

Antihyperglycemic and *In Vivo* Antioxidant Activities Of *Phyllanthus Watsonii* A. Shaw Roots In Streptozotocin Induced Type 2 Diabetic Rats

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ABSTRACT

Phyllanthus species have been used for treatment of many diseases. Many researchers reported their antidiabetic and antioxidant activities. *Phyllanthus watsonii* A. shaw was screened for antidiabetic and in vivo antioxidant activities in type 2 diabetic rats. *P. watsonii* roots extracts were prepared using successive solvent extraction process using petroleum ether, chloroform and ethanol. The extracts administered to the streptozotocin induced type 2 diabetic rats and evaluated for reduction of glucose levels. Both ethanolic and petroleum ether extracts produced significant blood glucose lowering effect. Extracts was administered to type 2 diabetic rats for 21 days at the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg and evaluated for changes in thiobarbituric acid reactive substances (TBARS), conjugated dienes, catalase and peroxidase levels. Significant increase in TBARS and conjugated dienes and reduction in catalase and peroxidase were observed. Ethanolic and petroleum ether extracts could significantly decrease TBARS and conjugated dienes; significantly increase catalase and peroxidase levels. Dose dependent antioxidant effect was observed. Chloroform extract could not produce any significant effect. Ethanolic extract produced effect has no significant difference with normal rats glucose, TBARS, Conjugated dienes, catalase and peroxidase levels.

Key words: *Phyllanthus watsonii* A. Shaw, Type 2 diabetes, blood glucose levels, *in vivo* antioxidant activity

INTRODUCTION

The indigenous drugs either alone or in combination have been widely used in the traditional system of medicine for the treatment of various ailments. Ayurveda has been the natural healthcare system which uses flora, fauna and minerals/metals for treatment of diseases in India since 5000 years¹. Many drugs used for treatment of various ailments are originated from plants and have reached widespread acceptability as therapeutic agents. The quest for development of new drugs are increasing and there is rising global interest in plant based drugs. India is enriched with enormous plant flora and fauna and these medicinal properties need to be evaluated scientifically. *Phyllanthus* comprises of around 600 plant species. *Phyllanthus* plant species are reported for pharmacological effects such as antiviral activity against Hepatitis B and related hepatitis viruses², antibacterial activity^{3,4}, anti-hepatotoxic or hepatoprotective activity^{5,6}, antidiabetic⁷, antihyperlipidemic^{8,9}, antioxidant¹⁰⁻¹², anti-tumour and anti-carcinogenic properties^{13,14}. *Phyllanthus watsonii* A. Shaw (family: Phyllanthaceae) is a small shrub, growing to about 1 m height, and usually found near fast-flowing

streams. It belongs to the family Phyllanthaceae^{15,16}. Various species of *Phyllanthus* genus had reported for antidiabetic and antihyperlipidemic activities. However, no reports are available on *P. watsonii* roots for its antidiabetic and antihyperlipidemic activities. Hence we have undertaken this study to evaluate its petroleum ether, chloroform and methanolic fractions for their antidiabetic and *in vivo* antioxidant activities in streptozotocin induced type 2 diabetic rats.

MATERIALS AND METHODS

Collection of plant material

The roots of *Phyllanthus watsonii* A. Shaw were collected and shade dried. They were pulverized using a ball mill until a coarse powder is obtained. Authentication of plant was carried out by our taxonomist and plant specimen is preserved in our herbarium.

Preparation of plant extracts

Plant powder was subjected to successive solvent extraction in Soxhlet apparatus for 72 hours with the solvents in the order of increasing polarity. We have used

