Chemical constituents, antihyperglycemic and antioxidant effects of Nepeta hindostana whole herb in alloxan and OGTT induced diabetes in rats

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ABSTRACT

The present study was aimed to evaluate the antihyperglycemic and antioxidant effects of Nepeta hindostana (NH) whole herb extract in alloxan and OGTT induced diabetes in rats. In this study, aqueous (NHA) and methanolic (NHM) extract of NH were prepared and qualitative and quantitative phytochemical screening was done to identify the different chemical constituents present in the extracts. Isolation of chemical constituents was carried via column chromatography using chloroform and ethyl acetate (10:4). In-vitro antioxidant potential of NHA and NHM extract was evaluated by employing DPHH, hydroxyl, superoxide radical and nitric oxide scavenging activity whereas in-vivo anti-oxidant activity was evaluated by measuring the reduced glutathione and catalase level. The antidiabetic activity was evaluated employing OGTT and alloxan (150 mg/kg, i.p.) induced diabetes in rats. The rats were divided into control, diabetic control and treatment group and different doses (100, 200 and 400 mg/kg) of NHA and NHM extract were administered. Various biochemical parameters i.e blood glucose, total cholesterol, triglyceride, LDL, VLDL and HDL level were estimated. In NHM extract, total flavonoids and total terpenoids were found to be present in major amount. The isolated compound (I) was identified as triterpenoidal aldehyde of lupine series. NHA and NHM extract significantly attenuated in-vitro and in-vivo oxidative stress. Moreover, hyperglycemia induced by OGTT and alloxan was also found to be significantly attenuated by different doses of NHA and NHM.

Keywords: Nepeta hindostana, antihyperglycemic, antioxidant, DPPH, alloxan etc.

INTRODUCTION

Diabetes Mellitus (DM) is a major metabolic syndrome characterized by derangement in carbohydrate metabolism associated with defect in insulin secretion or action [1]. It is dispersed worldwide along with prevalence from 171 million in 2000 to 366 million in 2030 [2]. DM is associated with oxidative reactions, oxidants (free radicals) and oxidative stress, which play a significant role in the pathogenesis of diabetes [3-4].

The currently available oral hypoglycemic and anti-hyperglycemic drugs used for the management of diabetes have their own limitations, adverse effects and secondary failures. Therefore, to reduce their cost, limitation and adverse effects; focus has been shifted towards the medicinal herbs for safe and effective use. Recently, numerous medicinal herbs are being investigated for their role in pharmacotherapy of diabetes [5].
Botanical description of Nepeta hindostana

Nepeta hindostana (Roth) Haines (Family: Lamiaceae) is an important medicinal plant of indo-pakistan subcontinent and commonly known as “Badrang-e-Boya. It is medium size velvety annual herb, 15-40 cm high, branched from base. Leaves are 1.3-5.0 by 1.3-8.0 cm, broadly ovate, obtuse, crenate-serrate, green or hoary, petioles 8-12 mm long. Flower are pedicellate, 6 mm, blue or purple, minutely dotted, one sided stalked and branched, clusters at interval. Fresh herb has a strong smell like that of mint [6-7].

Habit and habitat: found roadside and near streams in Europe, Asia (in himalayas) and in other hilly areas introduced in India. Flowering time of Nepeta hindostana is from spring to summer with blue purple colour flowers [8-9].

Traditional and pharmacological activities

Traditionally, this plant is used as carminative, stimulant, tonic, diaphoretic, emmenagogue, antispasmodic, aphrodisiac, hysteria, chlorosis, colic, amenorrhea and toothache [10] A number of pharmacological activities like cardioprotective [11-12], asthma [10], hypocholesteremic [13], hypotensive [14], antiphlogistic [15], antiplasmodal [16], antipyretic [17-18], anti-inflammatory [11], CNS depressant and sedative activities [11], antifungal [19] has been reported for Nepeta hindostana. The cardioprotective effect of the plant is reported due to the presence of terpenoid and flavonoids [11] but antioxidant and antidiabetic activity is not reported yet for Nepeta hindostana. So, the present study was designed to investigate in-vitro and in-vivo antioxidant and antihyperglycemic activity of aqueous and methanolic extract of Nepeta hindostana whole herb in OGTT and alloxan induced diabetes in rats.

