HYPOGLYCEMIC AND ANALGESIC ACTIVITY OF ROOT EXTRACT OF *RUMEX NEPALENSIS*

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ABSTRACT

Plants are an important source of novel pharmacologically active compounds. Ease of availability, least side effects and low cost make the herbal preparations the main key player of all available therapies, especially in rural areas. The aim of present study was to investigate in vitro α- amylase inhibition activity and in vivo hypoglycemic and analgesic activity of ethanolic root extract of *Rumex nepalensis*. In vivo hypoglycemic study was performed in normal and glucose loaded rats. Analgesic activity was determined by using hot plate method. Result revealed that *R. nepalensis* showed the dose dependent inhibition of α-amylase enzyme with IC₅₀ value 912.22 µg/ml. *R. nepalensis* (200 mg/kg) showed significant (p<0.05) decrease in blood glucose level at 60 and 120 min in normal rats and in glucose loaded rats at 30 and 120 minutes. Analgesic activity of *R. nepalensis* was found in dose dependent manner and was quite comparable to standard drug diclofenac. The highest nociperception of thermal stimulus was exhibited at a higher dose (200 mg/kg) with 68.72% analgesic activity at 120 min. Diclofenac (50 mg/kg) and *R. nepalensis* (100 mg/kg and 200 mg/kg) exhibit the significant increase in analgesic activity at 30, 60, 120 and 180 minutes. In conclusion, the ethanolic root extract of *R. nepalensis* showed potent in vitro α- amylase inhibitory activity and in vivo hypoglycemic and analgesic activity.

KEYWORDS: *Rumex nepalensis*, α- amylase, hypoglycemic, analgesic.
INTRODUCTION
Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia. It is considered to be a serious issue in many developing countries. Different treatment approaches such as insulin therapy, pharmacotherapy and diet therapy are available to control diabetes. There are several types of glucose-lowering drugs that exert anti-diabetic effects through different mechanisms. These mechanisms include stimulation of insulin secretion by sulfonylurea and meglitinides drugs, increasing the peripheral absorption of glucose by biguanides and thiazolidinediones, delay in the absorption of carbohydrates from the intestine by alpha-glucosidase and reduction of hepatic gluconeogenesis by biguanides. In the past three decades, despite of the significant progress that has made for treatment of diabetes, the results of treatment in patients is still far from perfection. Furthermore, these treatments have some disadvantages including drug resistance, side effects, and even toxicity.

Analgesics are drugs that selectively relieve pain by acting on the central nervous system or peripheral pain mechanism without altering consciousness. Opioid and non opioid analgesics are the most commonly employed agents for symptomatic relief of pain. Diclofenac is a phenyl acetic acid derivative belonging to the non steroidal anti-inflammatory group (NSAIDs). It is relatively non-selective as a COX inhibitor. It is the most commonly used NSAIDs which is approved for long term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It has analgesic, antipyretic and anti-inflammatory activities. Its potency against COX-2 is substantially greater than that of indomethacin, naproxen or several other NSAIDs. NSAIDs provide effective management of pain and inflammation, but are associated with risk of peptic ulcer, haemorrhage and perforation.

Natural products are the major mine for discovering promising lead candidates of drug. They play an important role in future drug development programs. Since centuries, many plants are considered a fundamental source of potent antidiabetic and analgesic drugs. Although oral hypoglycemic drug together with insulin are the main route for controlling diabetes and opioids and non opioids drugs are most commonly employed agents for symptomatic relief of pain, they exhibited prominent side effects and failed to reverse the course of its complications. This leads the major force for finding alternatives, mainly from plant kingdom that are of less or even no side effects. Therefore, the search for more safer, specific,
and effective antidiabetic and analgesic agents from readily available plant source offers great potential for discovery of new antidiabetic and analgesic drugs.[8]

*Rumex nepalensis* belongs to the family polygonaceae and is commonly called as “Halhale” in Nepali. It is a perennial herb with large roots, distributed throughout Nepal up to 3300 m and is common in open and moist places. It is one of the traditionally used medicinal plants of Nepal. The root is purgative. Decoction of the root is applied to dislocated bones. A paste of the root is applied to swollen gums. The leaves are used in the treatment of colic and headaches. A decoction of the plant is used to wash the body in order to alleviate body pain.[9] Various scientific investigation have reported its antihistaminic, anticholinergic, antibradykinin, antiprostaglandin,[10] anti-inflammatory,[11] antibacterial,[12] purgative,[13] and antipyretic[14] activities. The antibacterial, antifungal and insecticidal properties have also been reported from the extracts of this plant.[15] The main aim of present study was to investigate the *in vitro* α- amylase inhibitory activity and *in vivo* hypoglycemic and analgesic activity of ethanolic root extract of *Rumex nepalensis*.

