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RESEARCH ARTICLE

Comparative Hypoglycaemic Study of Methanolic Extract of Psidium guajava (Guava), Tamarindus indica (Tamarind) & Azadirachta indica (Neem) in Alloxan-induced Diabetic Rat with Reference to the Standard Drug Metformin

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ABSTRACT

Our aim of the study was to compare the anti-diabetic activity of already established methanolic leaf extract of Psidium guavaja, Tamarindus indica and Azadirachta indica over Alloxan-induced diabetic rat by using Metformin as a standard drug. Extract was prepared by proper drying, sieving followed by soxhelation and evaporation of the excess solvent by rotary vacuum evaporator. 2% solution of Alloxan was used for induction of diabetes to the animals both in long term (1, 7, 14, 21 and 28th day study) and short term study (1, 2, 3, 4 and 6 hours). In long term study, more significant decrease of blood sugar level had been observed in case of Azadirachta indica (86.7±0.62 mg/dL - High dose value on 28th day) than Tamarindus indica (98.3±0.86 mg/dL - High dose value on 28th day) and Psidium guajava (106±0.73 mg/dL - High dose value on 28th day) – where value for standard drug was reported as 73.9±0.2 mg/dL. In OGTT study also, more significant lowering of Blood glucose level had been observed in Azadirachta indica (81.9±0.79 mg/dL in 4 hrs high dose) in respect to Tamarindus indica (90.3±0.87 mg/dL in 4hrs high dose) and Psidium guajava (96.5±0.73 mg/dL in 4hrs high dose) – where Metformin shows value of 79.1±0.4 mg/dL (4 hrs). So, it can be concluded from the study that methanolic extract of Azadirachta indica shows better antidiabetic potential in comparison to methanolic extract of Tamarindus indica and Psidium guajava on Alloxan induced diabetic rat model in respect to standard drug Metformin.

KEYWORDS

Diabetes Mellitus, Psidium guavaja, Tamarindus indica, Azadirachta indica, Alloxan, OGTT, Comparative study

INTRODUCTION

Diabetes mellitus is basically a metabolic disorder – which is generally characterised by lack or complete absence of the hormone Insulin[1].

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There are a number of diseases – which are associated with diabetes mellitus, for example people suffering from diabetes mellitus may be affected with nephropathy, retinopathy, neuropathy, cardiovascular disease and stroke & ultimately it may lead to the death. It can be delayed or somehow can be prevented by the treatment of High blood sugar level[2-3].

According to WHO, It is one of the most prevalent diseases of the world and its prevalence is almost about 6.8% of the total

population[4]. In modern days also no such satisfactory therapy is available to cure Diabetes mellitus[5]; we can only prevent it by using medicines and indulines up to a certain limit. Drugs like biguanides, Sulphonyl- ureas are generally used to treat diabetes but on long time use, effectivity is quite poor[6,7]. In the last few years, plant medicines[8,9,10] have been found for the treatment against different diseases as herbal drugs are generally out of toxic effect[11,12] reported from research work conducted on experimental model animal. Anti-diabetic effect of various plants having "folk medicine reputation" has been screened through last few years[13].

Psidium guajava (Guava leaf) belongs to the family myrtaceae are generally found throughout the world and is widely used in the management of hypertension, Diabetes mellitus and obesity[14,15]. In the screening of plants, it has been found that leaves of Psidium guajava reduce the plasma blood sugar level in alloxan induced Diabetes mellitus[16]. Recent studies have revealed that Guavanoic acid obtained from P. guajava shows its anti-diabetic activity through inhibition of PTP1B pathway[17]. Tamarindus indica (local name tamarind / tetul) is the plant belongs to the family Fabaceae generally found in the tropical regions and is being considered as one of the most important plant resources in herbal medicine in different parts of the world[18].