EXPERIMENTAL SECTION

Chemicals

Alloxan (Sigma), d-glucose (Sigma), Aluminium chloride (nice chemicals), Ascorbic acid (Sigma), Atropine (Sigma), Cycloartenol (Sigma), Diosgenin (Hi media), DPPH (Sd fine), Folin ciocalteu reagent (Sigma), Gallic acid, Methanol (nice chemicals), Rutin (Sd fine), chloroform LR (nice chemicals), Silica gel 60-120 mesh (nice chemicals), ethyl acetate (nice chemicals) and instruments includes UV chamber,

\[ \text{1} \text{H} \text{NMR} \] spectra were recorded on Bruker Avance II 400 NMR spectrometer in appropriate deuterated solvents. Chemical shifts were reported as \( \delta \) (ppm) relative to tetramethylsilane (TMS) as internal standard. Infrared (IR) spectra were recorded as KBr pellet on Perkin Elmer FTIR spectrometer. The wave number is given in cm\(^{-1}\).

Plant Material

The fresh whole herb of Nepeta hindostana was collected on april 2013 from the Tau Devilal National herbal park, Khizrabad, Haryana, India and authenticated by Dr. Shiddamallayya N. National Ayurveda Dietetics Research Institute, Banglore, India (specimen number RRCBI-MUS-124).

Standardization of plant material

Various pharmacopoeial standard parameters were measured like ash value, pH value, moisture content, loss on drying, extractive values and solubility [20].

Preparation of extract

Dried course powder of whole herb of Nepeta hindostana was extracted by soxhlation using water and methanol at 60°C. The extract (both NHA and NHM) was concentrated using vacuum rotary evaporator at 40°C and dried in a hot-air oven at 40°C. The extract was stored in a refrigerator at 4°C during the study. The % yield of both aqueous and methanolic extract was calculated.

Qualitative and Quantitative estimation of Phytoconstituents

The preliminary qualitative screening of NHA and NHM was carried out by employing various phytochemical tests [21-22]. Furthermore, quantitative estimation of different phytoconstituents were carried with UV spectrophotometer using their standards like for total alkaloids (standard atropine) [23], total saponins (standard diosgenin) [23], total steroids (standard cycloartenol) [23], total tannin (standard rutin) [24], total phenolics (standard gallic acid) [24-25], total flavonoids (standard rutin) [26-27] and total terpenoids was estimated with simple extractive method that is 100g plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids [28-29].
**Isolation and identification of a compound from NHM**

Firstly TLC of crude methanolic extract was examined in chloroform as mobile phase, and then the column chromatography was proceeding. The column with internal diameter 2.5 cm and length 62 cm was packed with silica (60-120 mesh) using chloroform. The sample was packed with silica in ration 50:1. The column was equilibrated with pure chloroform. Initially run the column with pure chloroform and 1-6 fractions shows no compound elution confirmed with TLC. Then fraction 7-20 shows the spot in TLC at the polarity chloroform: ethyl acetate (99.5:0.5). The isolated compound shown in the present study was starts eluting at polarity chloroform: ethyl acetate (98:2). The fractions number (58-67) were collected in the same polarity solvent and evaporated. For identification of compound IR and $^1$H-NMR analysis was carried [30].