**MATERIALS AND METHODS**

**Chemicals and drugs**

α- amylase enzyme was purchased from Himedia Laboratories Pvt. Ltd., India. Starch and iodine were purchased from Qualigens Fine Chemicals, India. Drugs such as acarbose, metformin and diclofenac were purchased from Times Pharmaceutical Pvt. Ltd. All chemicals used were of analytical grade.

**Plant materials and extraction**

The root of *Rumex nepalensis* was collected from the Kaski district, Nepal and identified with the help of botanist and literature comparison. Freshly collected roots of *R. nepalensis* were thoroughly washed with water to remove the latex and other dirt and dried under shade. After complete drying of the plant samples, they were grounded into smaller pieces. The coarse powder was macerated in 70 % v/v ethanol. The macerate was collected by filtering and concentrated under reduced pressure by using rotary evaporator. The concentrated sample was labelled and stored in desiccator for further investigation.

**Animals**

Male albino wistar rats, weighing 150-180 g were used for this study. Rats of same sex were used in the experiment to avoid the hormonal disturbances that occur within the physiological
system of the animal which might affect the reliability of the result. They were housed in standard polypropylene cage under standard environment conditions (25±3°C, 55±5% humidity and 12 hours natural light and dark cycle). Animals were kept for acclimatization in laboratory of Pokhara University and fed for 3 weeks with standard diet and water ad. libitum. Animals were fasted for 14 hour before the experiment.

**Ethical Clearance**

An ethical clearance was obtained from Pokhara University Research Council (PURC) (Ref. no.: 131-073-074) and all activities were conducted in ethical manner.

**Preliminary phytochemical screening**

Presences of secondary metabolites in ethanolic root extract of *R. nepalensis* were determined by using different methods\(^{[16,17]}\) of phytochemical screening.

**In vitro α-amylase inhibition assay**

α- amylase inhibition assay was performed according to the method described by Uddin et al.\(^{[18]}\) with slight modification using acarbose as standard. 1 ml of α-amylase was added to plant extracts of different concentrations (200, 400, 600, 800, and 1000 μg/ml) and incubated for 10 min at 37 °C. After the incubation 1 ml of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 hour at 37 °C. 0.04 ml of 1 M HCl was added to stop the reaction followed by addition of 0.1 ml of iodine reagent. Absorbance was measured at 565 nm in UV-Visible spectroscopy. Percentage inhibition was calculated by using formula:

\[
\text{Percentage Inhibition} \% = \left( \frac{A_0 - A_1}{A_0} \right) \times 100\%
\]

\(A_1\) is Absorbance of control, \(A_0\) is Absorbance of sample

**In vivo hypoglycemic activity**

**Collection of blood and determination of blood glucose level**

Blood samples were collected from the tip of the tail at the defined time and fasting blood glucose level was determined by using clinical glucometer based on glucose-oxidase method. The percentage of glycemic changes was calculated as a function of time by applying the formula\(^{[19]}\) as follow:

\[
\text{% glycemic changes} = \left( \frac{G_X - G_O}{G_O} \right) \times 100\%.
\]

Where, \(G_X\) = glycemic values at x mins time interval, and \(G_O\) = initial glycemic values
Acute hypoglycemic effect on normal rats
Normoglycemic study was performed in 14 hours fasted normal rats having free access to water. Rats were divided into five groups (n=5). Group I was treated as a control group. Group II, III, IV as an experimental groups were treated with extracts of dose (50 mg/kg), (100 mg/kg) and (200 mg/kg) respectively and group V as standard group was treated with a standard drug metformin (50 mg/kg). Final volume of all the test samples given to each rat was 0.5 ml. Fasting blood glucose levels were determined at the beginning of the experiment. After the oral administration of test samples, blood glucose levels were measured at 30, 60, 120 min with the help of glucometer.

