM of tris cacodylic acid buffer (pH 8.2), 50 µL of the sample after suitable dilution and 20 µL of 0.2 mM of pyrogallol. In the blank, enzyme was substituted by equal quantity of distilled water. The increased absorbance due to auto-oxidation of pyrogallol was recorded at 420 nm using double beam UV-Vis spectrophotometer (DBS Model No. UV5704SS) of Electronic Corporation of India Limited. One unit of SOD activity was defined as the amount of enzyme which inhibited the auto-oxidation of pyrogallol by 50% under the given experimental condition and the values were expressed as units mg^{-1} of protein or haemoglobin for haemolysate.

Lipid peroxides level in the RBC haemolysate was determined following the methods of Placer *et al*. (1966). Briefly, 0.2 mL of RBC haemolysate was added to 1.3 mL of 0.2 M tris-KCl buffer of pH 7.4 and incubated at 37°C for 30 min, after which 1.5 mL of thiobarbituric acid (TBA) was added and the mixture heated in boiling water bath for 10 min using glass beads as condenser. After cooling, 3 mL of pyridine/n-butanol (3:1 v/v) and 1 mL of 1 N NaOH were added to it and mixed by shaking. Blank was prepared by taking 0.2 mL of distilled water instead of sample. The absorbance was read at 548 nm. The nmol MDA (malonaldehyde) per mg of protein or haemoglobin for haemolysate was calculated by using 1.56×10^6 as extinction co-efficient. Lipid peroxides level in the erythrocytes was expressed in nmol of MDA m mg^{-1} of haemoglobin.

Nitric oxide was assayed by conversion of nitrate to nitrite using reductase enzyme, then total nitrite was determined as a colored azo-dye product of Griess reaction (Green *et al*., 1982). Briefly 50 µL each of Griess Reagents (1% sulfanilic acid, 0.1% 1-naphthol, 5% H_3PO_4) were added to 100 µL sample and color was allowed to develop for 10 min at room temperature. Absorbance was read at 540 nm using the plate reader.

Histopathological examination: Pancreas and liver tissues which were collected and fixed in 10% buffered formalin were rinsed with water, dehydrated with graded concentration of ethanol and embedded in paraffin wax. The samples were sectioned into 5 µm thick and mounted on glass slides. The sections were then de-waxed using xylene and ethanol and stained with hematoxylin and eosin (H and E stain). A representative area was selected for qualitative light microscopic analysis under 40x magnification.

Statistical analysis: Data were analyzed by two-way analysis of variance (ANOVA) using statistical software package SPSS 16.0. Comparison was made between and within groups to study effect of ethanolic extract of *P. longifera* Rudolph on diabetic biomarkers and oxidative stress indices at three different times. Tukey test was used and comparison between values was established at level of significance, $p<0.05$.

RESULTS

Effect of treatment on diabetic biomarkers: Significantly ($p<0.05$) lower levels of glucose, GHb and fructosamine were observed in test group III, IV and V than diabetic control group II on different days of treatment (Fig. 1a-c). But non-significant ($p\geq0.05$) difference was noticed within test groups and group receiving standard antioxidant (VI); however, lower levels of glucose (263.18±3.22), GHb (6.35±0.1) and fructosamine (498.19±2.32) were noticed in group IV than group III (276.67±4.91, 5.23±0.31 and 673.21±1.96, respectively) and group V (281.09±2.61, 7.02±0.29 and 660.81±2.85) and standard antioxidant group VI (301.21±4.99, 8.36±0.19 and 745.32±1.96), 14 days after treatment. Non-significantly ($p\geq0.05$) higher levels of insulin were observed in test groups III (3.75±0.27), IV (3.89±0.19) and V (3.69±0.87) than diabetic control group II (1.94±0.99), which showed significantly ($p<0.05$) lower levels than standard antidiabetic drug group VII (4.78±0.16) (Fig. 1d).

