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Original Article

ANTIDIABETIC EFFECT AND PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *POLYALTHIA CERASOIDES* STEM BARK IN STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Objective: *Polyalthia cerasoides* stem bark is used in treatment the of various diseases. Some tribes of North Orissa used the stem bark in the treatment of diabetes. We investigated the effect of four different solvent (n-hexane, ethyl acetate, ethanol and aqueous) extracts of *Polyalthia cerasoides* stem bark on streptozotocintozocin (STZ) 45 mg/kg b.w.. induced diabetic rats.

Methods: Normal control and diabetic control groups were allowed with free access to water throughout the experiment. Oral administration of four different solvent (n-hexane, ethyl acetate, ethanol and aqueous extracts of *P. cerasoides* stem bark to 12 hrs fasted normal and STZ induced diabetic rats at a dose of 200, 400 and 600 mg/kg b.w. for acute studies. (Determination of effective extract and dose: Each group was maintained with six rats)

The fasting blood glucose FBG levels were checked for every 2 hrs (1h, 3h, 5h & 7h). Chronic study: (Each group was maintained with six rats)

The normal and diabetic groups were supplemented with optimum dosage 400mg ethanol extract of *P. cerasoides* (PcEE) stem bark and 20 mg of glibinclamide (Glb) a standard drug treated diabetic group for 21 days.

Results: Acute administration of *P. cerasoides* stem bark reduced FBG levels in ethanol extract atthe dosee 400 and 600 mg/ kg b.w. (48.5% and 32.4%, $P<0.05$) in the diabetic rats only. It does not affect the normal FBG levels in normal rats. In chronic treatment significant reduction in FBG levels in diabetic rats (51.6%). Body weight also increased in extract treated animals. The maximal antidiabetic effect was obtained in 400 mg PcEE which was similar to Glb treated group. The PcEE treated diabetic rats also confirmed the significant recovery of liver and kidney destruction.

Conclusion: Our study has revealed the therapeutic effect of PcEE for diabetes and its related complications.

Keywords: *Polyalthia cerasoides,* Antihyperglycemic, Antidiabetic, Streptozotocin, Glibinclamide, Histopathology.

INTRODUCTION

Diabetes mellitus is associated with several metabolic de-arrangements in carbohydrates, proteins and fat caused by insufficient secretion or inefficient action of insulin. β-cells of the pancreas produced the insulin hormone which enables the cells to absorb glucose from the blood and also helps in the utilization of the glucose in the cells by glycolysis, TCA cycle, HMP shunt etc. [1]. Diabetes mainly characterized by hyperglycemia, ketoacidosis, hypertriglyceridemia. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia and unexpected weight, loss [2]. The long term effects of diabetes include damage and dysfunction various organs especially the eyes, heart and blood vessels. According to recent estimates the prevalence of diabetes is 4% worldwide and that indicates 143 million persons are affected which will increase to 300 million by the year 2025 [3].

The clinical classification of diabetes is proposed by American Diabetes Association (ADA), according to this classification Type - I IDDM (insulin dependent diabetes mellitus) and Type-II NIDDM (non insulin dependent diabetes mellitus) are main groups [4]. Different types of oral glycemic drugs are available along with insulin for the treatment of diabetes, though there is a necessity for development of natural products with antidiabetic activity due to the side effects associated with these therapeutic agents. Treatment to diabetes by these therapeutic agents is expensive. So, herbal drugs are widely used in the treatment of diabetes [5]. Development of phytomedicine is relatively inexpensive and less time consuming. Plants are the traditional source for many chemicals used as pharmaceuticals, biochemicals and fragnences [6]. Most valuable phytochemicals are products of plant secondary metabolism and posses sufficient chemical or structural complexity, so that artificial synthesis is difficult.

Plant based products have been popular all over the world from centuries. Herbal remedies or plant extracts are prescribed widely because of their effectiveness and minimal side effects in clinical experiences and relatively low costs [7]. Ethnobotanical information indicates that more than 800 medicinal plants are used as traditional remedies for treatment of diabetes [8]. The hypoglycemic activity of a large number of these plants has been evaluated and confirmed in different animal models. The aim of the present work is to investigate the antidiabetic effect of various solvent extracts of *P. cerasoides* stem bark on STZ induced diabetic rats also investigated the hepato-toxocity and renal toxicity of the extract treated diabetic animals compared to the effect of Glb.