Acute hypoglycemic effect on glucose induced hyperglycemic rats / Oral glucose tolerance test
Oral glucose tolerance test (OGTT) was performed according to the method described by Sellamuthu et al.[20] with some modification. Group I was treated as normal control group. Group II, III and IV were treated with extracts (50 mg/kg), (100 mg/kg) and (200 mg/kg) respectively and group V as a standard was treated with standard drug metformin (50 mg/kg). After 30 min of extract administration, the rats of all groups were orally treated with 2 g/kg body weight of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration and at 30, 60 and 120 min after glucose loading. Blood glucose levels were measured by using glucometer.

In vivo analgesic activity
Hot plate method
Hot plate method was performed according to method described by Hijazi et al.[21] Group I was treated as normal control group. Group II, III and IV were treated with extracts (50 mg/kg), (100 mg/kg) and (200 mg/kg) respectively and group V as a standard was treated with standard drug diclofenac (50 mg/kg). At first, hot plate temperature was maintained at 55±1°C. Then animals orally treated with 0.5 ml of distilled water or extract (50 mg/kg, 100 mg/kg and 200 mg/kg) or standard drug diclofenac (50 mg/kg) were placed on the hotplate and covered with a glass beaker to avoid heat loss. Each mouse also acted as its own control. The latency time was recorded before and after 30 min, 60 min, 120 min and 180 min of extracts administration. The latency is defined as the reaction time taken by each mouse to respond to licking of the fore paws or jumping.

Percentage Analgesic Activity= \( \frac{(P_0 - P_1)}{P_0} \times 100\% \)
Where, \( P_0 \) = Post treatment latency, \( P_1 \) = Pre treatment latency
Statistical analysis

Results were expressed as mean ± Standard deviation (SD). Statistical analysis was performed using student’s t-test. \( P < 0.05 \) was considered statistically significant. All the statistical methods were carried out through Microsoft Excel 2007.

RESULTS

Preliminary phytochemical screening

Result revealed that ethanolic root extract of *R. nepalensis* showed the presence of phenol, flavonoid, anthraquinone, steroids, saponins, reducing sugar and tannins.

**In vitro \( \alpha \)-amylase inhibition assay**

Acarbose (at a concentration 1000 µg/ml) showed 80.75±0.02% \( \alpha \)-amylase inhibitory activity with an IC\(_{50}\) value 130.57 µg/ml. The ethanolic root extract of *R. nepalensis* (at a concentration 1000 µg/mL) exhibited 56.92±1.16 of \( \alpha \)-amylase inhibitory activity with an IC\(_{50}\) value 912.22 µg/ml. It also showed the dose dependent inhibition of alpha amylase enzyme.

<table>
<thead>
<tr>
<th>Extract/Standard</th>
<th>Concentration (µg/ml)</th>
<th>Percentage Inhibition (%)</th>
<th>( \text{IC}_{50} ) value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. nepalensis</em></td>
<td>200</td>
<td>20.54±1.42</td>
<td>912.22</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>26.75±1.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>35.79±0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>43.35±2.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>56.92±1.16</td>
<td></td>
</tr>
<tr>
<td>Acarbose</td>
<td>200</td>
<td>50.67±2.58</td>
<td>130.57</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>61.73±0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>68.34±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>73.33±0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>80.75±0.02</td>
<td></td>
</tr>
</tbody>
</table>

**In vivo hypoglycemic activity**

**Acute hypoglycemic effect on normal rats**

The effect of different doses of ethanolic root extract of *R. nepalensis* on blood glucose level of normal rats were assessed at different time intervals and depicted in Table 2. The blood glucose level at different time intervals (30, 60 and 120 min) was compared with initial (0 min) blood glucose level of their respective groups. *R. nepalensis* exhibit the dose dependent decrease in blood glucose level at various time intervals. The standard drug metformin (50
mg/kg) exhibits significant decrease in blood glucose levels at 30 min, 60 min and 120 min whereas *R. nepalensis* (200 mg/kg) exhibit the significant decrease in blood glucose level at 60 min and 120 min.

