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In vivo hypoglycemic study of *Manilkara zapota* leaf and seed extracts

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Abstract

The hypoglycemic activity of petroleum ether extracts of leaves and methanol extracts of seeds of *Manilkara zapota* was evaluated in the study. The oral glucose tolerance test was performed in mice treated with 2 mg/kg glucose solution and the blood glucose level was determined after 15, 30, 60, 90 and 120 min of administration. Alloxan (70 mg/kg) was injected intravenously to induce diabetes. The hypoglycemic study was carried out 7 days. In glucose tolerance test all extracts achieved significant values ($p<0.0001$) at 60, 90 and 120 min compared to the glucose control. In hypoglycemic study all extracts started to reduce the blood glucose level rapidly even starting from the 2nd day of treatment and significant values ($p<0.0001$) were achieved. So the study evinced the hypoglycemic potency of the leave and seed extracts of *M. zapota*.

Introduction

The overall health death rate in people with diabetes is about double than that of people without diabetes (Harrigan et al., 2011). To avoid side effects of existing hypoglycemic drugs, people are using natural products. It has been appraised that over 80% world population rely on herbal medicine for their therapeutic benefits (Tiwari, 2008) and more than 400 plant species have been identified in the literature with significant hypoglycemic activity (Belhekar et al., 2013).

Manilkara zapota (Family: Sapotaceae) commonly known as sapodila, is a long-lived evergreen tree. The ornamental leaves are medium green and glossy. Various studies have been performed to reveal the therapeutic effectiveness of different plant parts of *M. zapota*. Its fruit, pulp and peel are potent anti-oxidant (Woo et al., 2013; Gomathy et al., 2013; Jamuna et al., 2011). The flower contains anti-oxidant and antibacterial properties (Priya et al., 2014). Its leaf is a potent source of different types of therapeutic activities. The leaf has been reported to possess antimicrobial (Nahir and Chand, 2008; Osman et al., 2011), anti-oxidant (Kaneria and Chand, 2012), analgesic (Jain et al., 2011), anti-arthritic (Singh et al., 2011), anti-inflammatory and anti-pyretic (Ganguly et al., 2013) activities. The roots are also used for its antibacterial activity (Bhargavi et al., 2013). The seed has been studied to have anthelmintic (Kumar et al., 2012) and anti-oxidant (Mohanapriya et al., 2014) properties as well as a source of natural gum (Singh and Bothara, 2014). The bark contains cytotoxic (Awasare et al., 2012), antibacterial (Islam et al., 2013) properties and also used as a pesticide (Osman et al., 2011). The present study reports the hypoglycemic activity of petroleum ether extract of the leaves and methanol extract of the seeds of *M. zapota*.

Materials and Methods

Collection of plant materials

Fresh leaves and seeds of *M. zapota* were collected from the area of Gazipur and then the plant was taxonomically identified from Bangladesh National Herbarium, Mirpur, Dhaka. (Voucher specimen No: 40256)

Preparation of petroleum ether extract of Manilkara zapota leaves (PMZL)

Fresh leaves of the plant were first washed with water

to remove adhering dirt and then cut into small pieces, sun dried for 4 days and finally dried at 45°C for 36 hours in an electric oven. After complete drying, the entire portion was pulverized into coarse powder with the help of a grinding machine. About 100 g of powder samples were taken in a clean round bottomed flask and soaked in 1L petroleum ether. The container with its content was sealed by cotton plugs and aluminum foil and kept for a period of 7 days accompanying occasionally shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No. 1 filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator. The concentrated extract was then air dried to solid residues and dissolved in 2% tween solution.

Preparation of methanol extract of Manilkara zapota seeds (MMZS)

Fresh seeds were washed with distilled water thoroughly to remove traces of contaminants and shade dried for 7 days. Again, the seeds were dried in an oven for 3 days at 40°C, crushed in an electric grinder and then powdered. Extraction was performed by taking 50 g powder in 300 mL of 95% methanol with occasional shaking and stirring for 7 days. The extract was then filtered through filter paper (Double ring filter paper 102, 11.0 cm). The solvent was evaporated under reduced pressure and the extracted compound was dissolved in 2% tween solution.

