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Antioxidant and Hypoglycemic Properties of Seeds of Cucurbitaceae Family from Western Nepal

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ABSTRACT

Introduction: Diabetes mellitus is an endocrine and metabolic disorder characterized by the defect in insulin secretion or insulin action or both. The association between diabetes mellitus and oxidative stress is well established. Cucurbitaceae is the largest family containing 120 genera mostly grown for its sweet and juicy fruit in warm climates all over the world. In the present work ethanolic extracts of the seeds of *Trichosanthes cucumerina* Linn (Chichinno), *Lagenaria siceraria* (Lauka), *Cucurbita pepo* (Pharsi), *Luffa aegiptiaca* (Ghiraula), and *Benincasa hispida* (Kubindo) collected from Western Nepal has been evaluated for their in vitro antioxidant activity and *in vivo* hypoglycemic effects.

Methods: *In vitro* antioxidant activity was assessed by using DPPH free radical scavenging activities and their IC_{50} values were calculated. In vivo hypoglycemic effect was examined on normoglycemic rats. The clinical significance of ethanolic extract at the doses of 250 and 500 mg/kg body weight was investigated in 0, 30, 60, 120 and 180 minutes of oral administration. Metformin treated group was used as the positive control.

Results: For DPPH radical scavenging action, the IC_{50} values of *Trichosanthes cucumerina, Luffa aegiptiaca, Benincasa hispida, Cucurbita pepo, Lagenaria siceraria were* found to be 60.72, 127.73, 49.63, 98.16 and 52.46 µg/ml respectively which were compared to the IC_{50} value of ascorbic acid (Positive control) which was found to be 38.11 µg/ml. The extracts of *Benincasa hispida*, and were having higher antioxidant activity and tested for in vivo hypoglycemic activity. *In vivo* administration of two doses of ethanolic extract of *Lagenaria siceraria* reduced the level of blood glucose while the best result was obtained at 250 mg/kg.

Conclusions: Present study revealed promising antioxidant and hypoglycemic activity of ethanolic extract of *Lagenaria siceraria*. The further exploration of *Lagenaria siceraria* for its effective use in the traditional medicinal system is essential.

Keywords: *Cucurbitaceae Seeds, Antioxidant, Hypoglycemic Property, Western Nepal*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of multiple etiologies, characterized by hyperglycemia resulting from either defects in insulin secretion or insulin action, or in some cases both. Hyperglycemia resulting from defects in insulin secretion caused by pancreatic β-cell dysfunction or of resistance to the action of insulin in liver and muscles.[1]This results in the generation of reactive oxygen species (ROS) which cause lipidperoxidation and membrane damage and plays an important role in the development of secondary complications in diabetes mellitus such as cataract, neuropathy and nephropathy.[2] Medicines of plant origin are increasingly utilized to treat diabetes mellitus in spite of little knowledge regarding their mode of action.[3] Many adverse effects related to the presently used antidiabetic drugs raised the demand of using more safe medicines. According to WHO, there are 21,000 plants are being used for medicinal purposes around the world.[4]Among them 800 plants have been reported to show antidiabetic potential.[5] Cucurbitaceae fruits and seeds are used orally in many region of the world in order to treat diabetes.[5, 6, 7, 8] The treatment approach of diabetes focuses on the reducing or controlling the plasma blood glucose level by either of the four main ways which include stimulating the cells of pancreatic islets to release insulin, resisting the hormones which rise blood glucose, increasing the number of insulin receptors and decreasing the leading out of glycogen. The antidiabetic potential of medicinal plants is attributed to their ability to restore the function of pancreatic tissue by causing an increase in insulin output or inhibit the intestinal absorption of glucose or help the metabolites in insulin dependent process.[9]

In this study the seeds of *Trichosanthes cucumerina* Linn (Chichinno), *Lagenaria siceraria* (Lauka), *Cucurbita pepo* (Pharsi), *Luffa aegiptiaca* (Ghiraula), *and Benincasa hispida*