**Table 2: Acute hypoglycemic effect on normal rats.**

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Average blood glucose level (mg/dl) at various time interval (Percentage glycemic change)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>95±2.16</td>
<td>96.5±2.38 (1.57%)</td>
<td>93.25±0.95 (-1.84%)</td>
<td>92.75±2.06 (-2.36%)</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 50 mg/kg</td>
<td></td>
<td>91.75±3.86</td>
<td>96.5±4.43 (5.17%)</td>
<td>92.75±5.5 (1.08%)</td>
<td>90±4.24 (-1.907%)</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 100 mg/kg</td>
<td></td>
<td>94±8.75</td>
<td>100.5±7.59 (6.91%)</td>
<td>91±6.16 (-3.19%)</td>
<td>90±8.5 (-4.25%)</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 200 mg/kg</td>
<td></td>
<td>95.8±4.54</td>
<td>104±6.32 (8.55%)</td>
<td>85.6±6.3* (-10.64%)</td>
<td>83.6±7.16* (-12.73%)</td>
</tr>
<tr>
<td>Metformin 50 mg/kg</td>
<td></td>
<td>100±1.58</td>
<td>80.4±5.68* (-19.6%)</td>
<td>72.4±2.96* (-27.6%)</td>
<td>67.4±2.7* (-32.6%)</td>
</tr>
</tbody>
</table>

*Note: Data are expressed as mean ± standard deviation, *p*<0.05, when compared to control (baseline value). Values in parentheses represent % change in blood glucose level with respect to corresponding zero time and negative sign indicate the decrease in blood glucose level.*

**Acute hypoglycemic effect on glucose induced hyperglycemic rats (OGTT)**

The result of oral glucose tolerance test is shown in Table 3. The hypoglycemic activity of *R. nepalensis* was studied in glucose loaded rats and blood glucose level was measured at different time intervals. Results revealed that after 30 min of glucose (2 g/kg) intake, blood glucose level increased in all groups and after that decreases gradually. For all the groups the change in blood glucose levels was compared with respect to their baseline values. There was dose dependent decrease in blood glucose level. Decrease in blood glucose level in metformin (50 mg/kg) treated group and *R. nepalensis* (200 mg/kg) was 23.42% and 9.18% respectively at 120 min.
Table 3: Acute hypoglycemic effect in glucose induced hyperglycemic rats.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Average blood glucose level (mg/dl) at various time interval (Percentage glycemic change)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>86±5.59</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 50 mg/kg</td>
<td>94.25±4.11**</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 100 mg/kg</td>
<td>93.4±5.59</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 200 mg/kg</td>
<td>93.6±4.27</td>
</tr>
<tr>
<td>Metformin (50 mg/kg)</td>
<td>98.2±3.27</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean ± standard deviation, *p<0.05, **p<0.01, when compared to control (baseline value). Values in parentheses represent % change in blood glucose level with respect to corresponding zero time and negative sign indicate the decrease in blood glucose level.

In Vivo Analgesic Activity

Hot Plate Method

The results of analgesic activity of standard drug and plant extract are shown in Table 4 and Figure 1. Various doses of *R. nepalensis* increased the reaction time in a dose-dependent manner to the thermal stimulus. The highest nociperception of thermal stimulus was exhibited at a higher dose (200 mg/kg) with 68.72% analgesic activity at 120 min. Diclofenac (50 mg/kg), *R. nepalensis* (100 mg/kg) and *R. nepalensis* (200 mg/kg) exhibit the significant increase in analgesic activity at 30, 60, 120 and 180 minutes.

Table 4: Analgesic activity of *R. nepalensis* via hot plate method.

