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Complementary and Comparative Study on Hypoglycemic and Antihyperglycemic Activity of Various Extracts of *Eugenia jambolana* Seed, *Momordica charantia* Fruits, *Gymnema sylvestre*, and *Trigonella foenum graecum* Seeds in Rats

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Abstract In present study, we investigated hypoglycemic and antihyperglycemic potential of five extracts (water, ethanol, methanol, hexane, and chloroform) of four plants (i.e., seeds of *Eugenia jambolana*, fruits of *Momordica charantia*, leaves of *Gymnema sylvestre*, and seeds of *Trigonella foenum graecum*) alone and/or in combination with glimepiride in rats. Ethanol extract of *E. jambolana*, water extract of *M. charantia*, ethanol extract of *G. sylvestre*, and water extract of *T. graecum* exhibited highest hypoglycemic and antihyperglycemic activity (most active) in rats among all the extracts, while hexane extracts exhibited least activities. Most active extracts were further studied to dose-dependent (200, 100, and 50 mg/kg body weight (bw)) hypoglycemic and antihyperglycemic effects alone and in combination with glimepiride (20, 10, and 5 mg/kg bw). The combination of most active extracts (200 mg/kg bw) and lower dose of glimepiride (5 mg/kg bw) showed safer and potent hypoglycemic as well as antihyperglycemic activities without creating severe hypoglycemia in normal rats, while higher doses (200 mg/kg bw of most active extracts, and 10 and 20 mg/kg bw of glimepiride) were generated lethal hypoglycemia in normal rats. From this study, it may be

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concluded that the ethanol extract of *E. jambolana* seeds, water extract of *M. charantia* fruits, ethanol extract of *G. sylvestre* leaves, and water extract of *T. graecum* seeds have higher hypoglycemic and antihyperglycemic potential and may use as complementary medicine to treat the diabetic population by significantly reducing dose of standard drugs.

Keywords *Eugenia jambolana* · *Momordica charantia* · *Gymnema sylvestre* · *Trigonella foenum graecum* · Hypoglycemia · Glucose-infused diabetes · Herbal · Glimepiride

Introduction

Diabetes mellitus is a metabolic-cum-vascular syndrome of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in either insulin production or its action and/or both [1]. This disorder is frequently associated with long-term damage, which can lead to failure of organs like, eyes, kidneys, nerves, heart, and blood vessels [2, 3]. In recent years, India has witnessed a rapidly exploding epidemic of diabetes [4, 5]. Indeed, India, today, leads the world with its largest number of diabetics in any other country. World Health Organization estimated that there are 32 million people with diabetes in India in 2000, which is projected to rise to 80 million by the year of 2030 [6, 7]. With a long course and serious complications often resulting in high death rate, the treatment of diabetes spent vast amounts of resources including medicines, diets, physical training, and so on in all countries. Therefore, it is very important to search new therapeutic strategies which might be cheaper, safe, and convenient for treatment of diabetes.

In India, China, and other countries, use of herbal medicines is very common practice from ancient time, and it is considered as much safer and less expensive therapeutic strategies for treatment of various diseases [5, 7]. The use of herbal medicines for treatment of diabetes has been reported long ago. There is plethora of literature that is available for antidiabetic herbal plants [8, 9], but it is very rare to isolate and characterize a novel antidiabetic compound from these plants. Thereby, it is an urgent need to initiate very well-planned studies to isolate novel antidiabetic components from plants. Hence, in present study, we selected four medicinal plants named *Eugenia jambolana* (Myrtaceae family), *Momordica charantia* (Cucurbitaceae family), *Gymnema sylvestre* (Asclepiadaceae family), and *Trigonella foenum graecum* (Leguminaceae family), which are well-known antidiabetic plants [10–17]. Although a surfeit of literature is available for antidiabetic potential of these plants, the systemic studies has not been performed for their comparative and complementary effects with standard antidiabetic medicines. Therefore, the main objective of this study was to explore best active hypoglycemic and antihyperglycemic extracts and their complementary effects with standard antidiabetic medicine named glimepiride.