It has already been established that its seeds are having high antioxidant potentials[19,20] and the isolated antioxidant components are, 2-hydroxy-30, 40-di-hydroxy-acetophenone, methyl 3, 4- i-hydroxy-benzoate, 3, 4-di-hydroxy-phenyl-acetate and epicatechin in addition to oligomeric pro-anthocyanidins, phenolic compounds are pro-cyanidin B_2, epicatechin, procyanidin trimer, pro-cyanidin tetramer, pro-cyanidin pentamer, pro-cyanidin hexamer, polymeric tannins, polymeric tannins. It has been used in the treatment of Diabetes mellitus in human and animal trials[21,22]. It has been already established that aqueous extract of the seeds contain anti-hyperlipidemic activity in STZ induced diabetic rat model[23].

Azadirachta indica is commonly known as neem – which is widely used in the treatment of Diabetes mellitus. The therapeutic effect attributed to neem are as diverse as anti-plasmodial, larvicidal, antiviral, antiulcer, fungicidal, spermicidal, antibacterial, immune-contraceptive[24,25], anti-inflammatory etc. Its other already established activites include antipyretic[26] insecticidal, anti-helminthic, anti-implantation, immune-modulating[27,28], antioxidant[29,30], anti-diabetic[31], anticancer[32] etc.

The present study was directed to the compare of the anti-diabetic activity based of the leaf extracts of Psidium guajava, Tamarindus indica, Azagirachta indica (which already show inhibitory effect of glucose utilization and were used as hypoglycemic agent in traditional system of medicine) in Alloxan-induced diabetic rat with reference to the standard drug Metformin.

MATERIAL & METHODS

Preparation of Methanolic Leaf Extract of Psidium guajava

Freshly collected Psidium guajava leaves were cleaned and dried in room temperature for ten to fifteen days – which is followed by crushing to convert them in to coarse powder. Coarse powder was properly sieved and 60 gram of the sieved material was placed in the Soxhlet apparatus for extraction purpose by using methanol and water mixture. After complete extraction, extract was filtered and were placed in rotary vacuum evaporator at 45℃. Dried extract was stored in air tight containers in room temperature until further experiments were performed[33].

Preparation of Methanolic Leaf Extract of Tamarindus indica

Leaf collected from Tamarindus indica was properly dried under shade and was converted in to coarse powder by using grinder. Now the powder was placed in to soxhet apparatus with sufficient amount of aqueous methanol until complete extraction was completed. Extract was concentrated by using Rotary vaccum

evaporator and kept in air tight containers until the further experiments[31,34].

Preparation of Methanolic Leaf Extract of Azadirachta indica

Completely dried Azadirachta indica leaves were properly dried and placed for extraction by using aqueous methanol for three to four days followed by rotary evaporation under reduced pressure at 45℃. Extract was store in air tight containers for future use[35].

Chemicals and Reagents

Commonly used reagents were Alloxan (Sigma Aldrich, MW: 160.08), Glucose estimation Kit (Accu check instant kit) Metformin (Sigma Aldrich, MW: 165.62) etc. All chemicals and drugs were obtained commercially and were of high analytical grade. Equipments include Mouth gag, polythene feeding tube, tuberculin syringe and insulin syringe (each 1ml) etc.

Experimental Animals

Albino rats (weight between 250-300 g, either of sex) of wistar strain were used for the experimental purpose and they were provided standard laboratory food and water. Animal experiment was done by following guidelines followed by ICDDRB which were approved by the institutional animal ethical committee.

Induction of Diabetes Mellitus to the Experimental Animals

Freshly prepared 2% solution of Alloxan in 0.9% NaCl was used for the induction of Diabetes to the experimental animals. After overnight fasting, rats were injected the above drug in 150mg/kg body weight and continuous monitoring was done to check any presence of allergic symptoms, behavioural changes, convulsion etc. Fasting blood glucose was monitored every morning and the animals having stable fasting blood sugar level over 200 mg/dl were selected for the study[36-37]. Animals were divided in to nine groups (n=6).

Non –diabetic control group: Animals of this group were given 0.5ml of normal saline daily for 30 days. Blood sugar level was recorded on the day 1, 7, 14, 21 and 28.

Diabetic control: These animals were treated with 2% solution of alloxan intraperitoneally in a dose of 150 mg/kg body weight. This group also received 0.5ml of normal saline daily for 30 days. Blood sugar level was recorded on day 1, 7, 14, 21 and 28.