Polyalthia cerasoides (Roxb) bedd

P. cerasoides (Roxb) Bedd. belongs to the family Annonaceae, it's basionym is *Uvaria cerasoides* (Roxb). It is commonly known as Guttidudduga and deciduous tree grows up to 10 mts height and is distributed in India, China, Burma and Thailand. It has effective medicinal properties such as potent biological activity as an inhibitor of the mammalian mitochondrial respiratory chain [9], significant reactive oxygen species (ROS) scavenging activity [10], anti malarial and antibacterial activity [11]. Some of the tribal people of North Orissa used this stem bark *P. cerasoides* as folk medicine in management of diabetes [12].

MATERIALS AND METHODS

Collection of plant material

Stem bark of *P. cerasoides* was collected from the herbal garden in Dravidian university and surrounding areas of Kuppam A. P., India. The stem bark was dried in the shade, powdered and the powder

was used for the extraction of potential antidiabetic principles into three different non-polar organic solvents as ethanol, ethyl acetate, n-hexane and aqueous extract.

Preparation of extracts

The powdered plant material was extracted sequentially with increasing polarity solvents first with n-hexane (yield=1.3% w/w) and then filtrate was concentrated in a rotary vacuum evaporator and the residue was extracted with ethyl acetate (yield=2.6% w/w) and then concentrated in a rotary vacuum evaporator. The procedure was repeated with ethanol (6% w/w) and water (8% w/w).

Chemicals

STZ were purchased from Sigma Aldrich, St-Louis, USA. All the chemicals are analytical grade was purchased from SRL, Merck. Glb (Danoil) an oral hypoglycemic agent was obtained from the pharmacy.

Animal models

Male albino Wistar rats weighing 200-230 g were used for study. Rats were acclimatized to animal house conditions fed with standard pellet diet and provided water ad libitum. All the animal experiments were conducted according to the ethical norms approved the institutional ethical committee of SPMVV, Tirupati. (Ref.: 1677/PO/a/12/CPCSEA).

Induction of diabetes

Animals were allowed to fast overnight and were injected with STZ (dissolved in 0.1 M ice cold citrate buffer (pH=4.5) at a dose 45 mg/kg b.w.) intraperitoneously [13]. After 48 hrs rats with fasting blood glucose levels (> 250 mg/dl) were selected for the study.

Acute oral toxicity studies

The rats were divided into 24 groups (each group containing 6 rats) groups Group-1and 2 were normal (NC) and diabetic (DC) control groups, which received normal distilled water. Groups – 3 to 14 were normal rats treated with n- hexane, ethyl acetate, aqueous extracts of *P. cerasoides* stem bark at different doses respectively (200, 400, 600 mg/kg dissolved in water by adding 2 drops of Tween-20). Remaining 12 groups were diabetic rats treated with the same doses of plant extract 3 to 14. The anti hyperglycemic activity of plant extracts was assessed in normal and diabetic rats after a single administration, by measuring blood glucose level at 0 hr (before drug administration) and 1, 3, 5 and 7 hr respectively (after drug administration) [14].

Chronic study

In the present study, a total of 30 animals were used. The rats were divided into five groups consisting of 6 animals each group.

Group-1: Normal control (NC)

Group-2: Diabetic control (DC)

Group-3: Normal rats treated with PcEE (400 mg/kg b.w.)

Group-4: Diabetic rats treated with PcEE (400 mg/kg b.w.)

Group-5: Diabetic rats treated with Glb (20 mg/kg b.w.) for 21 days.

Every 7 days the body weights and glucose levels of animals were monitored and after 21 days of treatment the rats were sacrificed by cervical dislocation. The liver and kidney were collected in ice cold containers containing 10% formaldehyde (v/v) for histopathological analysis.

Phytochemical screening

Phytochemical screening was carried out for all the ethyl acetate and ethanolic extract as per procedure [15, 16].

Statistical analysis

Results were expressed as mean ± SD and the difference between the groups were tested by one-way analysis of variance (ANOVA) with Duncan multiple range test (DMRT) used to compare differences among groups. Data were statistically handled through SPSS software version (16.0) $p<0.05$ was considered as statistically significant.