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Reaction Time in Seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>3.44±0.7</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 50 mg/kg</td>
<td>3.88±1.1</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 100 mg/kg</td>
<td>4.00±0.4</td>
</tr>
<tr>
<td><em>R. nepalensis</em> 200 mg/kg</td>
<td>3.50±1.1</td>
</tr>
<tr>
<td>Diclofenac (50 mg/kg)</td>
<td>3.28±0.5</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean ± standard deviation, *p<0.05, **p<0.01, when compared to control (baseline value).
DISCUSSION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to insulin insufficiency and/or insulin resistance contributing to excess blood glucose. Management of the blood glucose level is an essential approach in the control of diabetes complications. Inhibitors of carbohydrate hydrolysing enzymes (α-amylase and α-glucosidase) has been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patients with type-2 diabetes mellitus. Inhibition of these enzymes holds of carbohydrate digestion and extends the total carbohydrate digestion time, leading to a decrease in the rate of glucose absorption and therefore reducing the post prandial glucose level. In present study, *R. nepalensis* showed dose dependent inhibition of α-amylase enzyme with IC$_{50}$ value 912.22 µg/ml. Preliminary phytochemical screening of *R. nepalensis* revealed the presence of anthraquinone, steroids, saponins, reducing sugar, flavonoid and tannin. α-amylase inhibitory activity of *R. nepalensis* may be due to the presence of these phytochemicals. Scientific studies on alpha-amylase inhibitors identified from medicinal herbs recommend that number of inhibitory capability belong to flavonoid class. In general the enzyme inhibitory activity of plant extracts not just rely on amount of specific phytochemicals but depend on the quality of phytochemicals. The 2,3-double bond, 5-OH, the linkage of the β ring at the 3 position, and the hydroxyl substitution on the β ring of flavonoid enhanced the inhibitory activity of α-amylase, while 3-OH reduced it.
In vivo hypoglycemic activity of root extracts of *R. nepalensis* at different concentration (50 mg/kg, 100 mg/kg and 200 mg/kg) and standard drug metformin (50 mg/kg) were investigated in normal rats and glucose loaded hyperglycemic rats. In normal rats, it was found that blood glucose level increases immediately after the administration of root extract of different concentration of *R. nepalensis* and at 60 and 120 min *R. nepalensis* (200 mg/kg) possessed significant decreased in blood glucose level. Rise in blood glucose level immediately after administration of plant extract may be due to presences of the reducing sugar content or as of a physiological phenomenon. In glucose loaded hyperglycemic rats *R. nepalensis* (200 mg/kg) showed 9.18% decreases in blood glucose level at 120 min. Significant decrease in blood glucose level may be due to the presences of phytochemical constituents such as anthraquinone, steroids, saponins, reducing sugar, tannins, flavonoid. Several studies had reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic principles.[27-30] One study has shown that saponins isolated from the root of *Panax notoginseng* showed significant anti-diabetic effects in KK-Ay mice.[27] Steroid demonstrated a significant anti-diabetic activity by reducing the elevated blood glucose levels and restoring the insulin levels in streptozotocin-induced diabetic rats.[28] Stigmasterol isolated from *Butea monosperma* has shown hypoglycemic activity.[29] Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues.[30]

In the present study, an investigation of in vivo analgesic activity of ethanolic extract of *R. nepalensis* was carried out by using hot- plate method where the result revealed the dose dependent increment in analgesic activity of *R. nepalensis*. Maximum analgesic activity (68.72%) was found at 200 mg/kg dose at 120 min. The result of hot plate test indicates that the extract may possess the ability to reduce centrally mediated pain. The presence of different phytoconstituents in *R. nepalensis* extract may have contributed to the analgesic activity. Different researcher has reported the role of different phytoconstituents in analgesic activity. It has been reported that flavonoids plays a role in analgesic activity primarily by targeting prostaglandins.[31] There are also reports on the role of tannins in anti-nociceptive activity by inhibiting cyclooxygenase enzyme (COX-1).[32] On biochemical pathway, it is known that prostaglandins release is mediated by cyclooxygenase. So, the inhibition of these cyclooxygenase enzymes (COX-1 and COX-2) by the active principles contained in the plant extract might reduce the production of prostaglandins and consequently results in the removal of pain. Furthermore, Devkota et al.[33] has reported the anti-inflammatory activity of
ethanolic root extract of *R. nepalensis* in carrageenan induced model. In another study, it was reported that root extracts of *R. nepalensis* possessed anti-inflammatory, COX-2, COX-1 inhibitory and free radical scavenging effects\(^{34}\) which might supports the possible reasons behind the analgesic activity of *R. nepalensis* in the present study.

**CONCLUSION**

Ethanolic root extract of *R. nepalensis* show potential hypoglycemic and analgesic activity. Presences of phytochemical constituents such as anthraquinone, steroids, saponins, reducing sugar, flavonoid and tannin in the plant extract may have contributed to these activities. However, further investigation must be carried out to find exact mechanism behind these activities.

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