Experimental animals

Young Swiss-albino mice of either sex aged 4-5 weeks, average weight 30-35 g were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All the mice were kept in standard environmental condition (12:12 hours) light and dark cycle at 25°C and relative humidity (60-70%) and fed ICDDR,B formula-ted rodent food and water. Before starting the experiment the animals were carefully marked on different parts of the body, these were later used as identification marks to identify animals, so that the response from a particular mouse prior and after the administration could be noted separately.

Chemicals and instruments

In the experiment, alloxan (Fluka, Germany), metformin hydrochloride (General Pharmaceutical Ltd.), glucose estimation kit (Human, Germany) and kitchen scale 1800 were used. All these were of analytical grades.

Ethical approval

Animal experiment was done by following guidelines followed by the ICDDR,B which were approved by the institutional animal ethics committee. The Southeast University Ethics Committee observed the whole experiment regarding animals (mice) and the experiment was performed according to the ethics of the institute.

Acute toxicity study

Normal, healthy mice were divided into five groups of six animals each. Different doses (100, 250, 500, 750 and 1,000 mg/kg body weight) of different extracts (petroleum ether, methanol) of plant *M. zapota* were administered orally. The mice were observed continuously for 2 hours of behavioral, neurological and autonomic profiles and after 24 and 72 hours for any lethality (Ecobichon, 1997).

Oral glucose tolerance test

Normal non-diabetic Swiss albino mice were fasted approximately for 18 hours before starting the experiment and divided into 6 groups of 5 mice each (n=5). Group 1 (Glucose control): Treated with distilled water; Group 2 (Positive control): Treated with metformin HCL at a dose of 100 mg/kg; Group 3 and 4 (Test groups): Treated with 150 mg/kg body weight of PMZL and MMZS separately; Group 5 and 6 (Test groups): Treated with 300 mg/kg body weight of PMZL and MMZS separately.

The non-diabetic mice of all groups were administered glucose solutions to the dose of 2 mg/kg body weight after 30 min of the administration of their respective dose. All the treatments were given orally. Blood was withdrawn from the tailvein just prior to the respective dose administered (fasting glucose level) and at 15, 30, 60, 90, and 120 min after glucose loading. The blood glucose level was measured by using glucometer.

Experimental design

To perform the hypoglycemic study, mice were divided into 7 groups, each consists of five mice (n=5). Group 1: Normal control mice treated with saline; Group 2: Diabetic control mice (saline treated alloxan induced mice); Group 3 and 4: Diabetes mice treated with 150 mg/kg body weight of PMZL and MMZS separately; Group 5 and 6: Diabetes mice treated with 300 mg/kg body weight of PMZL and MMZS separately; Group 7: Diabetic mice treated with 100 mg/kg body weight of metformin HCL.

Induction of diabetes by alloxan monohydrate

Diabetes was induced in Swiss albino mice by intravenous injection of aqueous alloxan monohydrate at a dose of 70 mg/kg (Kameswara et al., 1997). After two weeks, mice with a blood glucose level more than 11.1 mmol/L were considered for the experiment. To avoid fatal hypoglycemia mice were treated with 30% glucose solution orally at different time intervals after six hours of alloxan induction and 5% glucose solution was kept in the bottle in their cages for the next 24

hours to prevent hypoglycemia.

Collection of blood and determination of serum glucose

Blood samples were collected by cutting the tail veins of mice and blood glucose levels were checked by glucometer.

Determination of body weight

The body weight was determined by using balance (Kitchen scale 1800) at initial (0 day) and final (7th day). Body weight change was calculated by the following formula (Saba et al., 2010).

$$\%\text{Body weight change} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Statistical analysis

All the values (blood sugar, body weight, hypoglycemic change) were expressed as mean ± standard error of mean (SEM). One-way ANOVA followed by Dunnett's test was used to analyze the results of statistical significance.

Results

All extract-treated mice showed no discernible changes up to 1 g/kg by the oral route administration. No mortality or considerable symptoms of toxicity was found. Even no significant change in general behavior was observed in mice up to the study period.