**Evaluation of in vitro antioxidant activity of NHA and NHM**

**DPPH radical scavenging activity**

The free radical scavenging activity of NHA and NHM extract was calculated as hydrogen donating or radical-scavenging ability using the stable radical DPPH (1-1-diphenyl 2-picryl hydrazyl). Radical-scavenging activity was expressed as percentage inhibition of free radical by the sample and was calculated using the following formula:

$$\text{% inhibition} = \left(1 - \frac{A_{\text{blank}}}{A_{\text{sample}}}\right) * 100$$

where $A_{\text{blank}}$ was the absorbance of the control and $A_{\text{sample}}$ was the absorbance in the presence of extract. All the tests were performed in triplicate. IC$_{50}$ [25] and antioxidant activity index (AAI) was calculated [31].

$$\text{AAI} = \frac{\text{final concentration of DPPH in control}}{IC_{50}} \times 100$$

**Hydroxyl radical scavenging activity**

Hydroxyl radical generation by phenylhydrazine was measured by 2-deoxyribose degradation assay in 50 mM phosphate buffer (pH 7.4) containing 1 mM deoxyribose, 0.2 mM phenylhydrazine hydrochloride and % inhibition was calculated [24].

**Superoxide radical scavenging activity**

Superoxide radical scavenging activity was measured using NBT (Nitro blue tetrazolium reagent). The method is based on generation of superoxide radical (O$_2^-$) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, which gets reduced to nitrite. Nitrite in presence of EDTA gives a color that can be measured at 560 nm. Various concentrations (20-500 µg/ml) of test solutions were taken in test tube and reaction mixture consisting of 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 mM NBT 0.2 ml of 0.1 mM EDTA solution was added to the test tube and zero minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 ml of 1 mM hydroxylamine hydrochloride to the above solution. Reaction mixture was incubated at 25°C for 15 minute; the reduction of NBT was measured at 560 nm [24].

**Nitric oxide scavenging activity**

The interaction of extract with nitric oxide (NO) was assessed by the nitrite detection method. NO interact with oxygen to produce stable products, leading to the production of nitrates. After incubation for 60 min at 37°C, Griess reagent (a-naphthyl-ethylenediamine 0.1% in water and sulphanilic acid 1% in H$_3$PO$_4$ 5%) was added. The same reaction mixture without the extract but with equivalent amount of distilled water served as control. Ascorbic acid was used as positive control [32].

**Animals**

Wistar rats (both sex) weighing 220-250g were used in the study and experimental protocol was duly approved by Institutional Animal Ethics Committee (MMCP/IAEC/13/36). Animals were kept as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) in MM College of Pharmacy, Ambala, India. Animals were fed normal chow diet and ad libitum under controlled environmental condition of temperature (24-28°C), relative humidity 60-70% and natural light/dark cycle (12:12).

**Induction of diabetes**

Diabetes was induced in rats by oral glucose tolerance test (OGTT) and alloxan. In OGTT method, rats were divided into different groups (n = 6) and fasted overnight and 10% glucose solution per oral 30 min prior to different doses of extracts were administrated. Blood samples were collected at different intervals i.e 30, 60 and 120 min and glucose level in serum was measured immediately by GOD/POD method using ERBA Diagnostics kits [33-34]. Whereas, in alloxan induced diabetes method, hyperglycemia was induced by single intra-peritoneal injection of alloxan (150
mg/kg) [35]. The alloxan was freshly prepared by dissolving 150 mg of alloxan in 1ml of normal saline solution. To prevent the hypoglycaemic shock, 10% glucose was given orally before induction of diabetes in rats. Animals with plasma glucose of >200 mg/dl were included in study. Animals were divided into different groups consisting of six rats each. The blood samples withdrawn on 0th, 3rd and 7th day from the retro orbital plexus and serum was separated by centrifugation at a speed of 4000 rpm for 10 minutes. The serum was collected and blood glucose (GOD/POD method), triglycerides, total cholesterol (CHOD-PAP method), HDL (CHOD-PAP Method), LDL and VLDL with ERBA Diagnostics kits was measured [35]. Metformin was used as standard drug (400 mg/kg).

\[
\text{LDL} = \text{total cholesterol} - \text{HDL} - \left(\frac{\text{triglycerides}}{5}\right)
\]

\[
\text{VLDL} = \frac{\text{triglycerides}}{5}
\]

**In-vivo antioxidant effect of NHA and NHM in alloxan induced diabetic rats**

**Estimation of reduced Glutathione**

Serum glutathione level was determined by NWLSS Glutathione Assay. The absorbance was measured at 610 nm within 10 min to avoid bleaching. The glutathione concentration was then derived from the standard curve for glutathione [36-37].

