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Research Article

BENEFICIAL EFFECT OF ETHONOLIC EXTRACT OF BAEL FRUIT IN COMBINATION WITH METFORMIN HCL IN NORMAL RABBITS

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ABSTRACT

Diabetes mellitus is a chronic disease, Metformin an allopathic drug used in the treatment of diabetes as it causes side effects to reduce such side effects ethonolic bael extract a herbal drug which has no side effects is used with combination of metformin. The influence of single drug and multiple drug effect of the selected drugs on hypoglycemic activity were studied in normal rabbits. Rabbits were selected as suitable animal models for the study of the above parameters since adequate quantities of blood samples can be collected at the desired intervals of time. The selection of the rabbits in the study also represents non-rodent model apart from ease of collection of sufficient volume of blood sample. In this study we perform about the hypoglycemic effect of the ethonalic bael extract and metformin hydrochloride, individually and in combination in normal rabbits. Here we maintained same group of animals with appropriate wash out periods.

Keywords: Diabetes mellitus, Ethanolic bael extract, Hypoglycemia, Metformin hydrochloride.

INTRODUCTION

DIABETES MELLITUS

Diabetes mellitus, often simply referred to as **diabetes**—is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

There are three main types of diabetes

Type 1 Diabetes

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack.[1]

There is no known preventive measure against type 1 diabetes, which causes approximately 5%-10% of diabetes mellitus cases. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children.

Type 2 Diabetes

Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus due to a known defect are classified separately. Type 2 diabetes is the most common type. There is no known preventive measure against type 1 diabetes, which causes approximately 90%-95% of diabetes mellitus cases. In the early stage of type 2 diabetes, the predominant abnormality is reduced insulin sensitivity. At this stage hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

Aegle Marmelos (Rutaceae) is a popular medicinal plant in the Ayurvedic and Siddha systems of medicine and folk medicines used to treat a wide variety of ailments. The plant, popularly known as the bael tree, is native to the Indo-Malayan region and is currently cultivated in India, Pakistan, Bangladesh, Sri Lanka, Burma, and Thailand. The tree is a slender, aromatic perennial, 6.0–7.5 m tall and 90–120 cm in girth. It flowers from May to July and yields an annual average of 300–400 fruits (200–250 kg) per tree. Various parts of the tree, including the fruit, possess medicinal properties. The aqueous extract of leaf possesses a hypoglycemic effect. The incidence of diabetes is increasing. The effect of an aqueous extract of *Aegle marmelos* fruits on serum and tissue lipids in experimental diabetes.

Metformin is an oral ant diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function.[1][2][3] Evidence is also mounting for its efficacy in gestational diabetes, although safety concerns still preclude its widespread use in this setting. It is also used in the treatment of polycystic ovary syndrome, and has been investigated for other diseases where insulin resistance may be an important factor.

Metformin is primarily used for type 2 diabetes however is increasingly being used in polycystic ovary syndrome (PCOS),[10] non-alcoholic fatty liver disease (NAFLD)and premature puberty, three other diseases that feature insulin resistance; these indications are still considered experimental. The benefit of metformin in NAFLD has not been extensively studied and may be only temporary; although some randomized controlled trials have found significant improvement with its use, the evidence is still insufficient.

MATERIALS AND METHODS

The following drugs and other chemicals were used in the present study.

Metformin Hydrochloride Tablets 250mg	:	USV Pvt Ltd, Batch No (28004506)
Aegle marmelos(Bael tree) extract	:	Dr.O.P.Gouda, Maratha Mandal's College of pharmacy, Belgum, KARNATAKA.
Triple distilled water	:	Prepared in the laboratory
Cellulo se nitrate filters (0.45 μ)	:	Millipore, Bengaluru.
Blood Glucose Diagnoising kit	:	EUKARE Blood glucose testing kit

Animals

Albino rabbits of either sex weighing between 1.30 and 1.75 kg obtained from the animal house were used in the study. All the rabbits were maintained on uniform weight of leafy vegetable food and water. The animals were maintained at 25±1 ^{0}C in 12h/12h light/dark cycle. All the animals were fasted for 18 h before the experiment and water was withdrawn during the experiment.