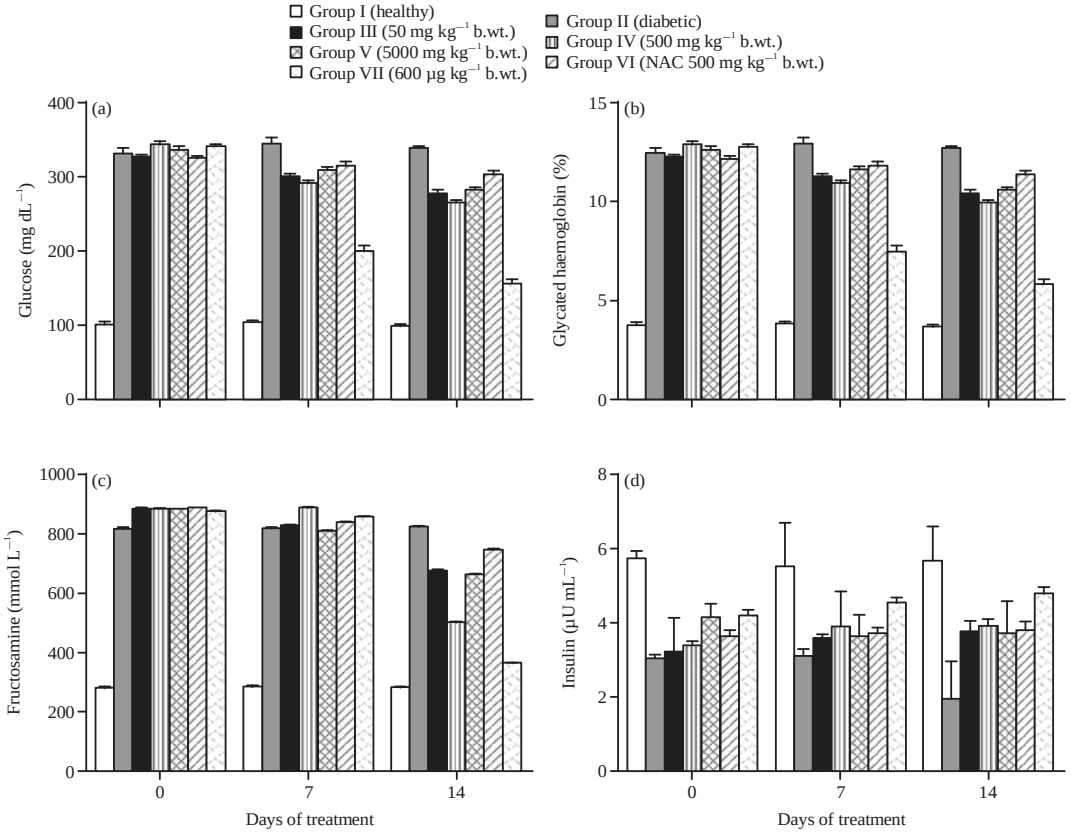

Fig. 1(a-d): Effect of treatment on (a) Glucose level, (b) Glycated haemoglobin, (c) Fructiosamine levels and (d) Insulin level

Table 1: Effect of treatment on oxidative stress indices in tissues of various groups on different days of treatment