**Estimation of Catalase Activity**

The catalase activity in serum was determined using the modified method as described. Serum (10 µL) was added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was measured at 240 nm for 5 min on UV spectrophotometer. A molar extinction coefficient of 0.041 mM⁻¹ cm⁻¹ was used to calculate catalase activity.

\[
2.3/\Delta t \times \ln\left(\frac{A_{\text{initial}}}{A_{\text{final}}}\right) \times 1.63 \times 10^3
\]

Where \(A_{\text{abs}}\) = absorbance at 240nm, \(\Delta t\) = time required for a decrease in the absorbance [38].

**Statistical analysis**

Statistical analysis was performed using Dunnett's Multiple Comparisons Test. Values are expressed as mean ± SEM and p<0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

**Standardization of plant material**

The parameters studied for quality assurance includes successive extractive value viz, petroleum ether 3.80±0.25, diethyl ether 8.24±0.71, chloroform 0.80±0.12, ethanol 19.78±0.85, aqueous 26.10±0.95, moisture content 6.28±0.02, total ash 8.17±0.14, acid insoluble ash 1.30 0.21, water soluble ash 2.31±0.55 and water soluble extractive 6.32±1.20, and alcohol soluble extractive 4.33±0.88, pH value at 1% 6.43 and at 10 % 5.80 and loss on drying at 105°C is 10.27±0.64.

**Qualitative and Quantitative estimation of Phytoconstituents**

The % yield of aqueous and methanolic extract was 5.8 and 5.2 respectively. The preliminary qualitative screening of NHA and NHM extracts of *Nepeta hindostana* herb revealed the presence of flavonoids, phenolic, tannins, alkaloids, steroids, saponins and terpenoids. Total flavonoids were found to be present in maximum amount in NHM extract whereas the other phytoconstituents were found to be present in the following order: total flavonoids > total terpenoids > total phenolic > total alkaloids > total saponin > total tannin > total steroids (Table 1).
Figure 1 (A): TLC of crude extract; (B): fraction (7-20); (C): isolated compound NH1 (fraction 58-67)

Table: 1 Quantitative estimation of phytoconstituents present in extracts of Nepeta hindostana

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Nepeta hindostana extracts</th>
<th>Standard</th>
<th>mg/g equivalent</th>
</tr>
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<tbody>
<tr>
<td>Total Phenolic</td>
<td>35.69±5.12</td>
<td>28.39±6.14</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>Total Flavonoid</td>
<td>54.10±0.61</td>
<td>49.45±0.71</td>
<td>Rutin</td>
</tr>
<tr>
<td>Total Tannin</td>
<td>8.40±0.25</td>
<td>3.66±0.35</td>
<td>Rutin</td>
</tr>
<tr>
<td>Total Alkaloid</td>
<td>19.11±3.28</td>
<td>15.16±1.37</td>
<td>Atropine</td>
</tr>
<tr>
<td>Total Saponin</td>
<td>16.14±1.78</td>
<td>14.80±2.45</td>
<td>Diosgenin</td>
</tr>
<tr>
<td>Total Steroid</td>
<td>1.35±0.35</td>
<td>0.84±0.54</td>
<td>Cycloartenol</td>
</tr>
<tr>
<td>Total Terpenoids</td>
<td>30.34±0.42</td>
<td>32.04±0.46</td>
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</tbody>
</table>

Isolation of a compound (NHI)

The initial isolated compound in fractions (7-20) with white amorphous state showed an aliphatic compound with CH₂O- group, but due to mixing proper identification was not occurring. The next compound (NH1) in fractions (58-67) was found as colourless crystal with weight 15mg in 1.5g NHM extract and melting point was found to be 210-215°C. IR and ¹H NMR data indicated the compound is a triterpenoidal aldehyde of lupine series and comparison of its physical and spectral data with published values confirmed the identity of compound (NHI) as Nepehinal (IUPAC name- 1beta,3beta,1 1alpha-trihydroxy-lup-20(29)-en-30-al) [39]. Figure 2 represents the structure of isolated compound.