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(Kubindo) collected from Pokhara, Kaski, Nepal have been evaluated for their in vitro antioxidant activity and in vivo hypoglycemic effects. *Trichosanthes cucumerina* Linn is traditionally used in bronchitis, headache and boils as well as anthelmintic. Its potential as antidiabetic and use in anantidiabetic polyherbal formulation is reported.[10] *Lagenaria siceraria* is a good source of Vitamin B complex, Choline, Vitamin C and β-Carotene. The aerial parts of the plant are reported to have antihyperglycemic activity which is may be due to the rich flavonoid content of the plant.[11] *Cucurbita pepo* is rich source of fatty acids, antioxidant vitamins and reported to possess lipid lowering, hepatoprotective, antimicrobial, anti carcinogenic as well as antidiabetic properties.[12] *Luffa aegiptiaca* is rich in various secondary metabolites such as alkaloids, tannins and saponins. The plant is reported to possess hepatoprotective, anti-hypertensive as well as anti-diabetic properties.[13] *Benincasa hispida* is rich in vitamins and dietary fibre. Amongst secondary metabolites it contains triterpenoids, flavonoids, glycosides and carotenes. It is reported to possess gastroprotective, anxiolytic, antihistaminic, angiogenic, antihelmentic activities apart from antidiabetic activity.[14] It is expected that this study highlights the importance of studied Cucurbitaceae seeds as potential antidiabetic agents

METHODS

Materials and instruments

The sample seeds were collected from Ranipauwa Nursery, Pokhara, Kaski, Nepal in September 2015. The reference standard drug metformin was obtained from Time Pharmaceuticals (P) Ltd. Nepal. Amongst the instruments the UV Spectrophotometer used was (model UV-1800) of Schimadzu, Japan and the rotary evaporator was (model Rotavepor R-215) of Buchi, Switzerland.

Animals and ethical consideration

Male wistar albino rats (total thirty) weighing 100-150 g were obtained from the Science House, Pokhara, Nepal. They were housed in standard polypropylene cage under normal environmental conditions and 12-hour natural light/dark cycle. They were fed with normal diet for 4 weeks and water *ad libitum*. They were fasted for 14 hours before the experiment but allowed free access to water. Throughout the experiment internationally accepted ethical guidelines for care of laboratory animals were followed and ethical aspects were considered as in accordance with the "Ethical Guidelines for Care and Used of Animals in Health Research in Nepal, 200." The research proposal was approved by the research committee of the school of health and Allied Sciences, Faculty of Health Sciences, Pokhara University for the partial fulfillment of the degree of bachelor of pharmaceutical sciences.

Sample extraction

Double maceration was carried out for the sample extraction. The ethanolic extracts were prepared by using 100 g of dry sample macerated using 500 ml of ethanol. After the double maceration the filtrate collected was then concentrated in rotary evaporator at 40°C, 40-80 rpm and 80 mmHg of pressure. The concentrated extracts were then transferred to preweighed clean vials and stored in desiccator for drying.

In vivo antioxidant activity by DPPH method

In order to determine the *in vivo* antioxidant activity, DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical free assay was carried out according to the method of Brand-Williams and colleague.[15] For the experiment DPPH solution of 60µM was prepared using methanol as solvent. The required concentrations of each sample 100µg/ml,10µg/ml,1µg/ml, were made through serial dilution method from initially prepared stock solution of 1mg/ml. The ascorbic acid samples with concentrations of 100µg/ml,10µg/ml,1µg/ml were also prepared in similar manner as standard. In brief, 2ml of each extract concentration (100µg/ml,10µg/ml,1µg/ml, of the sample was mixed with 2ml of DPPH solution (60µM). The mixture was allowed to stand for 30 minutes in the dark for completion of reaction. Finally, the absorbance was measured at 517 nm by using UV spectrophotometer.

Radical scavenging activity of each sample was calculated by using following formula:

Radical scavenging activity(%)=$[(A_0-A_S)/ A_0]\times100$

Where, A_0=Absorbance of control and

A_S=Absorbance of sample.

Test solution without sample was used as negative control whereas ascorbic acid was taken as positive control. IC_{50} of all the sample extracts was determined by trend method in Microsoft Excel 2013.

