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Antiinflammatory and hypoglycemic activities of aqueous alcoholic extract of *Punica granatum* fruit and its phytochemical studies

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Abstract

Diabetes is a well known cause of delayed inflammation healing yet, the impact of diabetes on inflammation healing is not widely appreciated. The phytochemical studies were carried out aqueous ethanolic extract of Punica granatum. Carrageenan was used to induced rats paw oedema and diclofenac sodium (100 mg/kg, i.p route), which has been served for positive control. Glucose Tolerance Test (GTT) was conducted in rats and glibenclamide oral dose of 20 mg/kg, which has been used for positive control. The phytocheical studies were indicated in the presence of saponin glycosides, steroids, tannins and terpenoids. The results revealed that the aqueous ethanolic extract of Punica granatum 200 mg/kg and 400mg/kg dose levels have showed significant decrease in the paw volume (P<0.001) when compared to control and positive control. The aqueous ethanolic extract of Punica granatum 200 and 400mg/kg dose levels were showed significantly reduced in the blood glucose level (P<0.001) when compared to control and positive control.

Keywords: *Punica granatum,*, anti inflammatory, hypoglycemic, carrageenan and diclofenac sodium.

INTRODUCTION

The ethanopharmacological use of herbal remedies for the treatment of diabetes mellitus is an area of study ripe with potential as a starting point in the development of alternative and expensive therapies for threating the disease. There are over 30 million people today suffering from one form of the diabetes or another and the numbers are increasing in rural and poor populations throughout the world. The researchers published an extensive review of plants with reported diabetic activity in order to provide the preliminary information needed to design research whose goal it is to develop "indigenous, renewable and plant resources as practical cost efficient alternatives (1-2).

Several literatures have indicated less number of different herbal extracts was screened against hyperglycemia and inflammation. Other literatures have been reported in 2 disadvantages of recent synthetic anti diabetes mellitus drugs [3]. The first disadvantage is poor anti inflammatory activity. The synthetic anti diabetes mellitus drugs second disadvantage is combination therapy produce more adverse effects in diabetes cum inflammation condition [4]. The *Punica granatum* plant portions of bark, flowers, seeds and fruits were used for several therapeutic purposes such as anti-allergic, anti-fungal, anti-inflammatory, anti-microbial, cardiotonic, hypoglycemic and hypolipidemic [5]. We have selected *Punica granatum* was belonging to the family *Punicaceae*. The fruits were collected from the southern part of Tamilnadu and authenticated from Botanical Survey of India (BSI), Coimbatore. Hence our present study was aimed to justify that the traditional claim of the entire fruit has used against inflammation and hypeglycemic condition.

MATERIALS AND METHODS

Preparation of extract (7)

Cold Maceration

The *Punica granatum* entire fruit powder 1000g mixed with 2000ml of 50% ethanol in round bottom flask which was kept under15 days and shaken regularly 2 times per day. The collected extract was dried under reduced pressure and stored in a desiccator.

Phytochemical Studies [6-7]

a. Chemical test for Saponin Glycosides: A few quantity of the extract was taken and shaken with little amount of water in a test tube.

b. Chemical test for Steroids:

1. Liebermann Burchard Reaction: The extract was dissolved in chloroform and few ml of acetic anhydride + few drops of concentrated sulphuric acid were added.
2. Salkowski Reaction: The extract was dissolved in chloroform and few drops of concentrated sulphuric acid were added.

c. Chemical test for Tannins:

1. The extract was treated with ferric chloride.
2. The extract was treated with bromine water.

d. Chemical test for Terpenoids: The extract was treated with vanillin in sulphuric acid.

Animals

Inbred adult male Sprague-Dawley rats (160-200 g) were obtained from the animal house Vinyaga of College of Pharmacy. The animals were maintained in a well ventilated room at a temperature of 35±2°C with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan lever, Bangalore) and tap water were provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory conditions 10 days prior to initiation of experiments. The project proposal was approved by Vinyaga Mission College of Pharmacy IAEC (Institutional Animal Ethical Committee) and the approval number being (IAEC No: Ph\3\2005).

Acute Toxicity Studies[8-10]

The animals were fasted over night prior to the (OECD Guidelines 432) experimental procedure. The Up and Down or 'Staircase, method was adopted, and according to doses of aqueous ethanolic extract of *Punica granatum* was fixed to 200 and 400 mg/kg body weight.

Experimental Design

Carrageenan Induced Paw Oedema Method in Rats [8&11-12]

Male sprague-dawley rats about weight range from 160 to180 gms were used. The animals were starved overnight. To ensured uniform hydration, the rats received water and *libitum*. The rats were divided into four groups and each group consists of six animals. Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Diclofenac Sodium (100mg/kg), Group III served as aqueous ethanolic extract of *Punica granatum* – (200mg/kg), Group IV served as aqueous ethanolic extract of *Punica granatum* – (400mg/kg). Making a mark on left hind paw just beyond the tibia tarsal junction, so that every time the paw was dipped in the mercury column upto fixed mark to ensure constant paw volume. All the groups of rats intial paw volume were measured by mercury displacement method using plethesmograph apparatus. The extracts and standard drug were administered orally by using oral feeding needle. After one hour inject 0.1ml of 1% (*w/v)* carrageenan in sub plantar region of the left hind paw. After the drug administration paw volume was again measured similarly different time intervals at 1, 2, 3, 4& 6 h, respectively.