RESULTS

Histopathology studies of liver and kidney of Pc EE treated normal and diabetic rats

Histopathology of liver and kidney was studied in normal, diabetic and treated groups. The normal histological liver section shows the well arranged cells and clear central vein. In the diabetic group it shows the complete destruction of hepatocytes de generation of central vein and also shows fatty degeneration. Histopathological changes are restored near to normal in the PcEE treated group and are near to the Glb drug treated group (Fig. 1).

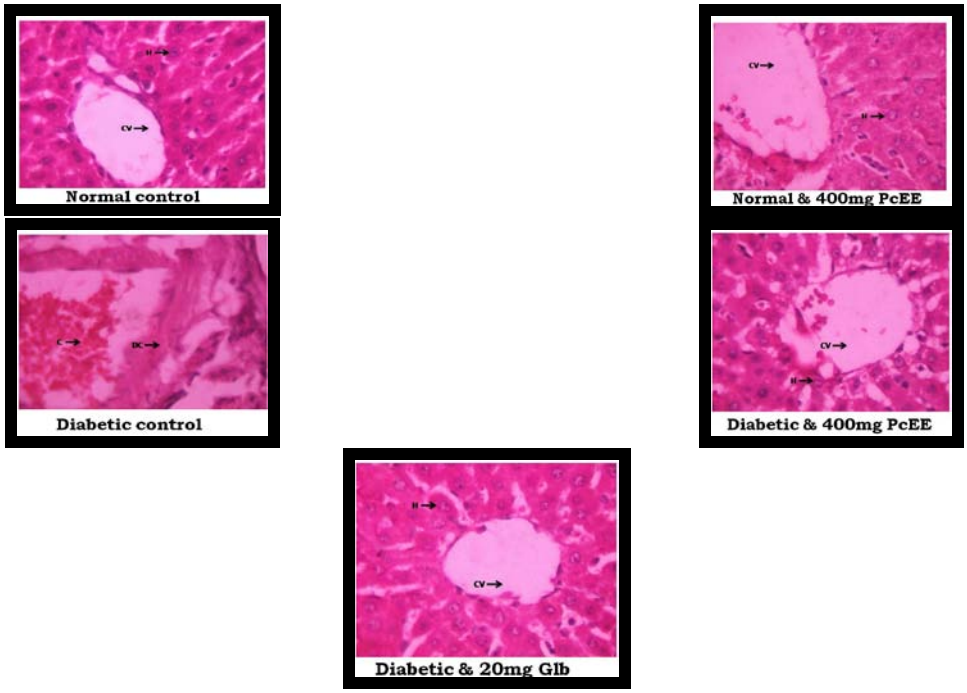

Fig. 1: Histopathological changes in liver of PcEE treated normal and diabetic rats.

Table 1: Effect of PcnhE, PcEAE, PcEE and PcAqE on blood glucose in normal rats (200, 400 and 600 mg/kg b.w.)

Groups	Blood glucose levels (mg/dl) at different hours after treatment				
	0 hr	1 hr	3 hr	5 hr	7 hr
NC	86.3±14.8	90.8±6.5	92.5±4.6	88.6±4.1	88.5±4.0
N+200mgPcnhE	91.8±4.7*	91.3±3.4*	87.6±5.4	88.3±4.5	89.1±5.4*
N+400mg	88.5±4.8*	86.7±5.4	90.0±3.4*	88.3±6.2**	87.6±4.4*
N+600mg	94.5±5.0*	91.8±6.4	88.1±6.9*	87.3±4.6*	92.0±4.1*
N+200mgPcEAE	89.8±4.5*	84.1±2.6*	82.6±5.7*	84.5±1.8*	81.3±4.6*
N+400mg	85.6±7.0	83.8±6.2*	77.8±4.9*	78.1±3.4*	77.5±4.4*
N+600mg	89.3±4.1*	84.5±5.7*	78.6±4.1*	78.5±3.5*	80.5±5.4*
N+200mgPcEE	94.6±9.3*	88.6±7.4*	83.8±5.1*	81.6±6.1*	84.1±2.4*
N+400mg	90.6±6.9**	91.1±5.8	82.1±3.1*	80.3±3.5**	78.8±4.6*
N+600mg	92.1±5.9*	90.8±5.0	83.0±3.7*	81.1±3.7**	81.0±2.1*
N+200mgPcAqE	92.3±8.5*	89.5±6.3*	88.5±8.5*	83.8±3.1*	80.5±5.3*
N+400mg	92.1±9.9	91.0±7.9*	88.8±4.4*	87.3±3.6*	84.8±6.6*
N+600mg	92.0±12.1*	87.5±10.5*	85.5±4.8*	85.3±5.3*	84.6±6.2*