Materials and Methods

Preparation of Extracts

Seeds of *E. jambolana*, fruits of *M. charantia*, and seeds of *T. graecum* were purchased from Gwalior market, and leaves of *G. sylvestre* were collected from Jiwaji University garden. All plant parts were verified with a botanist and air-dried before pounding into the powder. The powder was extracted with different polar (water, ethanol, and methanol) and nonpolar

(hexane and chloroform) solvents in soxhlet for 24 h. Organic solvents were evaporated under low pressure, and aqueous solvent (water) was evaporated by lyophilization.

Determination of Phytoconstituents

The chemical composition of total phenols, alkaloids, tannins, saponins, and flavonoids in different extracts of each plant were determined by methods described elsewhere [18].

Animals and Experimental Schedule

Male Wistar rats of 4–6 weeks old (122–128 g body weight (bw)) were housed in polypropylene cages at 22±3°C ambient temperature and 55±5% humidity in 12/12 light and dark cycle. This study was completed in two phases: phase 1: the best hypoglycemic extract was selected by orally injecting 200 mg/kg bw dose of each extract in 12-h-fasted normal animals. To study antihyperglycemic activity, 2 mg/kg bw (20% solution) dose of glucose was administered to 12-h-fasted animals (for induction of hyperglycemia) at the same time of extract ingestion. Phase 2: The most active hypoglycemic and antihyperglycemic extracts among other extracts were further studied for dose-dependent effects and its drug interactions with standard antidiabetic medicine, i.e., glimepiride. Three different doses of most active extract (200, 100, and 50 mg/kg bw) and glimepiride (20, 10, and 5 mg/kg bw) were tested for hypoglycemic and antihyperglycemic potential in both normal as well as glucose-overloaded rats.

Estimations of Blood Glucose

After oral administration of plant extracts and/or standard drug, the blood samples were collected from tail tip, and glucose was monitored using glucometer strips (Roche Diagnostics, Indiana, USA) at 1-h interval for 6 h.

Statistical Analysis

Data were represented as means ± standard deviation (SD) of six animals in each group, and analysis of variance was performed by using SPSS (SPSS Inc. Chicago). The significant differences among groups were analyzed with the help of multiple comparisons Student's two-tailed t test. The values with $p<0.05$ were considered statistically significant.

Results

Phytoconstituent in Different Extracts

All plant extracts of *E. jambolana* seeds were evaluated for different phytoconstituents, i.e., tannins, phenols, alkaloids, flavonoids, and saponin contents (Table 1). Alkaloid contents were higher in water, methanol, hexane, and chloroform extracts, while flavonoids and saponins were significantly higher in water, ethanol, and methanol. No change was observed in phenol and tannin contents among different extracts of *E. jambolana* seeds.

Alkaloids were highest in water extracts of *M. charantia* fruits, while it was moderately higher in ethanol and methanol extracts than those of hexane and chloroform extracts. Again, phenols, tannins, flavonoids, and saponins were significantly increased in water

Table 1 Phytoconstituents in various extracts of different parts of four plants (*E. jambolana*, *M. charantia*, *G. sylvestre*, and *T. graecum*)

