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Antihyperglycemic Perspective of Ethanolic Extract From Leaves of *Pistaciaintegerrima* Stew.Ex Brand

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ABSTRACT

Pistaciaintegerrima is used as antihyperglycemic agent associated with diabetes. In present study the antihyperglycemic study of ethanolic extract from leaves of *Pistaciaintegerrima* (EEPI) was studied in wister ratof either sex by giving 200mg/kg to rats. Antihyperglycemic study was observed by using alloxan induced hyperglycaemia. Lipidemic activity in the same animal was studied. One-way ANOVA followed by Donnette multiple Comparisons test was used. EEPI significantly decreased ($P<0.01$ and $P<0.001$) alloxan induced hyperglycaemia and lipidemic effect of the extract. Effect of alcoholic extract of EEPI on body weight in the alloxan induced hyperglycaemic rates after 12 days of treatment were also observed. These results showed that EEPI exerted an inhibitory effect on alloxan induced hyperglycaemia which may justify its traditional use as an antihyperglycemic agent. Thus, from the study it is concluded that the leaves of *P. integerrima* afford antihyperglycemic activity by preventing hyperglycaemia in diabetic animal model.

Keywords
Antihyperglycemic, Pistaciaintegerrima, Leaves, Ethanolic extract

INTRODUCTION

Pistaciaintegerrima Stewart ex. Brand. is less known high value plant belong to family Anacardiaceae. The plant is native to India and China. It consists of gall-like excrescences formed by insects on the leaves, petioles and branches of the plant *Pistaciachinensia*Burgo and *Pistaciaintegerrima* Stew. ex Brand is, *Rhussuccedanea* Linn. During autumn season, growing on the steps of Western Himalayas from Indus to Kumaon at an altitude of 350-2400 m, often cultivated in Punjab plains.Traditionally plant parts particularly its galls have been utilized for treatment of cold, cough, asthma, fever, vomiting, dysentery, liver disorder and for snake bite[1,2]. Plant mainly contain alkaloids, essential oil, tannins and resinous matters [2,3]. The galls of *Pistaciaintegerrima* Stewart have been reported to yield three new phytoconstituents namely *n*-decan-3′-ol-yl-*n*-eicosanoate, *n*-octadecan-9,11-diol-7-one and 3-oxo-9β-lanost-1,20(22)-dien-26-oic acid along with the known compound β-sitosteroland the structures of these phytoconstituents have also been elucidated on the basis of spectral data analysis and chemical reactions[4]. The volatile oil fraction obtained by hydrodistillation have been reported rich in 1-Tepinen-4-ol (28.82%), p-meth-10-en-8-ol, (43.38%), n-Octyl acetate (19.91%), and beta-Farnesene (7.88%). The concentration of α-terpinolene, limonene and α-thujene were reported less than 1%. The essential oils at the tested concentration (10,100 and 1000 µg/ml) were reported moderate to maximum phytotoxic effect. But here the maximum effect was also recorded with 1000 µg/ml (80%) followed by 100 µg/ml (60%) and 10 µg/ml (50%) [5]. The antibacterial[6,7,8] and antifungal[9] activity on different extracts of pistacia galls and leaves have also been reported. Hypoglycemic activity had also been reported in review article [10].

MATERIAL AND METHOD

Plant material- Selection of the plant was done from literature survey, information collected

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from standard books and also from traditional medicine system practitioners. Leaves from different age of plants in July were collected from Gharsi village hills of Solan of Himachal Pradesh in India. The collected leaves were authenticated by Dr. Manoj Joshi Ph.D. (Forestry) Environment Education Expert. H.P. state forest department, Himachal Pradesh.

Method of extraction- About 160g coarse powder of leaves were packed in soxhlet apparatus and extracted by using ethanol as solvent.The extract was then concentrated.

BIOLOGICAL STUDY

Animals- Wister rats (100-150 g) of either sex and of approximate 9-12-week-old, used in the present studies were procured from listed supplier Sri Venkateshwara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee after securitization. The animals received the drug treatment by oral gavaes tube.