In vivo hypoglycemic activity

The two samples that showed best antioxidant activity *Benincasa hispida* and *Lagenaria siceraria were* tested for their *in vivo* hypoglycemic activity. The ethanolic extract of the two samples were tested at two doses (250mg/kg and 500mg/kg). Metformin (100mg/kg) was used as standard. The test and standard solutions were prepared by suspending in isotonic saline solution (NaCl 0.9%), DMSO (10%) and Tween 80 (5%). All the test samples were prepared just before the administration. The total volume of 0.5 ml of respective solutions was administered to each rat of predetermined group. Stainless steel feeding tube was used for administration. Hypoglycemic study was performed in 14 hours fasted rats having free access to water. Rats were divided into six groups (n=6). Group I as a negative control received normal

saline. Group II as a positive control was given a standard drug Metformin. Group III and IV received ethanolic extract of sample *B. hispida* with dose of 250mg/kg and 500 mg/kg respectively. Group V and VI received ethanolic extract of sample *L. siceraria* with dose of 250mg/kg and 500mg/kg respectively. 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 0, 30, 60, 120, 180 minutes with the help of Clinical Glucometer. Blood samples were collected from the tip of the tail. The percentage of glycaemia changes was calculated as a function of time by applying the formula of Jimenz et al.[16] as follows:

$$\%\text{glycaemia changes}=[(Gx-Go)/Go]\times 100$$

Where, Gx=glycaemia values at x minutes' time interval and Go=initial glycaemia values.

Statistical Analysis:

The experimental data was expressed as mean ± SD.

RESULTS

The percentage yield *T. cucumerina, L. siceraria, L. aegyptiaca, B. hispida* and *C. pepo* was found to be of 8.5%, 9.2%, 8.3%, 9.5% and 8% respectively. The DPPH radical scavenging activity expressed as percentage inhibition was calculated as shown in the Table 1. The IC_{50} values were calculated and presented in Figure 1.

Table1: Percentage Inhibition of DPPH Free Radical by Sample at 517nm

Sample	%DPPH Radical Scavenging Activity		
Concentration (µg/ml)	1	10	100
Standard (ascorbic acid)	14.02±7.24	37.59±11.95	95.86±0.34
T. cucumerina	18.39±9.97	32.29±5.02	67.24±1.82
L. aegyptiaca	18.04±9.18	25.86±6.24	43.33±5.49
B. hispida	12.07±4.14	35.52±8.37	79.54±5.81
C. pepo	19.89±6.07	30.46±3.45	50.11±7.43
L. siceraria	29.66±2.07	49.31±3.98	60.23±1.55

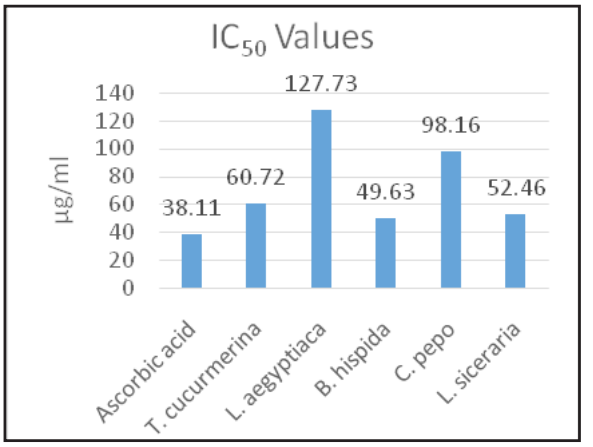

Figure 1: IC_{50} Values of Standard and Different Seeds Extracts

The result of the *in vivo* acute hypoglycemic effect on normal rats presented in Table 2.

Table2: Acute Hypoglycemic Effect on Normal Rats

Group (n= 6)	Average blood glucose levels after the t/t (mg/dl)				
	At 0 min	At 30 min	At 60 min	At 120 min	At 180 min
Negative Control	85.33±3.14	84.25±4.43 (1.26)	84.75±5.75 (0.67)	86.83±3.54 (1.76)	86.67±2.25 (1.57)
Metformin (100mg/kg)	97.66±8.28	98.66±8.77 (1.023)	75.5±3.33 (22)	72±25.87 (26)	52±21.19 (46.75)
B. hispida (250mg/kg)	89.16±14.9	94.83±15.0 (6.36)	82.16±14.1 (7.85)	96.5±10.31 (8.23)	94.33±11.5 (5.79)
B. hispida (500mg/kg)	97.33±22.21	84±3.16 (13.69)	93.83±11.63 (3.59)	88.33±10.76 (9.24)	90.33±4.96 (7.19)
L. siceraria (250mg/kg)	95±8.96	91.66±6.91 (3.51)	84.33±7.55 (11.23)	81.66±8.59 (14.04)	80.83±12.15 (14.91)
L. siceraria (500mg/kg)	81±16.46	97.33±15.86 (20)	89.66±12.59 (10.6)	87.66±10.03 (8.22)	96.5±13.08 (19.14)