Test group 1 - Methanolic leaf extract of Psidium guajava High Dose: Treated with 2% solution of alloxan intraperitoneally in a dose of 150 mg/kg body weight followed by 400mg/kg of the Psidium guajava diluted extract. Blood sugar level was recorded on day 1, 7, 14, 21 and 28.[38]

Test group 2 - Methanolic leaf extract of Psidium guajava low Dose: Treated with 2% solution of alloxan intraperitoneally in a dose of 150 mg/kg body weight followed by 200 mg/kg of the diluted Psidium guajava extract. Blood sugar level was recorded on day 1, 7, 14, 21 and 28.[38]

Test group 3 - Methanolic leaf extract of Tamarindus indica High Dose: Treated with 2% solution of alloxan intraperitoneally in a dose of 150 mg/kg body weight followed by 400 mg/kg of the diluted Tamarindus indica extract. Blood sugar level was recorded on day 1, 7, 14, 21 and 28.[38]

Test group 4 - Methanolic leaf extract of Tamarindus indica low Dose: Treated with 2% solution of alloxan intraperitoneally in a dose of 150 mg/kg body weight followed by 200 mg/kg of the diluted Tamarindus indica extract. Blood sugar level was recorded on day 1, 7, 14, 21 and 28.[38]

Test group 5 - Methanolic leaf extract of Azadirachta indica High Dose: Treated with 2% solution of alloxan intraperitoneally in a dose of 150 mg/kg body weight followed by 400mg/kg of the diluted Azadirachta indica extract. Blood sugar level was recorded on day 1, 7, 14, 21 and 28.[38]

Test group 6 - Methanolic leaf extract of Azadirachta indica low Dose: Treated with 2% solution of alloxan intraperitoneally in a dose of 150 mg/kg body weight followed by 200 mg/kg of the diluted Azadirachta indica extract. Blood

sugar level was recorded on day 1, 7, 14, 21 and 28.[38]

Standard control: These rats were treated by 2% solution of alloxan, intraperitoneally in a dose of 150 mg/kg body weight with Metformin (Standard drug) in a dose of 0.5mg/kg body weight suspended in normal saline, orally, daily for 30 days. Blood sugar level was recorded on the day 1, 7, 14, 21 and 28.

Evaluation of Short Term Anti-diabetic activity / Oral Glucose Tolerance Test (OGTT)

Rats were kept in overnight fasting for at least 12-18 hrs (only water was provided) and 0.1 ml of blood sample was taken at the end of fasting (considered as 0 h) to check the blood glucose level. All the nine group of animals (n=6) were treated with the specified samples (mentioned above) and level of blood sugar was monitored after 1, 2, 3, 4 and 6 hours after administration of single dose of samples[38,39].

Result obtained from the above study was put in a graph to compare the activity of the different extracts with the standard marketed drug.

Evaluation of Long Term Anti-Diabetic Activity

Selected rats of different groups (n=6) were treated with the samples (mentioned above) and blood sugar level was monitored on 1, 7, 14, 21 and 28th day by using glucometer (Glucose estimation kit) and put in to the graph in respect to the time to compare activity of the extracts in respect to the standard drug[39].

Data Analysis

All the data obtained from above experiments were expressed in the form of Mean ± sem. Data was evaluated using Student's paired t-test - an associated probability (p value) of less than 5% ($P<0.05$) was considered significant.

RESULTS

Result of Short Term Anti-diabetic Activity / Oral Glucose Tolerance Test (OGTT)

Results obtained after 1, 2, 3, 4, 6 hours were shown in the table as well as in the bar diagram below. ($p < 0.001$)

Blood Glucose Level (OGTT)									
Time (hr.)	**Control (Non-Diabetic)**	**Control (Diabetic)**	**A. indica (High Dose)**	**A. indica (Low Dose)**	**T. indica (High dose)**	**T. indica (Low dose)**	**P. guajava (High dose)**	**P. guajava (Low dose)**	**Standard Drug Metformin**
01	72.2±0.15	210.3±0.78	170.2±0.82	188.9±0.71	174.9±0.81	183.7±0.9	175.4±0.66	176.3±0.96	101.7±0.33
02	71.7±0.21	209.1±0.64	113.7±0.9	139.5±0.97	128.1±1.02	147.8±0.98	130.8±0.8	131.2±0.64	83.1±0.41
03	70.4±0.19	214.8±0.71	83.2±0.93	106.6±0.85	98.5±0.96	113.4±0.72	98.9±0.45	104.7±0.77	83.9±0.29
04	79.8±0.26	212±0.52	81.9±0.79	94.1±0.93	90.3±0.87	109.7±1.08	96.5±0.73	99.4±0.91	79.1±0.4
06	71.5±0.35	211.5±0.68	83.3±0.95	98.1±0.75	91.2±0.91	107.5±0.96	99.1±0.57	99±0.94	77.5±0.37