“Figure: 2 Compound (I) – Nepehinal”
1beta, 3beta,1alpha-trihydroxy-lup-20(29)-en-30-al (NHI); IR (KBr, cm⁻¹): 3548 (O-H stretching), 3050 (C-H stretching), 2967 (C-H stretching), 1530 (C-H bending), 1639 (C=O stretching), 1597 (C=C stretching); ¹H NMR (DMSO): 6 (ppm) 1.13 (s, 18H, CH₃), 1.18 (m, 2H, CH₂-, cyclopentyl), 1.59 (m, 2H, CH₂-, cyclohexane), 2.50 (s, 2H, CH₂-), 2.03 (t, 3H, OH), 6.95 (d, 1H, CH), 9.94 (s, 1H CHO).

Effect of NHA and NHM in in-vitro Antioxidant activity

The NHM extract was found to have strong antioxidant effect than NHA extract. The antioxidant effect was found in following order: ascorbic acid > NHM > NHA (figure 2). IC₅₀ values of ascorbic acid, NHM and NHA extract of Nepeta hindostana is shown in Table 2.

<table>
<thead>
<tr>
<th>In-vitro antioxidant assay</th>
<th>IC₅₀</th>
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</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td></td>
</tr>
<tr>
<td>NHM</td>
<td></td>
</tr>
<tr>
<td>NHA</td>
<td></td>
</tr>
<tr>
<td>DPPH radical scavenging assay</td>
<td>45.12</td>
</tr>
<tr>
<td>Hydroxyl scavenging assay</td>
<td>40.48</td>
</tr>
<tr>
<td>Superoxide radical scavenging assay</td>
<td>80.29</td>
</tr>
<tr>
<td>Nitric oxide radical assay</td>
<td>24.09</td>
</tr>
</tbody>
</table>

In DPPH assay 500µg/ml of NHM and NHA produced 72.66% and 65.53% scavenging activity. Whereas, in hydroxyl radical scavenging activity, 500µg/ml of NHM and NHA produced 66.89% and 60.23% activity. In superoxide radical scavenging assay ascorbic acid, NHM and NHA produced 76.15%, 63.18% and 59.37% inhibition respectively. In nitric oxide radical assay ascorbic acid, NHM and NHA have 86.20%, 66.39% and 62.50% inhibition respectively. Antioxidant effect of NHM and NHA extract in different assay is in following order: DPPH > Hydroxyl > nitric oxide > Superoxide.

Antioxidant Activity Index (AAI) of ascorbic acid, NHM and NHA was found to be 4.43>1.5>1.15 respectively.

![Figure 3 Effect of NHM and NHA on serum Catalase activity (µM/min/mg of protein)](image)

Values are represented as mean±SEM, n=6. Statistically analysis was done by using Dunnett’s Multiple Comparison Test and p<0.05 was considered to be statistically significant; b = vs diabetic control to test groups.
Effect of NHA and NHM on serum catalase and reduced glutathione level
Administration of alloxan produced oxidative stress which further attenuated the serum catalase and reduced glutathione level in rats. Administration of different doses of NHA and NHM significantly elevated the serum catalase level and serum reduced glutathione level. The increase in the amount of serum catalase was found to be dose dependent (Figure 3 and 4).
Effect of NHA and NHM on fasting blood glucose level in OGTT

The basal values of fasting blood glucose level were almost same and statistically no significant difference was observed while including the animals for experimentation. Fasting blood glucose level was measured at 0, 30, 60 and 120 min. Fasting blood glucose level of control group was found to be 79.0 to 90.89 mg/dl whereas significant increase in fasting blood glucose level was observed after administration of 10% glucose in rats i.e. 150.2 mg/dl as compared to control group (Figure 5).