Blood Glucose Testing Meter

The Eukare blood glucose testing strip is to be used with the EUKare blood glucometer to test glucose levels in the whole blood. When the blood sample is in contact with blood sample inlet of the test strip, it will flow in to the reaction area automatically and start the test. Only small amount of blood is needed (3µ/ one test). The testing range of blood glucose is 30-600mg/dl.

Testing Principles

1. Through the capillary action, the sample will be drawn into the reaction area from the inlet on the side of the test strip.
2. After filling up the reaction area, the sample will start automatically at the end of the in let
3. The sample amount under accurate control.
4. The test strip is an electro chemical detector after blood glucose reacts with the enzymes on the strip, electron produced from the reaction will accumulate on the surface.
5. Then the blood glucose meter releases an electric current at a regular voltage, the quantity of electrons accumulated on the surface of the test strip will be detected.
6. The current detected will be converted in to a special formula built inside the blood glucose meter to calculate the blood glucose level in the sample.

Testing Procedure

1. Insert the test strip into the test slot of the blood glucose meter. The meter will start automatically and show the current strip code
2. Prick the ear vein with the needle and remove the first blood drop and apply the second drop of blood to the strip from the red sample inlet. Through the capillary action , the sample compartment will be filled with 3µ 1 of blood and cover the reaction area a beep sound will let you know that the test has begun.
3. In 10 seconds, the blood glucose value will appear on the blood glucose meter and automatically store in the memory. After removing the strip the machine will turn off automatically.

Preparation of Drug Solutions

Bael solution

250mg /kg body weight of bael extract was weighed accurately and was dissolved in a 5ml/kg body weight using distilled water.

Metformin Hcl Tablet Solution

Rabbit Dose = Human therapeutic dose * Conversion factor (0.07)

The therapeutic dose of metformin for rabbit is reduced to ten times less because of considering the safety of rabbits. The dose was calculated according to equivalent factor of metformin hydrochloride tablets 11.16mg/kg body weight of tablet powder was dissolved in 5ml/kg body weight using distilled water.

Administration of Drugs

The drugs were administered by oral route with the help of oral feeding tube as described below. The rabbit was fixed in rabbit holder with its head protruding outside. Then a mouth gag was introduced in between the two jaws and held in position by holding the upper jaw and lower jaw with left hand. Infant feeding tube dipped in glycerin was introduced into the mouth through the central hole of the mouth gag. The tube was pushed slowly such that it enters the oesophagus and reaches the stomach. To the other end of the tube a syringe was attached. After administering 2 ml of distilled water to ensure free flow into the stomach, the required quantity of the drug solution was administered through the syringe and feeding tube. This was followed by 5 ml of distilled water to ensure the administration of the dose of the drug accurately. The tube was removed gently.

Single Drug Treatment with the Selected Drug (SD)

All the four rabbits were observed in single group. The experiment was conducted in four stages with one week washout period between each stage.

Stage-I Rabbits were administered with vehicle control and blood samples were collected at different time intervals (0-24hrs) and were estimated for blood glucose. These results were used as control. After this stage seven days washout period has been maintained and the rabbits were utilized for further experiment.

Stage-II Rabbits were treated with dose of Bael extract and blood samples were collected and analyzed for blood glucose serum levels. After this stage seven days washout period has been maintained and the rabbits were utilized for further experiment.

Stage-III After seven days washout period, the same groups of animals were treated with dose of metformin. After this stage seven days washout period has been maintained and the rabbits were utilized for further experiment.

Stage-IV After seven days washout period, the animals were treated with combination of drugs in individual dose with 30mins gap of time.