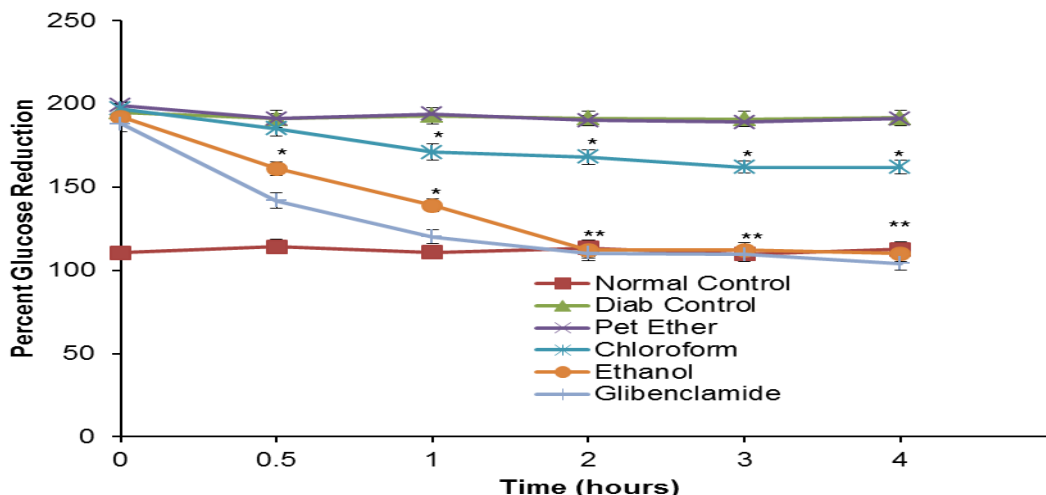


Figure 1: Effect of *Phyllanthus watsonii* roots extracts on streptozotocin-nicotinamide induced type II diabetic rats

*P<0.05 when compared with diabetic control

**P<0.01 when compared with diabetic control

Table 1: Effect of petroleum ether extract of *P. watsonii* roots on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated kidney tissues after 21 days of experimental study.

Parameters	Normal control	Diabetic control	<i>P. watsonii</i> (250 mg/kg)	<i>P. watsonii</i> (500 mg/kg)	<i>P. watsonii</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	3.44±0.09	5.08±0.29	4.67±0.14*	4.29±0.11**	3.96±0.18**
CD (nM/mg tissue)	318.60±8.51	512.90±19.58	468.15±18.55*	405.62±11.64**	340.91±21.62**
CAT (tissue/mg)	6.48±0.10	5.02±0.18	5.65±0.24*	6.02±0.23*	6.44±0.15**
Peroxidase (mM of H ₂ O ₂ consumption/gm of tissue)	9.44±0.16	5.93±0.27	6.84±0.22*	7.91±0.29*	9.28±0.38**

*p<0.05 when compared with diabetic control group.

**p<0.01 when compared with diabetic control group.

petroleum ether, chloroform and ethanol for extraction. Then the collected concentrates were filtered and the filtrates were evaporated to dryness using vacuum evaporator under reduced pressure. The obtained extracts were freeze dried and preserved in a vacuum desiccator.

Antihyperglycemic activity

Male Sprague-Dawly rats were used for this research study. All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethics Committee. Animals were fed with standard diet and water *ad libitum*. They were kept in clean and dry cages and maintained in well-ventilated animal house with 12 h light-12 h dark cycle. Type 2 diabetes was induced in overnight fasted rats by a single intraperitoneal (IP) injection of 65 mg/kg streptozotocin (STZ) was followed after 15 minutes with IP administration of 230 mg/kg nicotinamide¹⁷. STZ was dissolved in sodium citrate buffer

(pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycaemia was confirmed by the elevated glucose levels in plasma after 72 h. The animals having blood glucose concentration of more than 180 mg/dl were selected and used for the study. Animals were divided into five groups consisting of six rats in each group. Treatments were given as below

Group 1: Diabetic control rats received vehicle

Group 2: Diabetic rats received 500 mg/kg of petroleum ether extract

Group 3: Diabetic rats received 500 mg/kg of chloroform extract

Group 4: Diabetic rats received 500 mg/kg of ethanolic extract

Group 5: Diabetic rats received 1 mg/kg glibenclamide

Extracts were administered orally by preparing a suspension of each extract. Suitable volume was

Table 2: Effect of petroleum ether extract of *P. watsonii* roots on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated liver tissues after 21 days of experimental study.