Result of Long Term Anti-diabetic Activity

Results obtained after 1, 7, 14, 21 and 28 days were shown in the table as well as in the bar diagram below. ($p < 0.001$)

Blood Glucose Level (mg/dL)									
Day	**Control (Non-Diabetic)**	**Control (Diabetic)**	**A. indica (High Dose)**	**A. indica (Low Dose)**	**T. indica (High dose)**	**T. indica (Low dose)**	**P. guajava (High dose)**	**P. guajava (Low dose)**	**Standard Drug Metformin**
01	71.7±0.23	221.3±0.92	205.4±0.73	213.2±0.93	211.8±0.88	216.1±0.79	217.3±0.46	220±0.94	203.9±0.12
07	70.1±0.31	225.1±0.89	170.3±0.91	181.1±0.7	187.3±1.02	201.6±0.73	191.7±1.12	206.6±0.71	153±0.28
14	73.6±0.27	219.8±1.1	141.7±0.78	144±0.59	158.7±0.98	163.7±0.95	169.1±0.94	188±0.42	114.2±0.31
21	72.2±0.11	228.8±0.96	98.3±0.44	108.4±0.88	123.7±0.9	132.4±0.52	144.6±0.35	153.2±0.33	96.8±0.22
28	74.8±0.19	224.5±0.87	86.7±0.62	83.9±0.65	98.3±0.86	100±0.64	106±0.73	109.3±0.79	73.9±0.2