Days of Treatments	Group I (healthy)	Group II (diabetic control)	Group III) (50 mg kg^{-1} b.wt.)	Group IV (500 mg kg^{-1} b.wt.)	Group V (5000 mg kg^{-1} b.wt.)	Group VI (NAC @ 500) (mg kg^{-1} day^{-1})	Group VII (Glibenclamide @ 600 µg kg^{-1})
GSH (ηmoL mg^{-1} protein)							
Liver							
0	15.80±2.58^{aA}	7.21±0.86^{bA}	6.12±1.20^{bcA}	8.64±1.44^{bcdA}	6.72±2.91^{bcdeA}	7.27±1.99^{bcdefA}	8.38±2.02^{bcedfgA}
7	13.98±2.17^{aB}	5.73±1.13^{bB}	8.02±1.92^{cB}	8.41±2.14^{cdAB}	7.29±1.99^{eAB}	9.32±1.07^{fB}	7.62±1.87^{cdgAB}
14	15.02±1.90^{aAC}	4.08±1.01^{bC}	8.24±2.01^{cBC}	10.33±2.31^{dC}	7.80±2.04^{ceABC}	14.71±2.44^{fC}	6.12±1.98^{cegBC}
Pancreas							
0	12.18±2.01^{aA}	6.56±0.73^{bA}	8.64±1.10^{cA}	5.82±1.42^{bdA}	6.92±2.12^{bdeA}	7.41±1.99^{cfA}	8.81±1.32^{cfgA}
7	12.77±1.92^{aAB}	5.21±0.99^{bB}	8.99±0.10^{cAB}	7.21±1.03^{cdB}	7.10±1.91^{cdeAB}	9.21±2.18^{fAB}	7.61±1.90^{cdegAB}
14	10.37±2.22^{aABC}	5.01±1.32^{bBC}	9.91±0.21^{cC}	10.92±0.98^{adC}	7.99±2.10^{eABC}	10.77±1.89^{adfC}	8.06±2.12^{gABC}
SOD (units mg^{-1} protein)							
Liver							
0	29.43±2.18^{aA}	15.81±1.56^{bA}	14.04±2.01^{bcA}	15.54±1.86^{bcdA}	17.32±2.39^{eA}	15.78±1.99^{bcdfA}	17.85±1.80^{egA}
7	29.91±3.01^{aAB}	10.97±2.22^{bB}	14.99±2.20^{cAB}	16.92±1.19^{dB}	17.88±1.83^{deAB}	21.01±2.09^{fB}	14.01±2.21^{cgB}
14	28.02±1.99^{aABC}	6.05±1.87^{bC}	20.66±2.16^{cC}	21.07±0.97^{cdC}	19.12±1.29^{cdeABC}	26.37±1.04^{afC}	11.09±1.99^{gC}
Pancreas							
0	12.83±1. 81^{aA}	6.21±0.22^{bA}	5.99±0.93^{bcA}	5.01± 0.37^{dA}	6.02±2.09^{bceA}	5.47±0.78^{dfA}	4.96±0.16^{Ag}
7	11.09±1.76^{aAB}	3.31±0.37^{bB}	7.12±1.21^{cB}	7.99±0.24^{cdB}	6.98±1.38^{cdeAB}	7.91±0.92^{cdefB}	3.02±1.24^{gAB}
14	12.93±1.92^{aABC}	1.09±0.25^{bC}	8.98±1.76^{cC}	8.12±0.22^{BcdC}	7.67±1.27^{BcdeC}	9.20±0.21^{fC}	1.99±0.97^{gC}
LPO (nmoL mg^{-1} protein)							
Liver							
0	5.56±0.23^{aA}	11.32±1.23^{bA}	13.74±1.92^{cA}	15.58±0.32^{dA}	13.86±0.54^{ceA}	14.71±0.62^{cefA}	15.06±0.32^{dgA}
7	5.10±0.15^{aAB}	13.44±1.29^{bB}	10.65±2.11^{cB}	12.91±0.17^{bdB}	13.01±0.62^{bdeAB}	11.72±0.53^{cfB}	14.21±0.68^{gAB}
14	4.92±0.13^{aABC}	15.88±1.53^{bC}	9.96±1.87^{cC}	8.77±0.21^{cdC}	12.13±0.51^{eC}	8.64±0.42^{cdfC}	13.62±0.57^{gABC}
Pancreas							
0	1.92±0.12^{aA}	3.46±0.64^{bA}	4.21±0.75^{cA}	4.87±0.19^{cdA}	5.27±0.78^{cdeA}	6.42±0.31^{fA}	4.70±0.31^{cdgA}
7	2.43±0.11^{aAB}	4.63±0.233^{bB}	3.02±1.05^{cB}	4.28±0.21^{bdB}	4.86±0.55^{bdeB}	4.13±0.11^{bdefB}	4.92±0.45^{bdefgAB}
14	2.12±0.13^{aABC}	5.99±0.12^{bC}	3.99±0.13^{cAC}	3.07±0.16^{cdC}	4.25±0.14^{cdeBC}	2.72±0.15^{afC}	5.99±0.33^{bgC}
NO (µmol mL^{-1} homogenate)							
Liver							
0	5.78±1.72^{aA}	13.36±0.99^{bA}	10.78±0.72^{cA}	12.57±0.24^{dA}	11.68±0.96^{deA}	11.21±1.18^{defA}	10.52±1.07^{cgA}
7	6.99±3.11^{aAB}	11.99±1.91^{bB}	8.96±0.91^{cB}	8.21±0.13^{cdB}	9.92±1.02^{cdeB}	7.43±1.72^{fB}	10.99±0.86^{egAB}
14	4.11±2.31^{aABC}	10.01±1.22^{bC}	8.37±1.53^{cBC}	5.77±0.16^{dC}	9.17±1.10^{beBC}	5.02±1.86^{dfBC}	11.97±1.03^{bgABC}
Pancreas							
0	4.13±1.17^{aA}	11.72±1.22^{bA}	10.78±0.27^{bcA}	12.47±1.09^{bdA}	12.08±1.72^{bdeA}	11.63±1.19^{bdefA}	11.49±0.98^{bdefgA}
7	4.69±1.09^{aAB}	11.98±0.67^{bAB}	9.88±0.58^{cB}	8.71±0.99^{dB}	10.99±0.55^{eB}	7.91±1.23^{fB}	10.72±0.72^{egB}
14	5.02±1.10^{aC}	12.93±0.99^{bC}	9.03±0.63^{cBC}	8.21±0.39^{dBC}	9.18±0.72^{ceC}	7.22±1.14^{fC}	11.31±1.10^{bgAB}