The FBG levels (mg/dl) in normal rats were expressed in mean ± SD with control group. The values are statistically significant at $p<0.05$. In 12 hrs fasted normal control group decreased FBG levels with slight or no significance ($p\leq0.05$) when compared to 0hr was observed. N-hexane, ethyl acetate, ethanol and aqueous extracts treated normal groups (12 hrs fasted) showed decreased FBG levels after 7 hr with slight ($p\leq0.05$) or no significance when compared with 0hr.

Table 2: Effect of PcnhE, PcEAE, PcEE and PcAqE on blood glucose in Diabetic rats (200,400 and 600 mg/kg b.w.)

Groups	Blood glucose levels (mg/dl) at different hours after treatment				
	0 hr	1 hr	3 hr	5 hr	7 hr
DC	438.5±14.8*	446.5±13.1	470.8±8.9*	463.3±12.9*	463.5±14.2*
D+200mgPcnhE	346.1±14.1	344.0±13.8	346.8±17.7	341.8±10.9*	332.3±10.2(3.9)*
D+400mg	353.3±11.6	367.6±13.0	357.5±12.4	345.0±7.6(2.3)*	337.6±3.0(4.4)*
D+600mg	383.0±13.3	391.5±9.11	372.0±16.5	366.6±13.1(4.2)*	361.6±11.3(5.5)*
D+200mgPcEAE	337.0±11.8	334.0±7.7	314.5±14.8(6.6)*	302.8±6.8(10.1)	299.0±5.5(11.2)*
D+400mg	342.5±9.8	314.0±8.6*	306.0±9.2(10.6)*	294.8±6.1(13.9)*	288.0±7.2(15.9)*
D+600mg	349.6±6.1*	314.1±10.3*	321.6±4.4(8.0)*	304.8±7.4(12.8)*	301.3±9.9(13.8)*
D+200mgPcEE	347.5±9.4	309.5±7.8*	272.1±12.3(21.6)*	265.8±4.4(23.5)*	251.1±7.9(27.7)*
D+400mg	354.3±9.68*	328.1±11.7*	243.3±10.2(31.3)**	209.1±9.80(40.9)**	182.3±10.4(48.5)**
D+600mg	343.1±13.0*	313.0±10.02*	294.6±10.0(14.1)*	252.1±6.1(26.5)**	231.6±5.0(32.4)**
D+200mgPcAqE	349.8±6.5	344.1±11.3*	336.6±11.2(3.7)*	330.3±7.8(5.57)*	316.8±6.6(9.4)*
D+400mg	352.1±9.8*	360.0±9.2	335.3±8.8(4.7)*	322.5±3.3(8.4)*	312.5±7.8(11.2)*
D+600mg	353.3±11.8	345.8±8.5	337.6±7.1(4.4)*	322.5±3.4(8.7)*	317.8±8.5(10.1)*

The FBG levels (mg/dl) in diabetic rats were expressed in mean ± S. D. and its percentage (%) decreased when compared to 0 hr in control group (DC) was given in parentheses. The values are statistically significant, n=6; *and** =$p<0.05$. In 12 hrs fasted diabetic control rats FBG levels were almost maintained more or less equally when compared with 0 hr was observed. Among the four extracts each supplemented with graded dose to diabetic groups, ethanolic extract showed maximum activity at 400mg decreased FBG levels significantly in 5 and 7th hr. From the above results ethanolic extract was proved to be the best of the four extracts and 400mg was selected as optimum dosage.