Glucose Tolerance Test in Rats[13-15]

Male sprague-dawley rats about weight range from 180 to200 gms were used in hypoglycemic activity. The rats were divided into four groups and each group consists of six animals. Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (200 mg /kg), Group III served as aqueous ethanolic extract of *Punica granatum* – (200mg/kg), Group IV served as aqueous ethanolic extract of *Punica granatum* – (400mg/kg). All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been adminisratered to different groups and 30mins later all the groups of rats were treated with glucose orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the sino orbital puncture method. After drug administration blood samples have been collected different time intervals at 30, 60 and 120 mins. The blood glucose levels were determined immediately by using glucometeter.

Statistical Analysis

The values were expressed as Mean±SEM. Statistical Analysis were performed by one way ANOVA followed by Dunnet t test has indicated P<0.001 and P<0.05 were considered for significant activity.

RESULTS AND DISCUSSION

Phytochemical Studies

The preliminary phytochemicals screenings were performed the formation of 1cm layer of foam which was indicated the presence of saponin. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence and its indicated presence of steroid. Yellow precipitate was formed and it was indicated the presence of tannins. The formation of reddish violet colour indicated the presence of terpenoids. Whereas aromatic acids, fats, oils, proteins and alkaloids were absent in the 50% aqueous ethanolic extract of *Punica granatum*. The phytochemical screening results have shown Table. No-I.

Table – I : Details of qualitative phytochemical studies

Phytoconstituents	**Aqueous ethanolic extract of *Punica granatum***
Carbohydrates	-
Flavonoids	-
Saponin glycoside	+
Alkaloids	-
Steroids	+
Proteins & AA's	-
Tannins	+
Terpenoids	+

+ = Present; - = Absent

Antiinflammatory Activity

The results have been showed that the aqueous ethanolic extract of *Punica granatum* fruit at dose levels 200 and 400mg/kg body weight were significant decrease (*P<0.05) and (**0P<.001) in the paw oedema volume when compared with control group. The aqueous ethanolic extract of *Punica granatum* fruit at dose level 400mg/kg body weight was significantly dose dependent manner decreased in the paw oedema volume at 3hr (**0P<.001) when compared with (*P<0.05) 200mg/kg body weight. The diclofenac sodium treated group has shown more anti inflammatory activity when compared to each other groups The anti inflammatory activity results have shown Table No- II.

Table – II : Antiinflammatory activity of aqueous ethanolic extract of *Punica granatum*

Treatment	Dose mg/kg	Mean Paw Volume (ml)					
		0h	1 h	2h	3 h	4 h	6 h
CMC	0.5 %	0.96±0.68	1.4±0.06	2.1 ±0.68	2.4 ±0.11	2.6 ± 0.11	3.2 ± 0.12
DC	100	0.96±0.06	1.7±0.09	1.5±0.12**	1.4±0.08**	1.3±0.06**	1.3±0.08**
AEPG	200	0.86 ± 0.06	1.5±0.11	1.8 ±0.14*	2.1 ±0.15*	2.5 ±0.08*	3.1 ± 0.16*
AEPG	400	0.91±0.04	1.3±0.06	1.7± 0.14£*	1.4±0.11**£ £	1.3±0.14** £ £	1.3±0.18** £ £

CMC- Carboxy Methyl Cellulose(control)
DC- Diclofenac Sodium (positive control)
AEPG- Aqueous Ethanolic Extract of Punica granatum

The results of rats paw volume were expressed as Mean ± Standard Error (N=6), (P<0.05)* and (P<0.001) ** when compared to control and positive control groups and evaluated by using one way ANOVA followed by Dunnet 't' test and £- is indicated comparision between 200vs 400mg/kg body weight.

Hypoglycemic Activity

The GTT results have been expressed that the aqueous ethanolic extract of *Punica granatum* fruit at dose levels 200 and 400mg/kg body weight blood glucose levels were signicantly reduced (***P<0.0001) and (**0P<.001) when compared with conrol group. The aqueous ethanolic extract of *Punica granatum* fruit 400mg/kg body weight was significantly reduced (***P<.0001) in the blood glucose level when compared with (**P<0.001) 200mg/kg body weight group and this results indicated a significant dose dependant hypoglycemic activity for the aqueous ethanolic extract of *Punica granatum* fruit 400 and 200mg/kg. The glibenclamide treated group has shown more hypoglycemic activity when compared to each other groups. The hypoglycemic activity results have shown Table.No- III.

Table – III : Hypoglycemic effect of aqueous ethanolic extract of *Punica granatum*

Treatment	Dose mg/kg	Blood Glucose Concentration (mg/dl) at different time intervals (mins)			
		0	30	60	120
(CMC)	0.5%	67.05 ±2.2	108.6 ±2.2	90 ±2.8	78.5±2.51
GB	200	66.2±2.2	96.6±2.0	67.5±2.4***	72.3 ±1.6***
AEGP	200	68.5 ±1.0	96.6 ±2.0	87.1±2.1**	85.1 ±1.3**
AEGP	400	68.1 ±1.4	96.0 ±4.3	68.0 ±1.5***£ £	71.6±2.0***£ £

GB- Glibenclamide

The Glucose Tolerance Test results were expressed as Mean ± Standard Error (N=6), (P<0.05)* ,(P<0.001)** and (P<0.001)*** when compared to control and positive control groups and evaluated by one way ANOVA followed by Dunnet't' test and £ - is indicated comparision between 200vs 400mg/kg body weight.

CONCLUSION

The present study concluded the beneficial effect of *Punica granatum fruit* in the control of paw oedema volume in carrageenan induced paw oedema rats. The *Punica granatum fruit* has

been controlled by blood glucose level in glucose treated rats. This study confirms the rational basis for its use in traditional medicine for the treatment of diabetes cum inflammatory patients. Further phytochemical and pharmacological investigations are under way to characterize active phytoconstiuents and to establish exact mechanism of its hypoglycemic action. This work, we believe, will be useful for further diabetic research works.

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