Different doses of NHA and NHM (100, 200 and 400 mg/kg) were administered 30 min before the administration of 10% glucose and significant reduction in blood glucose level was observed in the treated diabetic rats. However, hypoglycemia was not observed after administration of NHA and NHM since as per CPCSEA normal blood glucose range of wistar rat is 50-135 mg/dl. Interestingly, at 30, 60 and 120 min. 200 and 400 mg/kg of NHM produced significant reduction in blood glucose. Similarly, NHA at dose 400 mg/kg has shown significant reduction in blood glucose level compared to diabetic control at 60 and 120 min. In the OGTT, the decrease in blood glucose level was found to be initiated after 30 min and maintained for 2 h. This indicates that it takes about 30 min for the active ingredients or their metabolites present in the extract to enter into the circulation and reach target tissues to bring about antidiabetic effect and duration was found to be 2 h. The levels of serum lipid were found to be non-significant in treated groups as compared to diabetic control in OGTT after 120 min (Figure 6).

Effect of NHA and NHM on fasting blood glucose level in Alloxan induced diabetes

A significant reduction in serum glucose level was observed at 3rd and 7th day in treated groups as compared to diabetic control group. In Alloxan induced diabetic rats serum glucose level was found to be increased on 3rd and 7th day. Administration of different doses (100, 200 and 400 mg/kg) of NHA and NHM significantly attenuated the serum glucose level at 3rd and 7th day from 210-300 to near normal range (110 mg/dl). NHM 400 mg/kg produced maximum decrease in glucose level (112.8 mg/dl) on 7th day. Metformin at a dose 400 mg/kg reduce serum glucose level at 3rd day and 7th day (P<0.001) significantly as compared with alloxan control (figure 7).
Effect of NHA and NHM on serum glucose level in alloxan induced diabetes

A significant increase was found in TG, LDL and VLDL level in diabetic control group as compared to control group. The administration of NHA, NHM and metformin for 7 days significantly attenuated TG, LDL and VLDL. Reduction in TG level was found to be maximum significant at dose 400 mg/kg of NHM. Moreover, in LDL level major attenuation was observed at dose 200 and 400 mg/kg of NHM as compared to diabetic control. Similarly,
significant attenuation was observed in LDL level at dose 400 mg/kg of NHA as compared to diabetic control. Moreover, the VLDL level was significantly attenuated at dose 200 and 400 mg/kg of NHM as compared to diabetic control (figure 8).

Effect of NHA and NHM on High density lipoprotein level (HDL)
HDL level was found to be significantly reduced in diabetic control group as compared to control group. The significant increase in HDL level was found to be at 200 and 400 mg/kg of NHM and 400 mg/kg of NHA after 7 days of intervention as compared to diabetic control group. The maximum significant increase in HDL level was found to be at 400 mg/kg of NHM extract.

CONCLUSION
The induction of diabetes by alloxan (150 mg/kg i.p) is well known model [40] Alloxan, a beta-cytotoxic, induces “chemical Diabetes” in a wide variety of animal species including rats by damaging the insulin secreting beta cells [41-42]. The increased levels of serum glucose may be due to the partial damage of the pancreatic beta cells. Alloxan is relatively toxic to insulin producing pancreatic β-cells because it preferentially accumulates in β-cells through uptake via the GLUT-2 glucose transporter. This cytotoxic action is mediated by dialuric acid, a reduction product of alloxan. These radicals undergo dismutation to H$_2$O$_2$. The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells, thereby decreasing the secretion of insulin, which in turn increases the blood glucose level [43]. The elevated blood glucose level in the diabetic animals was found to be more than 200 mg/dl, which resembles both type-II diabetes (150 to about 250 mg/dl) with partially functional pancreas as well type-I (above 280 mg/dl) with considerable amount of pancreas damaged [44]. Similar results shows that the administration of alloxan significantly increases the level of glucose when compared to control, which might account for the cytotoxic effect of alloxan on β-cells [45-47]. In OGTT model, attenuation in blood glucose level was observed after 30 min. The decline reached at its maximum at 2 h as compared to diabetic control. These results indicate the inhibition of glucose absorption by extracts.