Table 3: Effect of PcEE on body weight (g/day)

Groups	Body weight (g/day)			
	0 day	7 day	14 day	21 day
Group I	153.3±5.1	160.8±8.0	168.3±9.8	184.1±10.2
Group II	156.0±5.1*	165.8±9.1*	172.5±10.8*	176.6±8.1*
Group III	176.6±5.1*	163.3±8.1*	150.8±9.1*	141.6±8.9*
Group IV	179.1±4.9*	177.5±4.1*	181.6±7.5*	185.5±8.3**
Group V	177.5±6.1*	180.8±8.0*	185.8±7.3*	192.5±6.1**

Results are expressed in mean ± S. D.; n=6;**and *=$p<0.05$

Table 4: Chronic effect of PcEE on FBG levels (mg/dl) in normal and diabetic rats

Groups	Blood glucose levels (mg/dl)			
	0 day	7 day	14 day	21 day
Group I	89.1±6.7	88.5± 4.9	88.1± 4.5	87.1±8.1
Group II	378.3±14.8*	381.1± 12.5*	379.6± 11.9*	382.0±12.3*
Group III	81.3±7.5*	87.5± 4.3*	89.0± 3.4*	89.6±4.8*
Group IV	349.1±7.7*	321.5± 4.5*	284.8± 8.3**	168.8±6.4 (51.6)**
Group V	341.8±7.8*	313.1± 7.8*	270.5± 6.4**	159.3±6.3 (53.3)**

The FBG levels (mg/dl) in normal and diabetic rats were expressed in mean ± S. D. and its decreased percentage (%) when compared to 0 day was given in parentheses. The values are statistically significant; *and **=$p<0.05$.

Table 5: Phytochemical screening results *P. cerasoides* stem bark ethanolic and ethyl acetate extracts

Chemical constituent	Ethanolic extract	Ethyl acetate extract
Alkaloids	+	-
Flavonoids	-	-
Triterpenoids	+	+
Tannins	+	-
Saponins	+	+
Phenols	+	-
Amino acids	-	-

Histopathological Studies in kidney of PcEE treated normal and diabetic rats

The normal kidney section shows the well arranged cells. The glomaruler basement membrane is compact.

Diabetic group shows Necrotic changes, Vacuolization and Congestion. The tissues were recovered in PcEE treated group and it shows as similar to normal cyto architecture (Fig. 2).

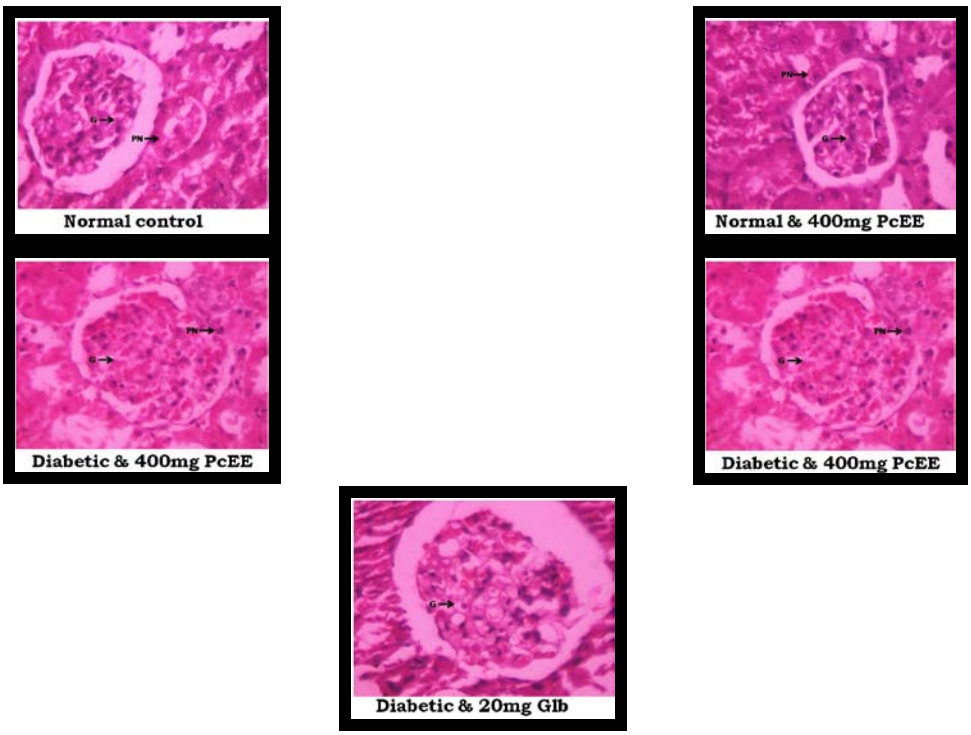

Fig. 2: Histopathological changes in kidney of PcEE treated normal and diabetic rats