Parameters	Normal control	Diabetic control	<i>P. watsonii</i> (250 mg/kg)	<i>P. watsonii</i> (500 mg/kg)	<i>P. watsonii</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	2.19±0.14	3.72±0.14	3.18±0.04*	2.70±0.18**	2.26±0.11**
CD (nM/mg tissue)	415.37±21.58	415.37±21.58	379.25±24.01*	318.02±18.64**	265.75±23.15**
CAT (tissue/mg)	4.24±0.38	4.24±0.38	6.88±0.62*	8.60±1.04**	9.95±1.55**
Peroxidase (mM of H ₂ O ₂ consumption/gm of tissue)	8.05±1.62	8.05±1.62	10.54±0.99*	12.86±1.02**	14.25±1.12**

**p*<0.05 when compared with diabetic control group.

***p*<0.01 when compared with diabetic control group.

Table 3: Effect of chloroform extract of *P. watsonii* roots on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated kidney tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>P. watsonii</i> (250 mg/kg)	<i>P. watsonii</i> (500 mg/kg)	<i>P. watsonii</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	5.08±0.29	5.01±0.09	4.99±0.05	4.99±0.11
CD (nM/mg tissue)	512.90±19.58	502.15±22.18	499.12±11.62	409.30±18.20
CAT (tissue/mg)	5.02±0.18	5.03±0.24	5.00±0.15	4.95±0.14
Peroxidase (mM of H ₂ O ₂ consumption/gm of tissue)	5.93±0.27	5.89±0.16	5.99±0.29	6.02±0.12

**p*<0.05 when compared with diabetic control group.

***p*<0.01 when compared with diabetic control group.

Table 4: Effect of chloroform extract of *P. watsonii* roots on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated liver tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>P. watsonii</i> (250 mg/kg)	<i>P. watsonii</i> (500 mg/kg)	<i>P. watsonii</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	3.72±0.14	3.69±0.19	3.62±0.24	3.62±0.15
CD (nM/mg tissue)	415.37±21.58	409.22±22.59	405.75±9.95	405.14±23.81
CAT (tissue/mg)	4.24±0.38	4.19±0.15	4.20±0.55	4.45±0.62
Peroxidase (mM of H ₂ O ₂ consumption/gm of tissue)	8.05±1.62	7.77±0.87	8.16±0.54	8.07±0.69

**p*<0.05 when compared with diabetic control group.

***p*<0.01 when compared with diabetic control group.

administered to the over-night fasting type 2 diabetic rats. Blood glucose levels were determined before administration of extract, 30 minutes and at hourly intervals until 4 hours after administration of extracts.

In vivo antioxidant activity

All the three extracts prepared were tested for *in vivo* antioxidant activity. Male Sprague-Dawley rats were used for this research study. All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethics Committee. Animals were divided into various groups of six each and were fed with standard diet and water *ad libitum*. They were kept in clean and dry cages and maintained in well-ventilated animal house with 12 h light-12 h dark cycle. Type 2 diabetes was

induced by the above given protocol and hyperglycemic rats were selected after 72 hours for this study.

Animals were divided into five groups of six rats each.

Group I: normal control rats received the vehicle (1% Gum acacia suspension).

Group II: Diabetic control rats received the vehicle (1% gum acacia suspension)

Group III: Diabetic rats were administered extract (250 mg/kg b.wt/day) in 1% Gum acacia suspension by p.o. route.

Group IV: Diabetic rats were administered extract (500 mg/kg b.wt/day) in 1% Gum acacia suspension by p.o. route.

Table 5: Effect of ethanolic extract of *P. watsonii* roots on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated kidney tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>P. watsonii</i> (250 mg/kg)	<i>P. watsonii</i> (500 mg/kg)	<i>P. watsonii</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	5.08±0.29	4.85 ±0.21*	4.17±0.19**	3.75±0.11**
CD (nM/mg tissue)	512.90±19.58	470.99±19.88*	429.33±15.41**	387.72±20.35**
CAT (tissue/mg)	5.02±0.18	5.94±0.17*	6.41±0.22**	6.98±0.12**
Peroxidase (mM of H ₂ O ₂ consumption/gm of tissue)	5.93±0.27	6.66±0.14*	7.04±0.25**	7.45±0.13**

* $p < 0.05$ when compared with diabetic control group.