The result of the oral glucose tolerance test shows that the extracts of different doses of plant reduced the fasting blood glucose level ($p<0.0001$) (Table I). Throughout the study of 2 hours, a general reduction of blood glucose level was obtained that meant insulin and drugs had played a role in converting glucose into glycogen stored in the liver and muscle. Starting with nearly same blood glucose level different groups ended in different values. At 15 min after the oral glucose administration all groups reached their peak values. Then the blood glucose level was suppressed at all checked points. The suppression rates of the rise of blood glucose level between 15 and 120 min were 56.2% (150 mg/kg of PMZL), 58.8% (150 mg/kg of MMZS),

Table I

Oral glucose tolerance test of 150 mg/kg and 300 mg/kg body weight of PMZL and MMZL

Group	Blood glucose level (mmol/L)					
	0 min	15 min	30 min	60 min	90 min	120 min
Glucose control	4.7 ± 0.1	15.4 ± 0.2	12.3 ± 0.1	12.3 ± 0.1	10.3 ± 0.1	6.7 ± 0.3
Positive control	4.8 ± 0.1	12.2 ± 0.1	9.3 ± 0.1	7.2 ± 0.1	5.1 ± 0.1	4.2 ± 0.1
PMZL (150 mg/kg)	4.9 ± 0.1	15.2 ± 0.2	11.7 ± 0.1	9.8 ± 0.1	8.0 ± 0.1	6.6 ± 0.1
MMZS (150 mg/kg)	4.6 ± 0.1	15.2 ± 0.1	11.3 ± 0.1	9.1 ± 0.1[a]	7.3 ± 0.1[a]	6.3 ± 0.1
PMZL (300 mg/kg)	4.6 ± 0.1	14.8 ± 0.1	10.6 ± 0.1[a]	8.3 ± 0.1[a]	6.7 ± 0.1[a]	5.7 ± 0.1
MMZS (300 mg/kg)	4.7 ± 0.1	14.4 ± 0.1	10.1 ± 0.1[a]	8.1 ± 0.1[a]	6.4 ± 0.1[a]	5.3 ± 0.1

Values are expressed as mean ± SEM (n = 5); [a]p< 0.0001, when compared with positive control

Table II

Effects of extracts of *Manilkara zapota* on blood glucose level in alloxan (70 mg/kg) induced diabetic mice

Group	Blood glucose level (mmol/L)						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Normal control	5.4 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.8 ± 0.1	5.6 ± 0.1
Diabetic control	12.7 ± 0.1	14.3 ± 0.1	16.4 ± 0.1	17.1 ± 0.1	18.5 ± 0.1	18.7 ± 0.1	18.9 ± 0.3
PMZL (150 mg/kg)	11.6 ± 0.1	10.3 ± 0.1[a]	9.1 ± 0.1[a]	8.5 ± 0.1[a]	7.8 ± 0.1[a]	7.5 ± 0.1[a]	7.3 ± 0.1[a]
MMZS (150 mg/kg)	12.1 ± 0.1	11.1 ± 0.1[a]	10.2 ± 0.1[a]	9.3 ± 0.1[a]	8.6 ± 0.1[a]	7.5 ± 0.1[a]	6.8 ± 0.1[a]
PMZL (300 mg/kg)	12.7 ± 0.1	11.3 ± 0.1[a]	10.4 ± 0.1[a]	8.8 ± 0.1[a]	7.5 ± 0.1[a]	6.7 ± 0.1[a]	6.5 ± 0.1[a]
MMZS (300 mg/kg)	12.6 ± 0.1	10.4 ± 0.1[a]	8.7 ± 0.1[a]	7.7 ± 0.1[a]	6.9 ± 0.1[a]	6.2 ± 0.1[a]	6.0 ± 0.1[a]
Metformin (100 mg/kg)	11.7 ± 0.1	8.3 ± 0.1[a]	6.4 ± 0.1[a]	5.5 ± 0.1[a]	4.5 ± 0.1[a]	4.2 ± 0.1[a]	3.8 ± 0.1[a]

Values are expressed as mean ± SEM (n = 5); [a]p< 0.0001, when compared with diabetic control

Table III

Effect of extracts of *Manilkara zapota* on body weight

Group	Body weight		Weight change (%)
	0 day	7th day	
Normal control	32.7 ± 0.7	34.3 ± 0.7	+4.9
Diabetic control	33.6 ± 0.8	28.5 ± 0.7	-15.3
PMZL (150 mg/kg)	34.4 ± 0.4	31.1 ± 0.6	-9.7
MMZS (150 mg/kg)	32.4 ± 0.4	30.6 ± 0.3	-5.5
PMZL (300 mg/kg)	33.6 ± 0.6	29.7 ± 0.9	-11.7
MMZS (300 mg/kg)	32.8 ± 0.7	29.9 ± 0.7	-8.8
Metformin (100 mg/kg)	35.3 ± 0.5	32.5 ± 0.8	-7.9

Values are expressed as mean ± SEM (n = 5); Negative (-) / Positive (+) signs represent decrease/increase in percent body weight change on the 7th day

61.2% (300 mg/kg of PMZL), 63.2% (300 mg/kg of MMZS) and 65.1% (100 mg/kg of metformin).