^{abcdefg}Values with different superscript differ significantly (p<0.05) between groups, ^{ABC}Values with different superscript differ significantly (p<0.05) within group, p<0.05 significant difference

Effect of treatment on oxidative stress indices in diabetic rats: Significantly (p<0.05) higher level of GSH and SOD activity was observed in test groups treated with ethanolic extract of Pedicularis than diabetic control group II, but non-significant (p≥0.05) difference was noticed between group IV and group receiving standard antioxidant (VI) in GSH levels and healthy control group I in SOD activity. However, GSH level and SOD activity of group IV (GSH 26.90±1.08 and SOD 34.07±1.76) and standard antioxidant group VI (GSH 27.10±0.91 and SOD 31.92±1.69) was higher than group III and group V (Fig. 2a, b).

Significantly (p<0.05) lower levels of LPO and NO were observed in test groups than diabetic control group (II) but non-significant (p≥0.05) difference was noticed between group IV and group receiving standard antioxidant (VI) in LPO levels. However, in test groups treatment with ethanolic extract of Pedicularis cannot decrease levels of LPO (16.77±3.69) and NO (27.45±1.69) than those in healthy control group I (LPO 10.17±2.14 and NO 20.18±1.36) (Fig. 2c, d).

Effect of treatment on oxidative stress indices (Mean±SE) in tissue homogenate (liver and pancreas) in different groups of rats on 0, 7 and 14 days of treatment is given Table 1. In liver tissue homogenate, significantly (p<0.05) lower levels of LPO and NO were observed in all test groups than diabetic control group (II) and groups receiving standard antidiabetic drug (VII). Non-significant (p≥0.05) difference was noticed within test groups (III, IV and V) in GSH level (8.24±2.01, 10.33±2.31 and 7.80±2.04, respectively) and SOD activity (20.66±2.16, 21.07±0.97 and 19.12±1.29, respectively), whereas, group receiving standard antioxidant (VI) had significantly (p<0.05) higher level of GSH (14.71±2.44) and SOD activity (26.37±1.04).

