

**ANTI-HYPERGLYCEMIC AND ANTI-HYPERLIPIDIMIC  
POTENTIAL OF A POLYHERBAL PREPARATION WITH A  
TYPE-2 DIABETES**



**Synopsis of the Ph.D Thesis**

**Submitted to the Sambalpur University in Partial Fulfilment of the  
Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY IN  
BIOTECHNOLOGY**

**by**

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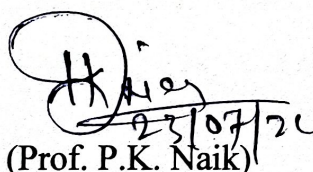
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768019, ODISHA**

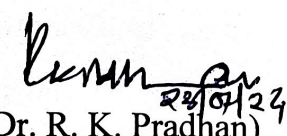
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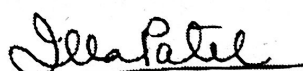


## SYNOPSIS OF THE THESIS SUBMITTED TO SAMBALPUR UNIVERSITY

<b>1. Title of the research topic</b>	<b>ANTI-HYPERGLYCEMIC AND ANTI-HYPERLIPIDEMIC POTENTIAL OF A POLYHERBAL PREPARATION WITH TYPE-2 DIABETES</b>
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<b>3. Registration NO</b>	232/2016/Bio-Tech
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## ABSTRACT OF THE DISSERTATION

The search for an effective drug, alone or in combination for the treatment of diabetes remains elusive. Polyherbal formulations used extensively in traditional systems of medicine may provide a suitable alternative for the treatment and management of Type-2 diabetes. In the present study, a polyherbal formulation was developed consisting of plant materials from 15 medicinal plants collected from the Gandhamardan such as *Tinospora cordifolia*, *Gymnema sylvestre*, *Mangifera indica*, *Syzygium cumini*, *Terminalia arjuna*, *Curcuma longa*, *Albania excelsa*, *Andrographis paniculate*, *Withania somnifera*, *Caesalpinia bonduca*, *Swertia chirayita*, *Holarrhena pubescens*, *Citrus lemon*, *Plumeria alba* & *Murraya koenigi*). The methanolic extract of this polyherbal formulation was used for the in vitro and in vivo evaluation of its antidiabetic potential.

The total flavonoids and phenols content of the polyherbal formulation were determined. The total Flavonoids content of the polyherbal formulation was found to be  $6.545 \pm 0.048$  mg/g. Whereas, the total phenol content of the polyherbal formulation was found to be  $11.071 \pm 0.184$  mg/g. The results revealed high content of both flavonoids and phenols. The antioxidant activity of the polyherbal formulation was investigated based on DPPH (2,2-diphenyl -1-picryl- hydrazylhydrate) free radical, ferric reducing ability of plasma (FRAP) and ABTS radical scavenging assay. The highest antioxidant activity of 92.22% was found in the polyherbal formulation based on DPPH assay. Similarly, the reducing antioxidant activity of  $4.84 \pm 0.0487\%$  was noted for the crude extract of polyherbal formulation based on FRAP assay. The high antioxidant activity was due to the presence of high amounts of phenols and flavonoids. The in vitro antidiabetes activity of the extract was investigated based on inhibition of  $\alpha$ -amylase &  $\alpha$ -glucosidase enzyme. The maximum inhibition of 56.21% for  $\alpha$ -amylase was obtained at a concentration of 100  $\mu$ g/ml solution of polyherbal formulation. In contrast, the standard drug, ascorbase has an inhibition of 88.92% at a concentration of 100  $\mu$ g/ml. The IC<sub>50</sub> value of  $\alpha$ -amylase activity for ascorbase and polyherbal extract was found to be 39.086  $\mu$ g/ml & 6.195  $\mu$ g/ml, respectively. Similarly the effective inhibition of  $\alpha$ -glucosidase with the treatment of standard and polyherbal extracts were carried out. The maximum percentage of inhibition of  $\alpha$ -glucosidase assay was found to be 76.42 % at a concentration of 100  $\mu$ g/ml of polyherbal extract. The standard drug ascorbase was shown a maximum inhibition of 81.91% of at a concentration of 100  $\mu$ g/mol. From the

result it was revealed that the polyherbal extract effectively inhibit the action of a  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme.