Acute Oral toxicity study (OECD-423)-The extract was given orally at the concentration of 5mg/kg, 50mg/kg, 300mg/kg, 2000mg/kg to animals as per OECD-423 guidelines. Motility was absorbed as requested in the guidelines. None of the animals showed any sign of toxicity up to 2000 mg/kg, therefore a dose of 200 mg/kg (1.10^{th}) was selected as the dose for present *in-vivo* investigation **(Table 2)**

Alloxan induced Diabetes- The experiment is 14 days model in which two days are to induce diabetes and 12 days for treatment. Rats were allowed to fast for 18 hours prior the injection of alloxan (po). Alloxan was dissolved (180mg/kg) in sterile water and injected (po.) to all animals accept group I. After 120 minutes the animals were divided in four groups. Group I was treated with standard pellet diet and water throughout the experimental period and known as normal control. Group II was treated with alloxan (150 mg/kg) with standard pellet diet and termed as diabetic control. Group III animals after receiving injection of alloxan were fed with standard pellet diet and water throughout the experimental period and were treated with glibenclamide(500 µg/kg) orally. The group IV were treated with EEPI (200mg/kg) and termed as test group. Blood samples were collected for the measurement of blood glucose level from the tail vein on 1st, 4th, 8th, 12th day. The blood glucose level was determined by Glucometer. The values were compared with that of the standard group which was treated with Glibenclamide (500mcg). The results are expressed in (**Table3)**.

Lipidemic activity

Animals which were used for the evaluation of antidiabetic activity for 12 days were employed for the determination of lipidemicactivity[10].

Material and method- After slight ether anesthesia, the blood samples were collected in lightly heparinised Eppendorf's micro centrifuge tubes, by retro orbital bleeding method and the serum was separated by centrifugation at 2500 rpm for 20 min, labelled appropriately and stored safely at 4ºC.

Estimation of total cholesterol- To 10 µl of serum, 1 micro litre of cholesterol reagent was added and incubated for 10 minutes. Then readings were observed in digital spectrophotometer at 520 nm.

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Estimation of HDL- The high-density lipoprotein was estimated by using digital spectrophotometer, 500 µl of reagent one and 500 µl of serum was mixed and centrifuged for 10 min. Then from the above precipitating mixture, 10 µl of serum was taken and mixed with 1 ml cholesterol reagent. It was incubated for 10 minutes. Then readings were observed in digital spectrophotometer at 520 nm.

Estimation of triglycerides- To 1ml of triglycerides reagent, 10µl of serum was added and it was incubated for 5 mins. Then readings were observed in digital spectrophotometer at 520 nm.

Estimation of VLDL- the very low-density lipoprotein was determined by using friedewald formula. Estimation of VLDL Cholesterol = Triglycerides/5**.**

Body Weight Test

The alcoholic extract of leaves of *Pistaciaintegerrima* were studied for change in body weight activity against alloxan induced diabetes[12]. The increase in body weight exhibited by the alcoholic extract was compared with standard drug (Glibenclamide). In alloxan induced diabetes rats there was a significant decrease in body weight compared to normal control, standard control and extract treated groups. The result reveals that the alcoholic extract of leaves of *Pistaciaintegerrima* exhibited significant increase in body weight compared to diabetic control rats.

RESULTS

Total 7.2% w/w percentage of alcoholic extract was obtained from 160 grams powder. In acute oral toxicity study the LD_{50} was recorded at 2000 mg and ED_{50} at 200mg.

Table 1: Effect of EEPI on blood glucose level of alloxan induced diabetic rats after 12th days of treatment

Groups/ Treatment	Base line mg/dl	1st Day mg/dl	4th Day mg/dl	8th Day mg/dl	12th Day mg/dl
Normal control	80.2±0.18	82.4±0.64	86.4±1.62	86.8±0.88	86.6±1.22
Diabetic control	376.8±0.26	382.6±0.86	396.2±1.16	406.0±0.42	422.0±0.16
Standard control (500 mg/kg)	366.6±0.18	348.0±0.16*	292.0±0.12*	268.0±0.22*	254.6±1.12*
Test control (200 mg/kg)	368.8±0.22	349.2±0.26**	300.2±0.22**	280.0±0.26**	272.4±0.42**

Normal control (distilled water); Diabetic control (Alloxan control); Std (glebenclamide); Test (alcoholic extract treated) Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Super script *, **, denotes statistically significance of $P < 0.01$, $P < 0.001$, when compared with respective diabetic control.