All the extracts reduced the blood glucose level in a significant way ($p<0.0001$) (Table II). The data showed that the normal control group had significantly lower blood glucose concentration compared to other groups, indicating the model of diabetic mice had been achieved. The plasma concentration was reduced to a day by day basis for all extracts in 7 days period and the lowest plasma concentration was recorded on the 7th day in the group of mice treated with 300 mg/kg of MMZS. As expected, the standard drug metformin reduced plasma concentration in a rapid and effective way, on the 7th day it became 3.8. There is a slight (3.9%) and dramatic increase (48.2%) of blood glucose level in normal and diabetic control group respectively. Significant glycemic change was monitored as -36.5, -43.6, -48.7, and -52.5% in mice treated with PMZL (150 mg/kg), MMZS (150 mg/kg), PMZL (300 mg/kg) and MMZS (300 mg/kg) respectively. Metformin exerted the highest glycemic change (-67.5%).

All groups except the normal control exhibited weight lose (Table III). The highest weight reduction was obtained in diabetic control group (15.3%). Metformin reduced the weight at a rate of 7.9%. The weight lost percentages were comparably high for both the doses of PMZL than that of MMZS.

Discussion

To search potent and better alternative drugs is the quest of science and scientists. Like past still plant origin is a vast unrevealed source to conduct these assiduous researches. Focusing the demand of natural, effective hypoglycemic drug of plant origin the current study was performed.

The results of oral glucose tolerance test indicated that all the extracts suppressed the rise in BGL after a heavy glucose meal. The outcome may be due to enhancement of gluconeo-genesis, which is characteristically activated at fasting state in diabetes animals or increase disposal of glucose by enhanced insulin sensitivity (Jahan et al., 2009).

All the extracts of *M. zapota* reduced the BGL. As β-cells were destroyed, it is clear that the observed hypoglycemic effect may be due to potential secretion of insulin from the few existing β-cells or there is any extra-pancreating mechanism. It is also possible that increase peripheral glucose utilization helped in blood glucose reduction (Kumavat et al; 2012).

Significant percent glycemic reductions in fasting BGL of all test groups were observed. Significance of p value ($p<0.0001$) was obtained for all plant extract groups. Comparatively seed extracts exhibited more hypoglycemic activity than leaf extracts.

During diabetic condition, body utilized triglycerides as an alternative energy source that is also accompanied by catabolism of tissue protein and results in loss of both fat and lean mass which lead to a significant loss in total body weight. In the study of 7 days period, the body weight reduced for all the extracts except the normal control. The percent changes of body weight loss for the entire test groups were less than the diabetic group as the body weight loss is highest in the diabetic group.

By scrutinizing the whole study, the hypoglycemic property of *M. zapota* (leaves, seeds) is revealed. The potency of the plant is quite competent to the standard drug. Moreover, it is safe and natural. So it can become a suitable alternative to manage diabetes.

References

Awasare S, Bhujbal S, Nanda R. *In vitro* cytotoxic activity of novel oleanane type of triterpenoid saponin from stem bark of *Manilkara zapota* Linn. Asian J Pharm Clin Res. 2012; 5: 183-88.

Belhekar SN, Chaudhari PD, Saryawanshi JS, Mail KK, Pandhare RB. Antidiabetic and antihyperlipidemic effects of *Thespesia populnea* fruit pulp extracts on alloxan-induced diabetic rats. Indian J Pharm Sci. 2013; 75: 217-21.

Bhargavi S, Kanakaiah B, Dantu K, Sowmya B, Ravi S, Nama. An evaluation of the antibacterial activity of root extracts of *Manilkara zapota* against *Satphylococcus aureus* and *Eschericha coli*. IJP 2013; 4: 171-73.

Ecobichon DJ. The basis of toxicology testing. New York, CRC Press, 1997, p 43.

Gomathy K, Baskar R, Kumaresan K. Comparison of antioxidant potential in pulp and peel extracts of *Manilkara zapota* (L.) P. Royen. Afr J Biotechnol. 2013; 12: 4936-43.