Collection of Blood Samples

The blood samples were collected by pricking the marginal ear vein. The veins were dilated by warming the ears on a low voltage lamp or by rubbing. The marginal ear vein was punctured with a surgical needle opposite to the direction of blood flow. The blood samples were collected in predose sample and15min, 30min, 1,2,4,6,8,12 and 24 h after drug administration. The blood samples were collected into clean disposable centrifuge tubes. The blood samples were analyzed immediately for blood glucose levels using glucometer.

RESULTS

Phase 0

The glyceimic levels of the rabbit with vehicle (water) used in experiment are observed.

The average mean & standard deviation values are mentioned in table number 1.0(A)

The percentage reduction of blood glucose levels are mentioned in Table number 1.0(B)

Phase 1

The glyceimic levels changes in the rabbit with Dose of bael fruit extract used are are observed.

The average mean& standard deviation values are mentioned in table number 1.1(A)

The percentage reduction of blood glucose levels are mentioned in Table number 1.1(B)

Phase **2**

The glyceimic levels changes in the rabbit with Dose of Metformin tablet powder used in experiment are are observed.

The average mean & standard deviation values are mentioned in table number 1.2(A)

The percentage reduction of blood glucose levels are mentioned in Table number 1.2(B)

Phase 3

The glyceimic levels changes in the rabbit with Dose of Metformin tablet powder and bael extract used in experiment are observed.

The average mean & standard deviation values are mentioned in table number

The percentage reduction of blood glucose levels are mentioned in Table number 1.3(A)

Reduction % Values of phase 0

Time(hr)	R1	R2	R3	R4	Mean	±	SD
0	0	0	0	0	0.00	±	0.00
0.25	-3.45	2.33	-2.10	-3.06	-1.57	±	2.66
0.5	-1.72	6.20	0.00	0.00	1.12	±	3.48
1	0.00	2.33	0.70	-2.04	0.25	±	1.81
2	-4.31	3.10	2.80	-3.06	-0.37	±	3.87
4	-2.59	3.88	1.40	-6.12	**-0.86**	±	4.40
6	-1.72	2.33	0.70	-8.16	-1.72	±	4.61
8	-2.59	0.78	-0.70	-4.08	-1.65	±	2.13
12	-3.45	2.33	0.70	-5.10	-1.38	±	3.47
24	-2.59	3.88	0.00	-3.06	-0.44	±	3.18

Table no: 1.0(A)

Reduction% Values of phase 1

Time(hr)	R1	R2	R3	R4	Mean	±	SD
0	0	0	0	0	0.00	±	0.00
0.25	-72.41	-8.53	5.59	-54.08	-32.36	±	36.90
0.5	-50.86	8.53	-25.87	33.67	-8.63	±	37.26
1	-47.41	5.43	-11.89	-39.80	-23.42	±	24.56
2	-12.93	14.73	6.29	-16.33	-2.06	±	14.98
4	-12.07	17.05	13.29	-3.06	**3.80**	±	13.72
6	3.45	19.38	7.69	-33.67	-0.79	±	22.94
8	1.72	17.83	11.89	-8.16	5.82	±	11.45
12	7.76	-5.43	17.48	-13.27	1.64	±	13.67
24	5.17	3.88	21.68	-3.06	6.92	±	10.48

Table no: 1.1(A)