** $p < 0.01$ when compared with diabetic control group.

Group V: Diabetic rats were administered extract (1000 mg/kg b.wt/day) in 1% Gum acacia suspension by p.o. route.

Each extract was tested according to the above protocol and doses for 21 days. Only petroleum ether extract was administered using Tween 80 as additional surfactant to enable the extract miscible in the prepared suspension.

All the rats were fasted for 16 hrs before experimentation, but allowed free access to water. Twenty-four hours after the last treatment animals were sacrificed. Liver and kidneys were dissected out and stored at -20°C for the quantification of the levels of the products of free radicals conjugated diene (CD)¹⁸ and thiobarbituric acid reactive substances (TBARS)¹⁹ and for the assessment of the activities of the antioxidant enzymes catalase (CAT)²⁰ and peroxidase²¹.

Statistical analysis

Data is expressed as mean \pm standard error of mean. Statistical analysis was done using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnet's t-test. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

P. watsonii dried roots were fractionated successfully using various solvents. The yield of the extracts was 9.2%, 11.5% and 10.3%/Kg of the diabetic roots respectively. Antihyperglycemic study results are shown in Figure 1. The Figure 1 shows that ethanolic extract of *Phyllanthus watsonii* roots had produced antidiabetic activity ($P < 0.05$) when treated with a single dose in streptozotocin treated type II diabetic rats. The extract had lowered blood glucose levels significantly after one hour of treatment ($P < 0.05$). Maximum effect was observed after 4 hours when treated with ethanolic extract ($P < 0.01$). No significant difference was observed between the maximum effect produced by the extract and standard drug produced effect ($P < 0.05$). The chloroform extract could produce significant lowering of blood glucose levels after one hour of treatment ($P < 0.05$) and increased effect was produced was after 4 hours of administration ($P < 0.05$). Petroleum ether extract treatment could not show any significant lowering of blood glucose levels. The results of the *in vivo* antioxidant effect of *P. watsonii* roots petroleum ether extract on type 2 diabetic rats are presented in tables 1 & 2. Levels of TBARS and conjugated dienes were increased

significantly and catalase and peroxidase levels were decreased significantly in diabetic rats. The extract produced a dose dependent decrease in the MDA levels (TBARS) in the serum. The serum MDA levels of the group treated with 250 mg/kg of extract was significantly ($p < 0.05$) lower when compared to normal control group. The extract produced dose dependent decrease in serum levels of MDA and maximum effect ($p < 0.01$) was observed in 1000 mg/kg treated rats. Conjugated diene levels were reduced significantly in extract treated rats and maximum effect was observed when treated with 1000 mg/kg dose. Significant decrease ($p < 0.05$) in the serum level of catalase dismutase levels was observed in 250 mg/kg dose treated rats. Dose dependent reduction in serum catalase dismutase was observed and was significantly ($p < 0.01$) higher in rats treated with 1000 mg/kg dose when compared with control group. Significant increase ($p < 0.05$) in the serum level of catalase activity was observed in 250 mg/kg dose treated rats. Dose dependent serum catalase activity was observed and was significantly ($p < 0.01$) higher in rats treated with 1000 mg/kg dose when compared with control group. Significant increase ($p < 0.05$) in the serum peroxidase levels was observed in 250 mg/kg dose treated rats. Dose dependent rise in peroxidase levels was observed and was significantly ($p < 0.01$) higher in rats treated with 1000 mg/kg dose when compared with control group. These effects are prominently observed in both liver and kidney tissues. The results of the *in vivo* antioxidant effect of *P. watsonii* roots chloroform extract on type 2 diabetic rats are presented in tables 3 & 4. The extract could not produce any significant effect on MDA levels (TBARS), conjugated dienes, catalase and peroxidase levels in the serum. The results of the *in vivo* antioxidant effect of *P. watsonii* roots ethanolic extract on type 2 diabetic rats are presented in tables 5 & 6. The extract produced a dose dependent decrease in the MDA levels (TBARS) in the serum. The serum MDA levels of the group treated with 250 mg/kg of extract was significantly ($p < 0.05$) lower when compared to normal control group. The extract produced dose dependent decrease in serum levels of MDA and maximum effect ($p < 0.01$) was observed in 1000 mg/kg treated rats. Significant decrease ($p < 0.05$) in the serum level of catalase dismutase levels was observed in 250 mg/kg dose treated rats. Dose dependent reduction in serum catalase dismutase was observed and was

Table 6: Effect of ethanolic extract of *P. watsonii* roots on levels of Thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), catalase (CAT) and peroxidase in isolated liver tissue after 21 days of experimental study.