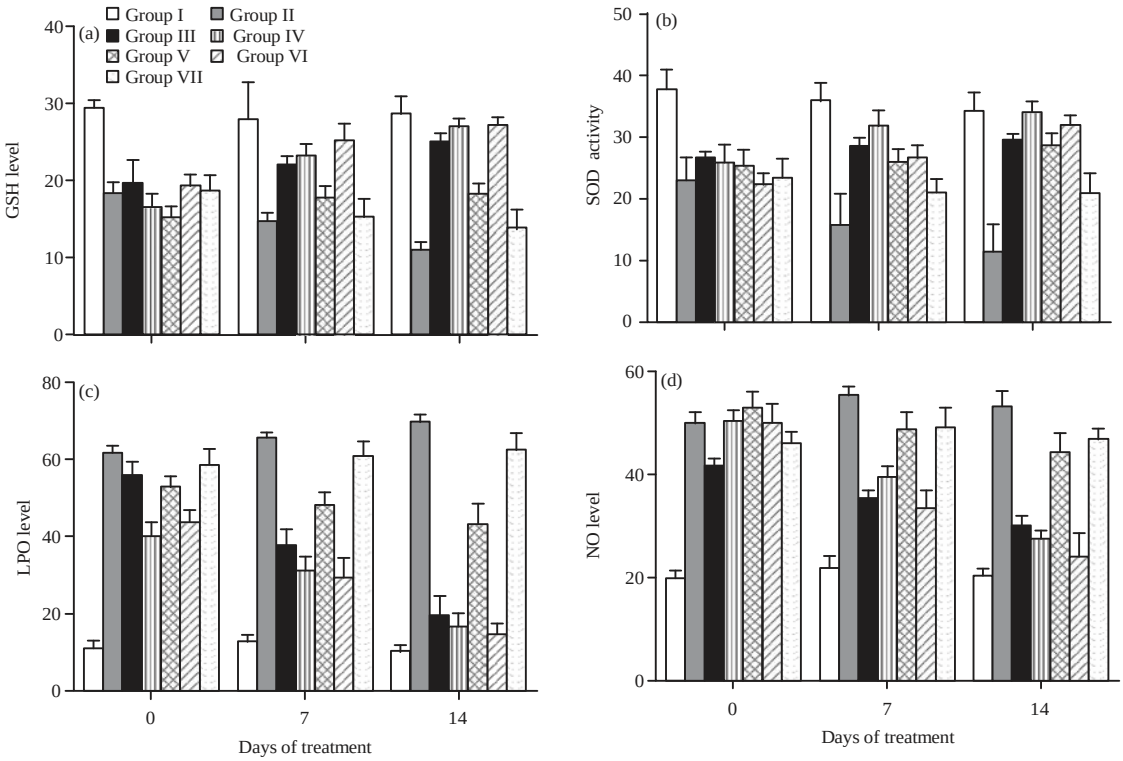

Fig. 2(a-d): Effect of treatment on (a) GSH levels (ηmol mg^{-1} Hb), (b) SOD activity (units mL^{-1}), (c) LPO (nmol mL^{-1}) and (d) NO levels (μmol mL^{-1})

In pancreas tissue homogenate, significantly ($p<0.05$) higher levels of GSH and lower levels of LPO were observed in all test groups than diabetic control group II and group receiving standard antidiabetic drug (VII). Significantly ($p<0.05$) higher GSH levels were noticed in test group IV than group III and group V. Significantly ($p<0.05$) lower levels of LPO were observed in test group IV (3.07±0.16) than diabetic control group II (5.99±0.12), test group III (3.99±0.13) and V (4.25±0.14). Non-significant ($p \geq 0.05$) difference was noticed in SOD activity within test groups but were significantly ($p<0.05$) lower than group receiving standard antioxidant (VI) (9.20±0.21). The NO levels showed non-significant ($p \geq 0.05$) difference between test groups III (9.03±0.63), IV (8.21±0.39) and V (9.18±0.72), but levels were significantly ($p<0.05$) higher than standard antioxidant group VI (7.22±1.14). However, fall in NO levels was significant ($p<0.05$) on day 14 in all test groups.

Histopathology examination: Histopathological examination of pancreas revealed amyloidosis, degeneration, infiltration and pyknosis of beta cells of the islets of Langerhans in diabetic control group (Fig. 3b), comparatively normal islets in diabetic challenged extract treated groups (Fig. 3c-e) in relation to healthy group (Fig. 3a) and standard antioxidant group (Fig. 3f), which showed normal islets of Langerhans and beta cells and standard antidiabetic drug group (Fig. 3g), which showed amyloid degeneration and infiltration of islets of Langerhans.

Similarly liver tissue revealed significant tissue damage as evidenced by degeneration of hepatocytes and inflammatory cell infiltration of sinusoids in diabetic control group (Fig. 4b) and comparable recovery in diabetic challenged extract treated groups (Fig. 4c-e) in comparison to healthy group (Fig. 4a).

DISCUSSION

Application of medicinal herbs for amelioration of diabetes mellitus and oxidative stress is widely reported (Marles and Farnsworth, 1995; Gao *et al*., 2011a; Khodaie *et al*., 2012a). These herbs contain biochemical constituents which can act as antidiabetics and/or antioxidants (Gao *et al*., 2011a; Khodaie *et al*., 2012a; Liu *et al*., 2013). Reactive Oxygen Species (ROS) or oxidants (LPO, NO) are produced in normal physiological processes and are catalyzed by antioxidants (GSH, SOD) to prevent cell injury. However, due to imbalance