(Data are expressed as mean±SD, when compared to the control. Values in the brackets represent percentage glycaemia change)

DISCUSSION

Free radicals are well known for their damaging effect to the body. Thus the scavenging of free radicals is often desired. When the natural system of body become weak, we need external antioxidants. The free radical scavenging activity is associated with the presence of phenolics in addition to flavonoids. It could be considered that compounds in the polar solvent extract were good electron donors and could terminate the oxidation chain reaction by reducing the oxidized intermediates into the stable form.[17] In the present study, the DPPH scavenging activity of the studied seeds reveal that the extracts of *L. siceraria, B. hispida* and *T. cucumerina* were potently active. This suggests that these extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for oxidative stress. The secondary metabolites of the plants are the phenolic compounds and polyphenols that are important virtue of their antioxidative action. They act by chelating redox-active metal ions, inactivating lipid free radical chains and preventing

hydroperoxide conversions into reactive oxygen species.[18] ROS and oxidative stress appears to be an important factor in the production of secondary complications in diabetes mellitus.[19] Active oxygen metabolism plays an important role in the normal function of the β cells of the pancreas. Hyperglycemia generates reactive oxygen species, which in turn cause lipid peroxidation as well as destruction of pancreatic cells. Thus the oxygen metabolism and hyperglycemia are related. Hence this suggests correlation between anti diabetic and antioxidant activities.[19]

Diabetes is characterized by hyperglycemia and glycosuria due to absolute or relative lack of insulin, the fasting plasma glucose cut-off level being 7.0 mmol/l.[20] In spite of availability of various types of anti diabetic drugs, due to associated side effects and reduced response after prolonged use, the interest in use of medicinal plants has been heightened. A part from currently available therapeutic options, many medicinal plants have been recommended for the treatment of diabetes even though the evidence is lacking.[19] Various plants that belong to the cucurbitaceae family are used as vegetables. But they are equally used as the home remedies for lowering blood glucose level traditionally. The plants such as *Momordica charantia* (Karela) have along history of being used to control blood glucose level in diabetic patient as home remedies and also has scientific evidence for the exhibition of hypoglycemic activity.[21] Similarly, *Lagenaria siceraria* (Lauka) from the same family is found to be really effective in controlling blood glucose level, due to which the juice of the fruit of this plant is used often by the diabetic patients traditionally. In practice, the fruits of cucurbitaceae are used to treat the hyperglycemia while seeds are discarded. Present study aims to highlight the effect of seeds. The antioxidative effect and antihypertensive effects of these seeds may be due to the polyphenol compounds.

A number of studies have shown that the solvents used in the extraction of plant can qualitatively or quantitatively affect the biologically active principles of the plants.[21] Thus in this study we have used ethanol as the solvent for extraction due to the presence of higher amounts of polyphenols as compared to other extracts.

In accordance with known mechanisms of action of oral hypoglycemic agents,[22,23] the hypoglycemic activity of *L. Siceraria* could be due to one or more of the following mechanisms: (a) induction of the β-cells of the islets of Langerhans to secrete insulin (b) direct increase in the peripheral utilization of glucose (c) inhibition of intestinal glucose absorption or (d) inactivation of an endogenous insulin inhibitor.

These findings give basis for the use of seeds of Cucurbitaceae plants in diabetes. However, further research and studies are necessary to elucidate the mechanism of action of the formulation at the cellular and molecular levels.

CONCLUSION

Present study revealed promising antioxidant and hypoglycemic activity of ethanolic extract of *B. hispida* and *L. siceraria.* Our investigation showed that oral administration of two doses of ethanolic extract of *L. siceraria* reduced the level of blood glucose while the best result was obtained at 250 mg/kg. Further investigations and explorations are needed to elucidate the exact mechanism of action, particularly the bioactivity guided fractionation, isolation-identification and enzymatic study of constituents of the plant extract responsible for the observed pharmacologic activities.

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Contribution Details:

AK: Concept, design, literature search, monitoring of the experiment, manuscript preparation, manuscript editing
AGC: Concept, literature search, experiment, data acquisition, data analysis, manuscript review
AB: Concept, literature search, experiment, data acquisition, data analysis, manuscript review
KP: Concept, literature search, experiment, data acquisition, data analysis, manuscript review
NA: Concept, literature search, experiment, data acquisition, data analysis, manuscript review
SK: Concept, literature search, experiment, data acquisition, data analysis, manuscript review

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