Ganguly A, Mahmud ZA, Uddin MMN, Rahman SMA. In-vivo anti-inflammatory and anti-pyretic acticities of *Manilkara zapota* leaves in albino Wistar rats. APJTD. 2013; 3: 301-07.

Harrigan RA, Nathan MS, Beattie P. Oral agents for the treatment of type 2 diabetes mellitus: Pharmacology, toxicity, and treatment. Ann Emerg Med. 2011; 38: 68-78.

Islam MR, Parvin MS, Banu MR, Jahan N, Das N, Islam ME. Antibacterial and phytochemical screening of ethanol extracts of *Manilkara zapota* leaves and bark. Int J Pharma Sci. 2013; 3: 394-97.

Jamuna KS, Ramesh CK, Srinivasa TR, Raghu KL. *In vitro* antioxidant studies in some common fruits. Int J Pharm Pharm Sci. 2011; 31: 60-63.

Jahan S, Saeed N, Ijlal F, Khan MA, Ahmad M, Zafar M, et al. Histomorphological study to evaluate anti-fertility effect of *Abrus precatories* L. in adult male mice. J Med Plants Res. 2009; 3: 1029-33.

Jain PK, Soni P, Upmanyu N, Shivhare Y. Evaluation of analgesic activity of *Manilkara zapota* (leaves). Eur J Exp Biol. 2011; 1: 14-17.

Kaneria M, Chanda S. Evaluation of anti-oxidant and antimicrobial properties of *Manilkara zapota* L. (chiku) leaves by sequential soxhlet extraction method. APJTB. 2012; 1526-33.

Kumar YDR, Hema V, Agrawal M, Sruthy PC, Vedamurthy AB, Krishna V, et al. *Manilkara zapota* seed embryo extract: A potent anthelminthic agent. Asian J Pharm Clin Res. 2012; 5: 159-61.

Kameswara RB, Kesavulu MM, Giri R, Apparao CH. Hypoglycemic and hypolipidemic effects of *Momordica cymbalaria* Hook. Fruit powder in alloxan-induced diabetes rats. J Ethnopharmacol. 1997; 58: 149-55.

Kumavat UC, Shimpi SN, Jagdale SP. Evaluation of hypoglycaemic activity of *Cassia nodosa* leaves in normal and streptozotocin-induced diabetic rats. Int J Green Pharm. 2012; 6: 9-13.

Mohanapriya C, Uma S, Modilal RD, Nithyalakshmi V. Phytochemical screening and *in vitro* anti-oxidant studies on acetone extract of *Manilkara zapota* L. seeds. IJPSR. 2014; 5: 2354-61.

Nair R, Chanda S. Antimicrobial activity of *Terminalia catappa, Manilkara zapota* and Piper betel leaf extract. Indian J Pharm Sci. 2008; 70: 390-93.

Osman MA, Aziz MA, Habib MR, Karim MR. Antimicrobial investigation on *Manilkara zapota* (L.) P. Royen. Int J Drug Dev Res. 2011; 3: 185-90.

Osman MA, Aziz MA, Habib MR, Karim MR. Pesticidal evaluation of *Manilkara zapota* (L.) against *Tribolium castaneum* (Herbest). Agric Conspec Sci. 2011; 76: 143-46.

Priya P, Shoba FG, Parimala M, Sathya J. Anti-oxidant and antibacterial properties of *Manilkara zapota* (L.) Royen flower. IJPCR. 2014; 6: 174-78.

Singh S, Bothara SB. *Manilkara zapota* (Linn.) seeds: A potent source of natural gum. ISRN Pharmaceutics. 2014.

Saba AB, Oyagbemi AA, Azeez OI. Antidiabetic and haematinic effects of *Paraquetina nigrescens* on alloxan-induced type-1 diabetes and normocytic normochromic anaemia in Wistar rats. Afr Health Sci. 2010; 10: 276-82.

Singh M, Soni P, Upmanyu N, Shivhare Y. *In vitro* anti-arthritic activity of *Manilkara zapota* Linn. Asian J Pharm Tech. 2011; 1: 123-24.

Tiwari S. Plant: A rich source of herbal medicine. J Nat Prod. 2008; 1: 27-35.

Woo PF, Yim HS, Khoo HE, Sia CM, Ang YK. Effects of extraction conditions on anti-oxidant properties of sapodilla fruit (*Manilkara zapota*). IFRJ. 2013; 20: 2065-72.

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