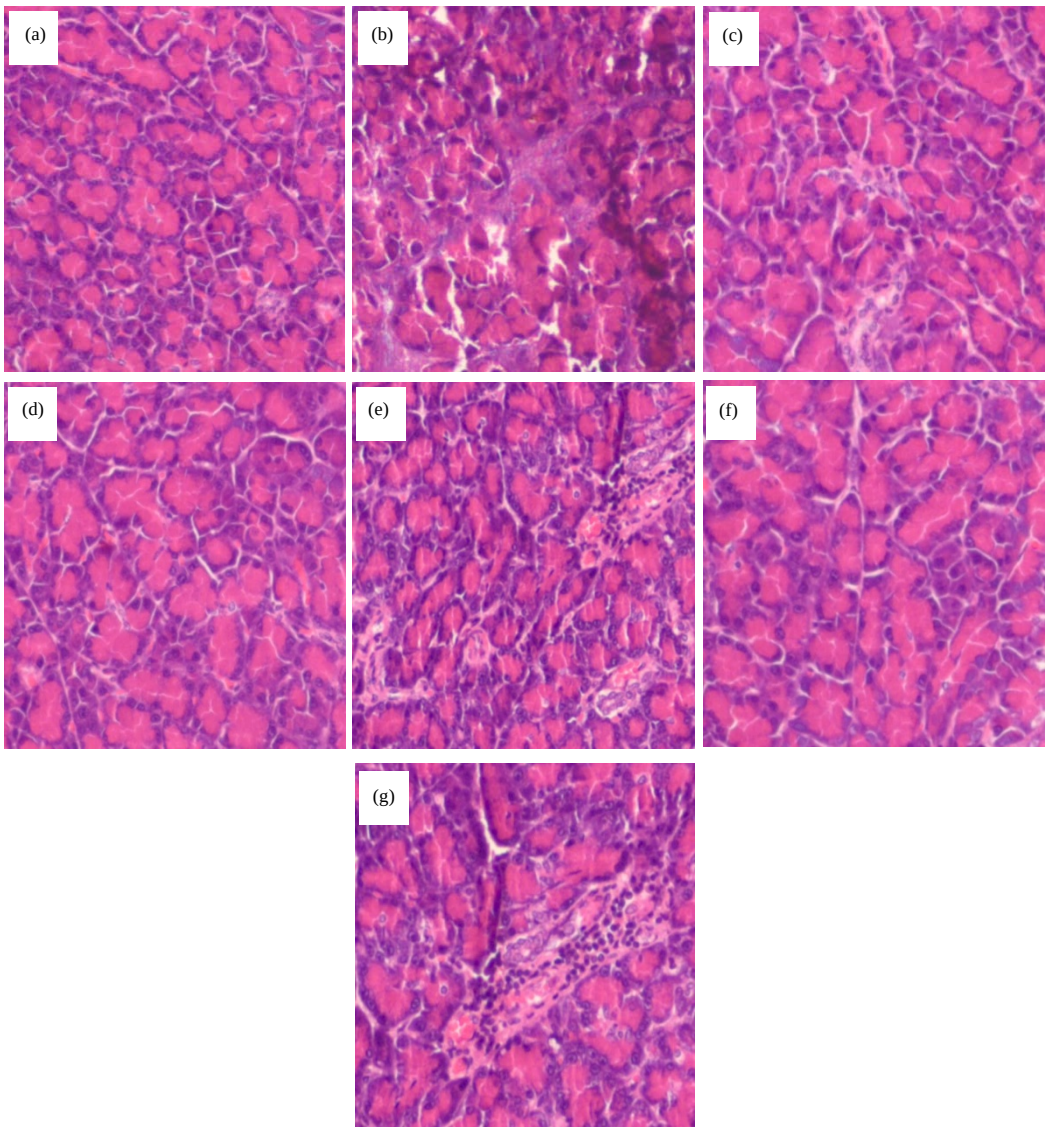

Fig. 3(a-g): Histophalogy of pancreas (H and E stain, 40x) after 14 days of experiment (a-g), (a) Healthy group having normal islets of Langerhans and beta cells, (b) Diabetic group with amyloidosis, degeneration, infiltration and pyknosis of beta cells of the islets of langerhans, (c-e) Test group recovering islets with comparatively normal tissue structure, (f) Standard antioxidant group having normal islets of Langerhans and beta cells comparable to Group I, (g) Standard drug group with amyloid degeneration and infiltration of islets of Langerhans