Flavonoids, sterols, triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles [48]. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats [49]. Phenolics are found to be effective as antihyperglycemic agents [50]. As well as, a number of phytoconstituents were present in Nepeta hindostana plant with flavonoid, phenolic and terpenoids present in major amount. The present study indicated that NHA and NHM extract have strong antioxidant activity. So, it can be considered that antioxidant effect is produced due to these phytoconstituents. Phenolic constituents are very important because their scavenging activity due to their hydroxyl radicals. Flavonoids have been recently suggested as playing primary antioxidant functions in the responses of plants to a wide range of abiotic stresses [51].

GSH is an intra-cellular reductant and plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds. Glutathione also plays an important role in the reabsorption of amino acids [52]. A significant decrease in catalase activity was observed in diabetes control rats as compared to control group, while treatment with the NHM resulted in the increase in the enzyme activity, suggesting that the antioxidant properties of NHM extract may be related to the increased antioxidant enzymatic activity.

On the other hand, treatment of NHA and NHM extracts (100, 200 and 400 mg/kg b.w) for 7 days; significantly attenuated the serum glucose level. Flavonoids also have a role to play in the treatment of diabetes [53-54] and protect against hyperglycemic and alloxan-induced oxidative stress in experimental animal models [55].

Hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis. It is well known that hyperlipidemia has an association with atherosclerosis and the incidence of atherosclerosis is increased in diabetics [56-57]. Lipid plays an important role in the pathogenesis of complications associated with diabetes mellitus. The elevated level of serum cholesterol and reduced level of serum HDL cholesterol in diabetic condition, poses to be a risk factor for developing microvascular complication leading to atherosclerosis and further cardiovascular diseases like coronary heart disease. The abnormal high concentration of serum lipid in diabetic mainly due to increased mobilization of free fatty acids from peripheral fat depots, and insulin deficiency or insulin resistance may be responsible for dyslipidemia [4, 58]. Whereas the NHA and NHM treated group showed significant improvement in the lipid profile comparable to alloxan control group. Interestingly, 200 and 400 mg/kg
of NHM produced significant attenuation in TG and LDL as compared to alloxan control group. In various studies diabetic rats were found to be possessing high lipid level [59] and similar results were found in our study.
The study reveals the antihyperglycemic and antihyperlipidemic activity of NH whole herb in rats. Also, shows that *Nepeta hindostana* extract might be useful both in type-II and type-I diabetes, irrespective of whether the pancreas is partly functional or almost totally destroyed. However the precise mechanism by which NH reduced blood glucose level in diabetic rats requires further study. Therefore, future research and clinical trials in this area may lead to the use of NH as a new type of therapeutic agent in treatment of diabetes. As well as, further pharmacological studies of isolated compounds may provide a new therapeutic agent.

**ABBREVIATIONS**

AAI - Antioxidant activity index  
CPCSEA - Committee for the purpose of control and supervision of experiments on animals  
DM - Diabetes Mellitus  
EDITA - Ethylene diamine tetra acetic acid  
GSH - Glutathione  
HDL - High density lipoprotein  
IAEC - Institutional animal ethics committee  
IC50 - Inhibitory concentration 50  
LDL - Low density lipoprotein  
NBT - Nitro blue tetrazolium  
NH - Nepeta hindostana  
NHA - Nepeta hindostana aqueous extract  
NHM - Nepeta hindostana methanol extract  
NO - Nitric oxide  
OGTT - Oral glucose tolerance test  
SEM - Standard error of means  
TG - Triglyceride  
VLDL - Very low density lipoprotein

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**REFERENCES**