The marker components in the polyherbal formulation methanol extract were identified and confirmed by their respective mass ion, fragmentation pattern, offline and online mass spectral database, and related literature. Data acquisitions were executed under positive (+ve) and negative (-ve) mode of ionization utilizing a full spectrum scan. A total of 17 major compounds such as Cordifolide A, Gymnemagenin, Naringenin 4'-glucoside, 5-hydroxy-2-methyl-9 dihydroxyphenyl)-5,7 -dihydroxy-3,4- dihydro-2h-1-benzopyran-3-yl]oxy}butanedioic acid, Mangiferin, Pterocarpol A, 3,7,4'-Trihydroxy flavonone, -({[(2r,3r,4s,5s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy} methyl)-8h,11h-oxepino [2,3-h] chromen-4-one, (2r)-2-{{[(2r,3r)-2-(3,4- Cernuine, Curcumin-L, 2-O- $\beta$ -d-glucosyloxy-4-methoxy benzenepropanoic acid, and 14- Deoxy-11,12-didehydroandrographolide were identified from the polyherbal formulation using LC-HRMS.

The efficacy of the polyherbal formulation in reducing the serum blood glucose level was determined using in vivo diabetic animal model. Wistar rats were divided into four different groups, each are having six animals. Diabetes was induced in overnight, fasted rats by administrating a single intraperitoneal (i.p.) injection of freshly prepared alloxan with a single dose of 100mg/kg BW. Diabetes was confirmed in the alloxan treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of alloxan injection, the rats were given 5% w/v of glucose solution to prevent the mortality. The rats were fasted overnight, collection of blood samples and sera glucose determination were drawn from their tail tips. Sera glucose estimation was done by one touch electronic glucometer using glucose test strips, and the glucose level more than 250 mg/dl was used for the study. As per different groups of animal study (**Group I:** Non diabetic normal control rats, **Group II:** Negative control, **Groups III:** Diabetic rats administered with standard drug metformin (50 mg), **Groups IV:** Diabetic rats administered with standard drug voglibose (1 mg), and **Groups V:** Diabetic rats was given the polyherbal extract of 250 mg/kg BW. Blood glucose level of diabetic animals decreased tremendously from Day I to Day 10 from 497 to 101 with the Polyherbal Treated group (Unit mg/dL)) as compared to normal untreated group and Metformin Treated group. At the end of the experiment day- 14, the rats were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and analysed. The polyherbal formulation also reduced elevated levels of selected biochemical parameters and

prevented other complications of hyperglycemia. These findings provide scientific evidence for antidiabetes use of a traditional formulation and suggest that administration of polyherbal formulation to alloxan/metformin induced diabetes rats, in a dosage used safely by humans, reduces the production of various diabetes causing biochemical parameters and it may prevent the development of type-2 diabetes in established animal models.

The acute toxicity study of the polyherbal formulation was carried out on Wistar rats as per the Organization for Economic Co-operation and Development (OECD) guidelines. Wistar rats were used for the study and fasted for 12 hours prior to dosing. Each animal was given a single dose of the polyherbal extract 5000 mg/kg body weight. After dosing they were observed first 30 mins for any behavioural changes, then were observed for another 24 hours and 72 hours subsequently. The polyherbal formulation was found non-toxic at a concentration >5000mg/kg body weight. The animals were divided into four groups normal control, and three treatment groups, each containing three animals. Group-I was set as control and this group was given normal food and water. The other three groups received doses of 150mg/kg b.w/day, 250mg/kg b.w/day, and 500mg/kg b.w/day, respectively. The weight of the animals was measured daily and their behavioural and morphological changes were observed on the 28th day of treatment. The animals were anesthetized in the anaesthesia chamber containing isoflurane. Cardiac puncture was performed to collect blood sample and was analysed, histopathological study was done for different organs. The histopathology and haematology study revealed no toxicity to the vital organs and organ function among the treated and control untreated groups.

Therefore, we strongly believe that the polyherbal formulation developed in the study has a greater potential as for the treatment and management of type-2 diabetic without any side effects.

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The search for an effective drug, alone or in combination for the treatment of diabetes remains elusive. Polyherbal formulations used extensively in traditional systems of medicine may provide a suitable alternative for the treatment and management of Type-2 diabetes. In the present study, a polyherbal formulation was developed consisting of plant materials from 15 medicinal plants collected from the Gandhamardan such as *Tinospora cordifolia*, *Gymnema sylvestre*, *Mangifera indica*, *Syzygium cumini*, *Terminalia arjuna*, *Curcuma longa*, *Alanthu sexcelsa*, *Andrographis paniculate*, *Withania somnifera*, *Caesalpinia bonduc*, *Swertia chirayita*, *Holarrhena pubescens*, *Citrus lemon*, *Plumeria alba* & *Murraya koenigi*). The methanolic extract of this polyherbal formulation was used for the in vitro and in vivo evaluation of its antidiabetic potential.

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