caused by excess production of oxidants many vital organs are vulnerable to cellular damage due to oxidative stress. Pancreas and liver being of particular importance, damage to beta cells of pancreas and hepatocytes of liver leads to diabetes mellitus (Gyamfi *et al*., 1999; Lee, 2006). Hence, many antioxidants especially of herbal origin have been found useful in diabetes. But such studies regarding *Pedicularis longiflora* Rudolph are lacking. Still it is used in traditional medicine by Amchis in Ladakh (India). So in present study, antidiabetic and antioxidant potential of ethanolic extract of *P. longiflora* was evaluated. Lowering of glucose, GHb and fructosamine in test groups can be attributed to antidiabetic effect of ethanolic extract of *P. longiflora* (Khodaie *et al*., 2012a). It may have protective effect on pancreas and liver as noted in histopathological sections (Fig. 3c-e and 4c-e). Normal beta cells of pancreas produce insulin, which helps in uptake and utilization of glucose. Rise of insulin in test groups may be due to its insulin secreting potential. Similarly normal hepatocytes utilize glucose and store excess in the form of glycogen (Gao *et al*., 2011a, b). However, Gao *et al*. (2011a, b)

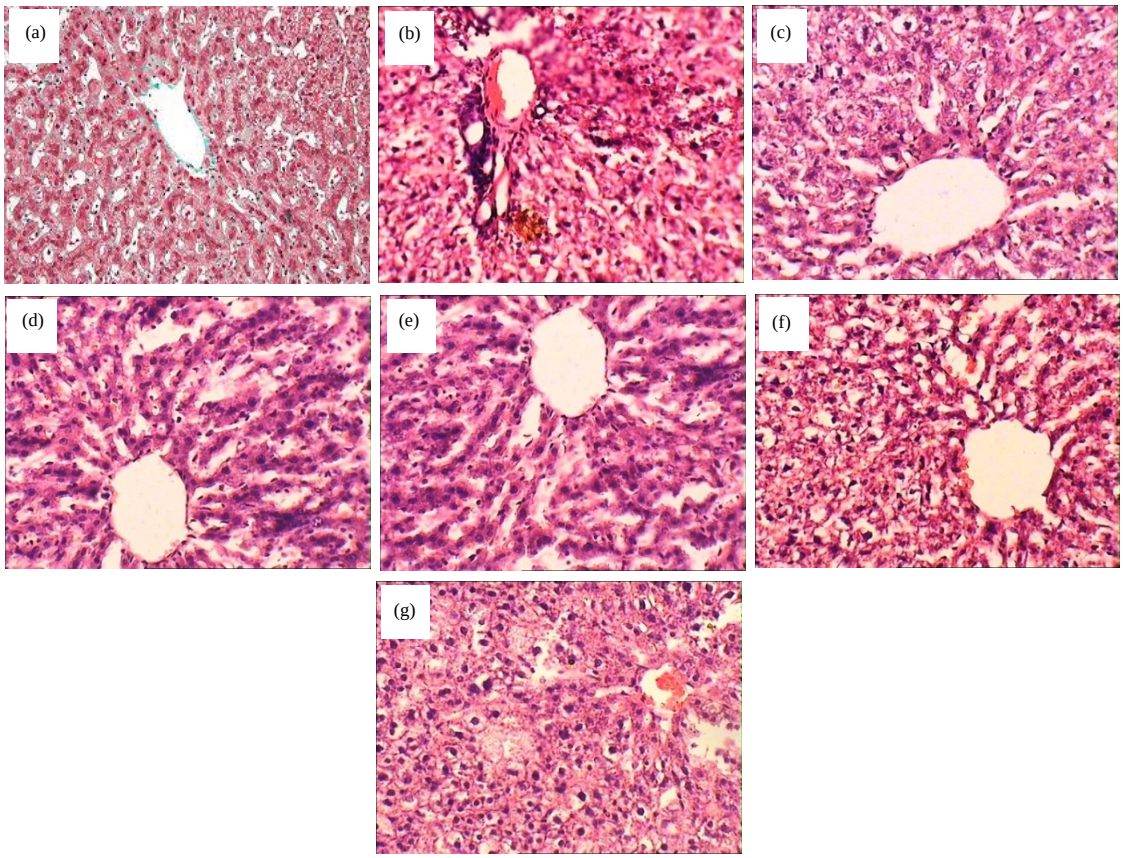

Fig. 4(a-g): Histophalogy of liver (H and E stain, 40x) after 14 days of experiment (a-g), (a) Healthy rats with normal hepatocytes and sinusoids, (b) Diabetic rats, degenerated and clumped hepatocytes with constriction of cytoplasm and irrigular sinusoids, (c-e) Diabetic ethanolic extract treated rats, comparatively normal hepatocytes and centrilobular vessels, (f) Diabetic N-acetyl cysteine treated rats with normal hepatocytes and centrilobular vessels, (g) Diabetic glibenclimide treated rats with degenerated and irregular hepatocytes