DISCUSSION

Plants are principle source of many drugs used in modern medicine due to fewer side effects. For example, quinine, opium alkaloids, atropine, cardiac glycosides and many more. Diabetes is the collection of disorders, which results from the deficiency of insulin secretion, action of insulin or both. Now a days diabetes was induced by using alloxan, ditizona, STZ and anti-insulin serum in animals which are widely used for experimental model [17]. In the present study diabetes was induced by single intraperitonial injection of STZ (45 mg/kg b.w.). By using these experimental diabetic rats the antidiabetic and hepatoprotective activity of PcEE were determined.

The administration of STZ to the normal rats experimental diabetes was developed a severe hyperglycemia and decreased body weight. The β-cells of pancreas undergo destruction of necrosis due to STZ [18]. The lower plasma insulin levels increased the blood glucose due to metabolic changes in liver [19]. Oral hypoglycemic drugs like biguanide, sulphonyl urea had the severe adverse effect in the treatment of diabetes [20]. Therefore, herbal remedies are gaining importance in treatment of diabetes. The present study antidiabetic activity of PcEE results indicates that the plant extract was found to reduce the FBG levels in STZ induced diabetic rats. In this study, we used three different non-polar organic solvent (ethanol, ethyl acetate and n-hexane) extract and aqueous extract of *P. cerasoides* stem bark with different doses (200,400 and 600 mg/kg b.w.) to treat the STZ induced diabetic rats and normal rats. Among four extracts PcEE and PcEAE (400 mg/kg b.w.) showed effects on blood glucose levels. But PcEAE showed the least effect on the reduction of blood glucose compared with PcEE. The remaining two extracts does not show any positive effect in FBG levels. It may be due to lack of certain phyto constituents in that solvent extracts. The four solvent extracts does not show any side effects in normal metabolisms and FBG levels in normal rats. Thus the optimized dosage 400 mg of PcEE was checked for the chronic effect by supplementing to the diabetic and normal rats for 21 days. After 21 days of treatment the FBG levels were decreased significantly when compared with 0 day. Thus PcEE was effective in reduction of FBG levels in STZ induced diabetic rats and it may permit to recovery of partially destroyed β-cells.

We observed the significant reduction of FBG levels in PcEE (400 mg/kg b.w.) when compared remaining three types of extracts. The reduction of blood glucose levels in PcEE is near to Glb drug treated rats were compared to diabetic control by both acute and chronic studies, it is due to insulin secretion from islets of langerhans.

Increased insulin levels in PcEE treated rats showed the possible mechanism of glucose uptake. The obtained results were similar to *Andrographis paniculata* [21], *Helianthus annuus* [22], *Azadirecta indica* [23] and *Pterocarpus marsupium* [24] which was well known for their antidiabetic activities. We observed increased body weight in PcEE and Glb drug treated diabetic rats compared with diabetic control. The reduced blood glucose, increased body weights in diabetic rats may correlate with decreased gluconeogenic activity [25]. This may be one of the reasons for increasing body weights [26]. In the phytochemical screening results, PcEE showed alkaloids, triterpenoids, tannins, phenols and saponins. The PcEAE showed triterpenoids and saponins. In histopathological studies PcEE showed the maximum recovery of liver and kidney in STZ induced diabetic rats, which are similar to Glb treated groups.

CONCLUSION

Thus the results of the present study showed that PcEE brings the reduced FBG levels in STZ induced diabetic rats. In histopathological studies PcEE showed the protective effect on tissues (liver and kidney). Further research to refine the extraction procedure of *P. cerasoides* stem bark could lead to improved pharmaceutical products.

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ABBREVIATION

STZ – Strepotozotocin; Glb – Glibinclamide; PcEE – Ethanolic extract of *P. cerasoides*; PcEAE – Ethyl acetate extract of *P. c.e.rasoides*; PcnhE – n-hexane extract of *P. cerasoides*; PcAqE – Aqueous extract of *P. cerasoides.*

CONFLICT OF INTEREST

Declared None

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