Table 2: Lipidemic effect of EEPI on alloxan induced diabetic rats

Groups	TC	TG	PL	HDL	LDL	VLDL
Control	138.4±11.8	116.5±10.6	148.4±12.2	54.2±3.8	24.9±1.9	64.10±4.8
Diabetic Control	278.8±2.4	174.8±14.6	286.8±24.2	38.4±2.4	34.8±2.8	204.6±16.2
STD 500 mcg	136.6±10.6*	112.6±11.8*	148.4±9.2*	51.4±3.8*	23.10±2.2*	76.8±5.6*

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Glibenclamide						
Alcoholic Extract 250 mg	142.6±12.6**	118.4±9.6**	154.8±12.4**	51.6±3.8**	26.6±2.4**	64.8±4.8**

No. of. Animals - 6, Values are expressed as mean ± SEM
*P < 0.001, **p < 0.01 vs Control (One-way ANOVA followed by Dennett's multiple comparison test).
TC = Total cholesterol, TG = Triglyceride, PL = Platelet, HDL= high density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low density lipoprotein

Table 3:Effect of EEPI on body weight in gms. ofalloxan induced diabetic rats after 12 days of treatment

Groups	Treatments	1st	4th	8th	12th
Normal Control	Distilled water(10ml/kg)	132.16±4.84	136.00±4.91	139.5±5.15	144.83±5.25
Diabetic control	Distilled water(10ml/kg)	111.66±3.85**	114.33±3.85*	116.16±3.93**	115.5±3.44**
Standard	Glibenclamide 500mcg/kg)PO	114.5±4.08	102.83±4.15*	100.5±3.60**	71.5±2.86
Test	Alc. extract 250 mg/kg PO	118.3±4.99	109.16±4.49	105.0±4.39*	103.0±4.39

DISCUSSION

The total yield of EEPI was reported as 7.2 % w/v (Table 1). The LD_{50} of the extract was found to be 2000 mg/kg and hence its ED_{50} is 200 mg/kg **(Table2).**
The antidiabetic activity of the extract was compared with diabetic and the standard control on 1st, 4th, 8th and 12th day of treatment at day 1st the decrease in elevated blood glucose level from 382.6 ± 0.86 to 348.0 ± 0.16 and 349.2 ± 0.26 for standard and test group looked little near but showed more difference upto12th day at 272.4 ± 0.42 for standard and test group respectively. But at day 4th& 8th also both groups were giving little similar results at 292.0 ± 0.12 , 300.2 ± 0 and 254.6 ± 1, 12,280.0 ± 0.26 for 4th and 8th day respectively. In an activity researcher reported the antidiabetic activity of *P. integerrima* as a glucosidaseinhibitor[11]. In this study they reported strong α-glucosidase inhibitory potential of *P.integerrima* using molecular docking simulations against yeast α-glucosidase at (IC(50): 89.12 ± 0.12 μm) and rat intestine α-glucosidase at (IC (50): 62.47 ± 0.09 μm). This activity was reported due to isolated pistagremic acid from the pistacia galls.
In case of antidiabeticactivity,the decreased blood lipidemic factors were examined insame experimental animals. Significant ($P \leq 0.01$ and $P \leq 0.001$) results were recorded as compare to control group. Lipid level for HDL and VLDL were recorded to be significant at the concentration of 51.6 ± 3.8 and 64.8 ± 4.8 respectively. Rest the sigficant level of triglyceride 118.4 ± 9, 6 were reported. LDL level was recorded at a significant level of 26.6 ± 2.4 as compare to diabetic control at 34.8 ± 2.8.
The effect of alcoholic extract of *P. integerrima* on rate body weights compared to diabetic group, were significantly reported at 4th, 8th and 12th day as 109.16 ± 4.49, 105.0 ± 4.39* and

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103.0 ± 4.39.