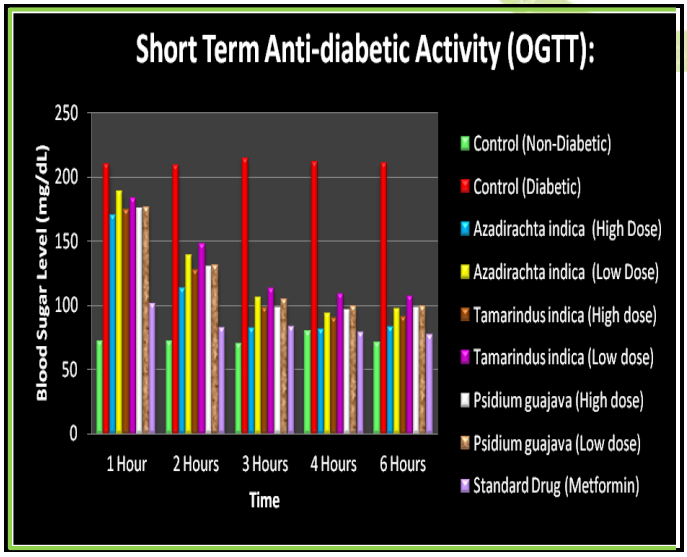

Figure 1

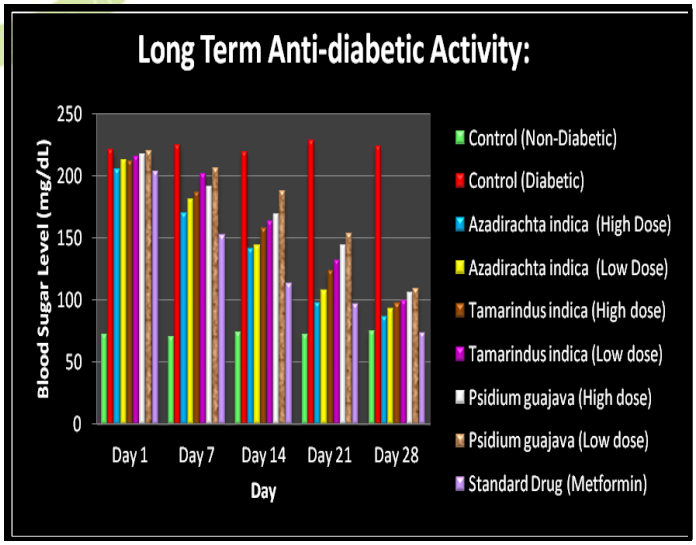

Figure 2

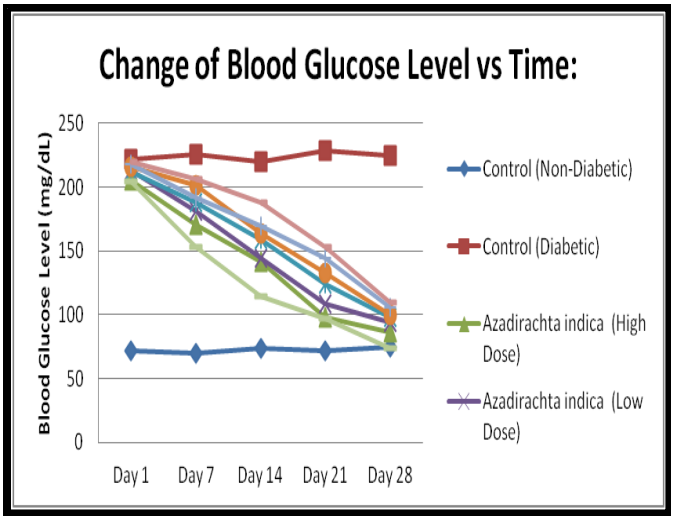

Figure 3

DISCUSSION AND CONCLUSION

A number of studies and clinical trials had already established that the high level of blood sugar is the main cause of this diseases[40] and this is why glucose loaded hyperglycaemic model was selected to compare the effect of different plant extracts[41]. Alloxan was widely used to induce diabetes in health rats and this model had been widely used as an anti-diabetic model on plant product extracts[42]. Alloxan induces Diabetes mellitus to the animals by destroying the beta cells of Islets of Langerhans – which are responsible for the production of insulin in body[43]. diabetogenic effect of alloxan is due to the excess production of reactive oxygen species leading to cytotoxicity in pancreatic beta cells, which reduces the synthesis and release of insulin[44]. Medicinal plants were selected based on the availability and already established anti-diabetic activity. Metformin was selected as standard anti-diabtic drug because it decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects.

Result shows, in long term study, more significant lowering of blood sugar level had been found in case of Azadirachta indica (86.7 ± 0.62 mg/dL - High dose value on 28th day, 83.9 ± 0.65mg/dL – Low dose value on 28th day) than Tamarindus indica (98.3 ± 0.86 mg/dL - High dose value on 28th day and 100 ± 0.64 mg/dL – Low dose value on 28th day) and Psidium guajava (106 ± 0.73 mg/dL - High dose value on 28th day and 109.3 ± 0.79 mg/dL – Low dose value on 28th day) – where value for standard drug was reported as 73.9 ± 0.2 mg/dL. In respect to Oral Glucose Tolerance Test (OGTT), more significant Hypoglycemic activity had been observed in Azadirachta indica (81.9±0.79 mg/dL in 4 hrs high dose and 94.1 ± 0.93 mg/dL in 4 hrs low dose) in respect to Tamarindus indica (90.3 ± 0.87 mg/dL in 4hrs high dose and 109.7 ± 1.08 mg/dL in 4 hrs low dose) and Psidium guajava (96.5 ± 0.73 mg/dL in 4hrs high dose and 99.4 ± 0.91mg/dL in 4 hrs low dose) – where Metformin shows value of 79.1 ± 0.4 mg/dL (4 hrs).

So, it can be concluded from the study that methanolic extract of Azadirachta indica shows better antidiabetic potential in comparison to methanolic extract of Tamarindus indica and Psidium guajava on Alloxan induced diabetic rat model in respect to standard drug Metformin. Still further studies are in progress to elucidate mechanisms of anti-hyperglycaemic activity of the plant extracts.

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