Parameters	Diabetic control	<i>P. watsonii</i> (250 mg/kg)	<i>P. watsonii</i> (500 mg/kg)	<i>P. watsonii</i> (1000 mg/kg)
TBARS (expressed as nM MDA formed/15 min/mg tissue)	3.72±0.14	3.07±0.10*	2.54±0.04**	2.05±0.19**
CD (nM/mg tissue)	415.37±21.58	358.72±19.46*	301.77±15.21**	242.33±14.78**
CAT (tissue/mg)	4.24±0.38	7.05±0.62*	8.49±0.84**	10.28±0.32**
Peroxidase (mM of H ₂ O ₂ consumption/gm of tissue)	8.05±1.62	11.95±1.36*	14.82±1.12**	15.65±1.14**

* $p < 0.05$ when compared with diabetic control group.

** $p < 0.01$ when compared with diabetic control group.

significantly ($p < 0.01$) higher in rats treated with 1000 mg/kg dose when compared with control group. Significant increase ($p < 0.05$) in the serum level of catalase activity was observed in 250 mg/kg dose treated rats. Dose dependent serum catalase activity was observed and was significantly ($p < 0.01$) higher in rats treated with 1000 mg/kg dose when compared with control group. Significant increase ($p < 0.05$) in the serum peroxidase levels was observed in 250 mg/kg dose treated rats. Dose dependent rise in peroxidase levels was observed and was significantly ($p < 0.01$) higher in rats treated with 1000 mg/kg dose when compared with control group. These effects are prominently observed in both liver and kidney tissues. Thiobarbituric acid reactive substances (TBARS) and conjugated dienes are the indicators of oxidative injury and lipid peroxidation. The results shown by the petroleum ether and ethanolic extracts indicated protective role of oxidative damage in both kidney and liver tissues²². Diabetic complications such as atherogenesis, coronary heart failure, and nephropathy display causal relationship with oxidative stress and lipid peroxidation. The extracts could reduce the levels of TBARS and conjugated dienes in both kidney and liver tissues. Catalase (CAT) considered as most important H₂O₂ removing enzyme and also a key component of antioxidative defense system. H₂O₂ is the product of conversion of oxygen radical (O^{2•}) by superoxide dismutase. H₂O₂, a secondary type of reactive oxygen species are still toxic. It is also more stable than O^{2•} and it can pass through cell membranes more easily than O^{2•}. H₂O₂ can also react with O^{2•} to form the OH⁻ ion, which is more toxic than H₂O₂ and O^{2•}²³. CAT is needed for the conversion of H₂O₂ to a non-toxic substance (water), to protect the cell from the negative effects of ROS. Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage²⁴. Hence the extracts improved significantly CAT and peroxidase levels in kidney and liver tissues and these effects are important for prevention of detrimental effects of reactive oxygen species. Among the extracts ethanolic extract produced effect has no significant difference with normal rats values ($P < 0.05$). In this study, *P. watsonii* possessed both in vivo antioxidant and antihyperglycaemic activities. It will be beneficial for preventing oxidative stress induced free radical damage in diabetic condition. This mechanism will be useful for further deterioration of

pancreatic cells. In addition, the extracts can be beneficial not only for maintain blood glucose levels to near normal but also for prevention of free radical mediated diabetic associated complications such as diabetic nephropathy, retinopathy and cardiomyopathy.

CONCLUSION

Phyllanthus watsonii A. Shaw ethanolic extract and petroleum ether extracts produced significant blood glucose lowering effect in type 2 diabetic rats. Ethanolic extract could lower the glucose levels to near normal. Both petroleum ether and ethanolic extracts produced significant lowering of TBARS and conjugated dienes. They could also increase the levels of catalase and peroxidase. Ethanolic extract could produce significantly higher effect in type 2 diabetic rats.

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