reported increase in blood glucose and liver glycogen in diabetic and stressed mice treated with *Pedicularis decora* Franch root extracts. Decrease in GHb and fructosamine levels may also be indicative of normal functioning of pancreas and liver as GHb and fructosamine levels reflect excess glycation of serum proteins due to hyperglycemia (Liu *et al*., 2013) and increased levels of GHb (Yue *et al*., 1982; Lyons and Basu, 2012) and fructosamine (Youssef *et al*., 2008; Reusch *et al*., 1993) are indicative of diabetes (De Michele *et al*., 2008; Sako *et al*., 2008; Thomson *et al*., 2013). Higher levels of diabetic biomarkers in diabetic control group and standard antioxidant group are due to the diabetic effect of alloxan (Hoftiezer and Carpenter, 1973). Alloxan destroys beta cells of pancreas resulting in insulin deficiency. Glibenclimide causes increase of insulin thereby lowering level of other diabetic biomarkers in standard antidiabetic drug group. Glucose and fructosamine showed significant decrease in group IV (23.08 and 43.46%) than group III (15.06 and 23.95%) and group V (15.99 and 24.84%), whereas, glycated haemoglobin (GHb) showed 33.85% decrease in group IV, 46.13% in group III and 27.77% in group V. However, insulin showed significant increase in group IV (15.43%) and group V (5.12%) but not than group III (16.82%) on day 14 of treatment. As group IV treated with 500 mg kg^{-1} b.wt. of ethanolic extract of *P. longiflora* Rudolph, showed better antidiabetic response among test groups, hence, this dose can be considered as ideal for antidiabetic effect.

Increase of antioxidants (GSH, SOD) and decrease of oxidants (LPO, NO) in test groups may be due to antioxidant properties of *P. longiflora.* It may be due to the presence of phenyl propanoid (Zhang *et al*., 2011), flavonoid (Fujii *et al*., 1995; Khodaie *et al*., 2012a), iridoid (Fujii *et al*., 1995), triterpenoid (Zhang *et al*., 2011) and polyphenolic compounds (Khodaie *et al*., 2012a), which have antioxidant properties as reported in *P. longiflora* (Zheng and Wang, 2001) or many other variants of *Pedicularis* sp. (Chu *et al*., 2009; Gao *et al*.,

2011b; Khodaie *et al*., 2012b). Rise of GSH, SOD and lowering of LPO and NO in test groups is in corroboration with Gao *et al*. (2011a). This also indicates that ethanolic extract of *P. longiflora* minimizes tissue injury to pancreas and liver due to oxidative stress and thus has protective role on these organs as evidenced from histopathological sections (Fig. 3 and 4). Gao *et al*. (2011b), have reported increase in SOD and decrease in LPO in ethanolic and aquous extract treated exercise induced stressed mice. Antihyperglycemic effect of *P. longiflora* may also prevent oxidative stress as hyperglycemia increases levels of oxidants (Fiorentino *et al*., 2013). Better amelioration of oxidative stress in group IV treated with 500 mg kg^{-1} b.wt. of ethanolic extract of *P. longiflora* Rudolph, compared to other test groups indicates effectiveness of this dose as an antioxidant. Increase of oxidants and decrease of antioxidants in diabetic control group II is due to severe tissue injury by alloxan as it damages beta cells of pancreas (Fig. 3b) and hepatocytes (Fig. 4b) (Gao *et al*., 2011a; Szkudelski, 2001; Karimov *et al*., 1996). Such damage may have been ameliorated in standard antioxidant group VI by N-acetyl cysteine as antioxidant minimizes oxidative stress and thus protects pancreatic (Fig. 3f) and hepatic (Fig. 4f) cellular integrity and function (Fiordaliso *et al*., 2004; Garg *et al*., 2005).

CONCLUSION

Decrease in levels of glucose, GHb, fructosamine and increase in levels of insulin reflects antidiabetic potential of ethanolic extract of *P. longiflora* Rudolph. Increase in levels of GSH, SOD activity and decrease in levels of LPO and NO suggests that ethanolic extract of *P. longiflora* Rudolph has antioxidant potential. It may also have protective effect on liver and pancreas and thus improves their function. However, further investigation regarding molecular characterization of chemical constituents and toxicity may elucidate its use as natural medicine.

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