Reduction% Values of phase 2

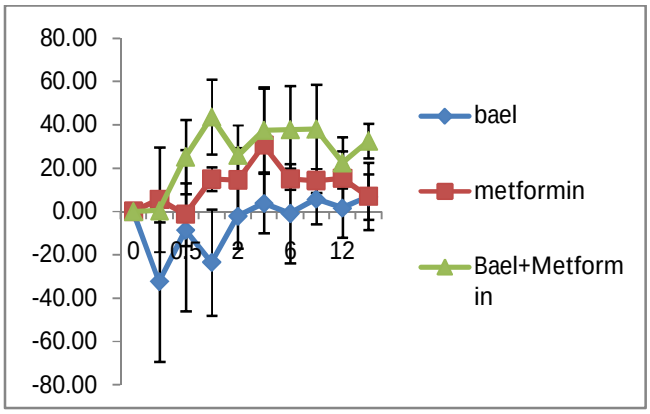
Time(hr)	R1	R2	R3	R4	Mean	±	SD
0	0	0	0	0	0.00	±	0.00
0.25	12.16	32.62	3.17	-25.62	5.59	±	24.18
0.5	1.35	17.73	-15.87	-8.26	-1.26	±	14.49
1	19.59	13.48	7.94	19.01	15.00	±	5.46
2	14.86	34.04	-1.59	11.57	14.72	±	14.71
4	43.92	12.06	61.90	4.96	**30.71**	±	26.82
6	19.59	14.89	17.46	8.26	15.05	±	4.92
8	22.30	11.35	13.49	9.92	14.26	±	5.55
12	22.30	14.18	26.98	-1.65	15.45	±	12.57
24	14.19	4.26	23.02	-13.22	7.06	±	15.54

Table no: 1.2(B)

Reduction% Values of phase 3

Time (hr)	**R1**	**R2**	**R3**	**R4**	**Mean**	**±**	**SD**
0	0	0	0	0	0.00	±	0.00
0.25	-0.69	8.39	-2.43	-3.50	0.44	±	5.42
0.5	5.52	27.97	20.87	46.85	25.30	±	17.15
1	31.03	68.53	32.52	42.66	43.69	±	17.35
2	13.79	15.38	42.72	32.17	26.02	±	13.90
4	18.62	29.37	64.08	37.76	37.46	±	19.40
6	24.14	21.68	66.02	39.16	37.75	±	20.37
8	23.45	23.08	66.99	38.46	37.99	±	20.62
12	9.66	18.18	37.38	25.17	22.60	±	11.72
24	31.03	26.57	44.17	28.67	32.61	±	7.92

Table no: 1.3(B)



Graphical representation of comparison of% reduction of blood sugar levels of the rabbits

DISCUSSION

Diabetes Mellitus is a chronic disorder demands lifelong medication to maintain the body's glucose homeostasis with the aid of single/multiple drug therapy. It is well known that the allopathic medications on long run usage tend to cause side effects and even in some cases they proved to worsen the condition. Hence there is growing interest and demand for the herbal drugs as supplements to the therapy because of their no or less side effects even in chronic administration. Apart from their fewer side effects they usually posses multiple beneficiary effects such as antioxidant and anti-inflammatory etc. Hence in many parts of the rural areas of india it has become practice to use the herbal drugs in combination with allopathic drug with or without the consultation of the physicians for many other ailing conditions. The present study focused to give sufficient scientific evidence in the usage of ethonolic extract of bael fruit in combination with metformin in normal rabbits as a preliminary investigation. The ethonolic extraction of *Aegle marmelos* has no hypoglycemic effect in the rabbits. Metformin an allopathic drug had shown a considerable amount of blood glucose reduction in normal rabbits.

When ethonolic extract of bael fruit was administered in combination with metformin quite interestingly it shown synergistic action of metformin and even it had shown the sustained activity by increasing the rate of absorption of metformin up to 24 hrs.

CONCLUSION

The present study in normal rabbits reveals that the ethonolic extract of bael fruit haven't shown any effect on blood glucose level on its own, but when given in combination with metformin, it tend to enhance its effect and even extended its therapeutic value. So from the present preliminary study we can conclude that, ethonolic extract of bael fruit is useful in combination with metformin in chronic therapy like diabetes mellitus by which one can reduce the dose of metformin required to maintain glucose homeostasis alone or in combination with other antihyperglycaemic agents. To validate its beneficiary effect in combination with metformin in diabetic animal models may prove it's worthy to be used in long run.

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