CONCLUSION

From the present investigation, it can be concluded that EEPI possess significant antihyperglycemic activity due toglucosidase inhibitor activity[11] because of presence of pistagremic acid. Overall, can say that that EEPI is efficient to exert an inhibitory effect on alloxan induced hyperglycemia which may justify its traditional use as an antihyperglycemic agent. Thus the study concluded that leaves of P. Integerrima afford antihyperglycemia activity by preventing hyperglycemia in diabetic animal model and can be used to formulate effective antidiabetic remedy.

Ethics approval and consent to approval
Biological activity was carried out at LR Institute of Pharmacy, Solan (HP) India.

Human and animal right
No human patient was used in this study. Animal care and experiments were performed in accordance with OECD guideline no. 423.

Conflict of interest
The authors report no conflict of interest in this work in this work.

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Declared none

REFERENCES

[1] Zahoor M, Zafar R, Rahman NU. Isolation and identification of phenolic antioxidants from Pistaciaintegerrima gall and their anticholine esterase activities. Heliyon. 2018; 4(12):1007.
[2] Bibi Y, Zia M, Qayyum A. An overview of Pistaciaintegerrima a medicinal plant species: ethnobotany, biological activities and phytochemistry. Pakistan journal of pharmaceutical sciences. 2015; 28(3):1009-13.
[3] Jindal A, Vashist H, Gupta A, Jalhan S. Physicochemical and phytochemical evaluation of Pistaciaintegerrima Stew ex Brand. Journal of Global Pharma Technology. 2012; 4(7):24-27.
[4] Ahmad S, Ali M, Ansari SH, Ahmed F. Phytoconstituents from the galls of Pistaciaintegerrima Stewart. Journal of Saudi chemical society. 2010 Oct 1;14(4):409-12.
[5] Rauf A, Uddin G, Arfan M, Muhammad N. Chemical composition and biological screening of essential oils from Pistaciaintegerrima. African Journal of Pharmacy and Pharmacology. 2013; 7(20):1220-24.
[6] Uddin G, Rauf A. In vitro antimicrobial profile of Pistaciaintegerrima galls Stewart. Middle-East Journal of Medicinal Plants Research. 2012; 1(2):36-40.
[7] Ramachandra YL, Ravi Shankara BE, SujanGanapathy S, SundarRajan S. In-vitro antimicrobial activity of Pistaaciaintegerrima leaf gall extracts. Pharmacophore. 2010; 1(2):149-54.

[8] Ahmad R, Pieters L, Riaz M. Antimicrobial and antioxidant activity of crude extracts of two medicinal plants** Pistaciaintegerrima** and** Debregeasiasalicifolia. International journal of pharmaceutical sciences: review and research. 2013; 18(1):13-17.
[9] Falahati M, Sepahvand A, Mahmoudvand H, Baharvand P, Jabbarnia S, Ghojoghi A, Yarahmadi M. Evaluation of the antifungal activities of various extracts from PistaciaatlanticaDesf. Current medical mycology. 2015; 1(3):25.
[10] Malviya N, Jain S, Malviya SA. Antidiabetic potential of medicinal plants. Acta Pol Pharm. 2010; 67(2):113-18.
[11] Uddin G, Rauf A, Al-Othman AM, Collina S, Arfan M, Ali G, Khan I. Pistagremic acid, a glucosidase inhibitor from Pistaciaintegerrima. Fitoterapia. 2012; 83(8):1648-52.
[12] Radenković M, Stojanović M, Prostran M. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. Journal of pharmacological and toxicological methods. 2016; 78:13-31.
[13] Komeili G, Hashemi M, Bameri-Niafar M. Evaluation of antidiabetic and antihyperlipidemic effects of peganumharmala seeds in diabetic rats. Cholesterol. 2016.