Phytoconstituent(s)	Water extract	Ethanol extract	Methanol extract	Hexane extract	Chloroform extract
E. jambolana					
Alkaloids (%)	5.69±0.13[a]	0.44±0.12[a]	3.56±0.10[b]	4.84±0.18[c]	3.40±0.21[d]
Phenols (%)	0.48±0.09[a]	0.49±0.16[a]	0.59±0.12[a]	0.55±0.13[a]	0.53±0.10[a]
Tannins (%)	1.21±0.14[a]	1.43±0.34[a]	1.12±1.3[a]	2.02±1.30[a]	1.43±0.50[a]
Flavonoids (%)	7.42±0.26[a]	12.22±0.32[a]	3.53±0.08[a]	2.34±0.14[b]	1.55±0.16[c]
Saponin (%)	6.77±0.76[a]	5.57±0.54[b]	1.43±0.46[b]	0.43±0.54[c]	0.77±0.33[c]
M. charantia					
Alkaloids (%)	15.59±0.28[a]	9.44±0.22[b]	5.50±0.29[b]	2.34±0.19[c]	1.69±0.15[d]
Phenols (%)	5.47±0.08[a]	3.46±0.16[b]	2.39±0.18[c]	1.55±0.13[d]	1.43±0.14[d]
Tannins (%)	6.21±0.24[a]	7.03±0.52[a]	2.12±0.5[c]	2.32±0.32[c]	4.40±0.46[a]
Flavonoids (%)	2.02±0.46[a]	1.20±0.38[a]	1.50±0.16[a]	2.34±0.21[b]	3.55±0.14[c]
Saponin (%)	8.77±0.76[a]	5.57±0.54[b]	1.43±0.46[b]	0.43±0.54[c]	0.77±0.33[c]
G. sylvestre					
Alkaloids (%)	6.59±0.29[a]	4.40±0.20[a]	4.50±0.39[b]	2.34±0.19[c]	1.79±0.10[d]
Phenols (%)	5.47±0.18[a]	3.48±0.16[a]	2.30±0.18[a]	1.50±0.13[a]	1.43±0.14[a]
Tannins (%)	6.22±1.26[a]	7.40±0.50[b]	2.10 ±1.80[c]	2.32±1.30[d]	1.40±0.40[d]
Flavonoids (%)	2.01±0.46[a]	1.10±0.38[a]	1.52±0.18[a]	2.30±0.20[b]	3.50±0.56[c]
Saponin (%)	10.85±0.86[a]	16.87±0.64[b]	7.45±0.48[b]	3.43±0.56[c]	1.78±0.35[c]
T. graecum					
Alkaloids (%)	0.59±0.13[a]	0.44±0.12[a]	5.56±0.19[b]	7.34±0.15[c]	8.49±0.11[d]
Phenols (%)	0.47±0.08[a]	0.46±0.06[a]	0.39±0.10[a]	0.55±0.13[a]	0.43±0.09[a]
Tannins (%)	21.21±0.24[a]	11.43±0.54[b]	5.12±1.3[c]	2.32±1.32[d]	1.43±0.56[d]
Flavonoids (%)	1.42±0.36[a]	1.22±0.32[a]	1.53±0.06[a]	4.34±0.11[b]	3.55±0.14[c]
Saponin (%)	6.77±0.76[a]	1.57±0.54[b]	1.43±0.46[b]	0.43±0.54[c]	0.77±0.33[c]

Values are means ± SD of three independent measurements of each extract. Values with different superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$

extract of *M. charantia* fruits as compared to all other four extracts. Similarly, alkaloid, phenols, tannins, and flavonoids were significantly increased in water extract of *G. sylvestre*, except saponins that was higher in ethanol extract of this plant. Interestingly, alkaloid and flavonoids were significantly higher in methanol, hexane, and chloroform extracts of *T. graecum* seeds, while tannins and saponins were significantly higher in water extracts than those of the other solvent extracts of *T. graecum*.

Hypoglycemic and Antihyperglycemic Extract from *E. jambolana*

While evaluating hypoglycemic and antihyperglycemic activities of *E. jambolana* seed extracts, it has been found that ethanol extract was most active for these activities among all other extracts (Fig. 1a, b). The oral administration of ethanol extract reduced 41% and 44% blood glucose in normal rats after 1 and 2 h, respectively, and after 3 h, blood glucose increased, and values became normal (similar to 0 h) after 4 h, but we continued to measure blood glucose up to 6 h to see any episodic hypoglycemic effects of extracts after their oral ingestion. Water extract of *E. jambolana* seeds also reduced blood glucose by 26% and

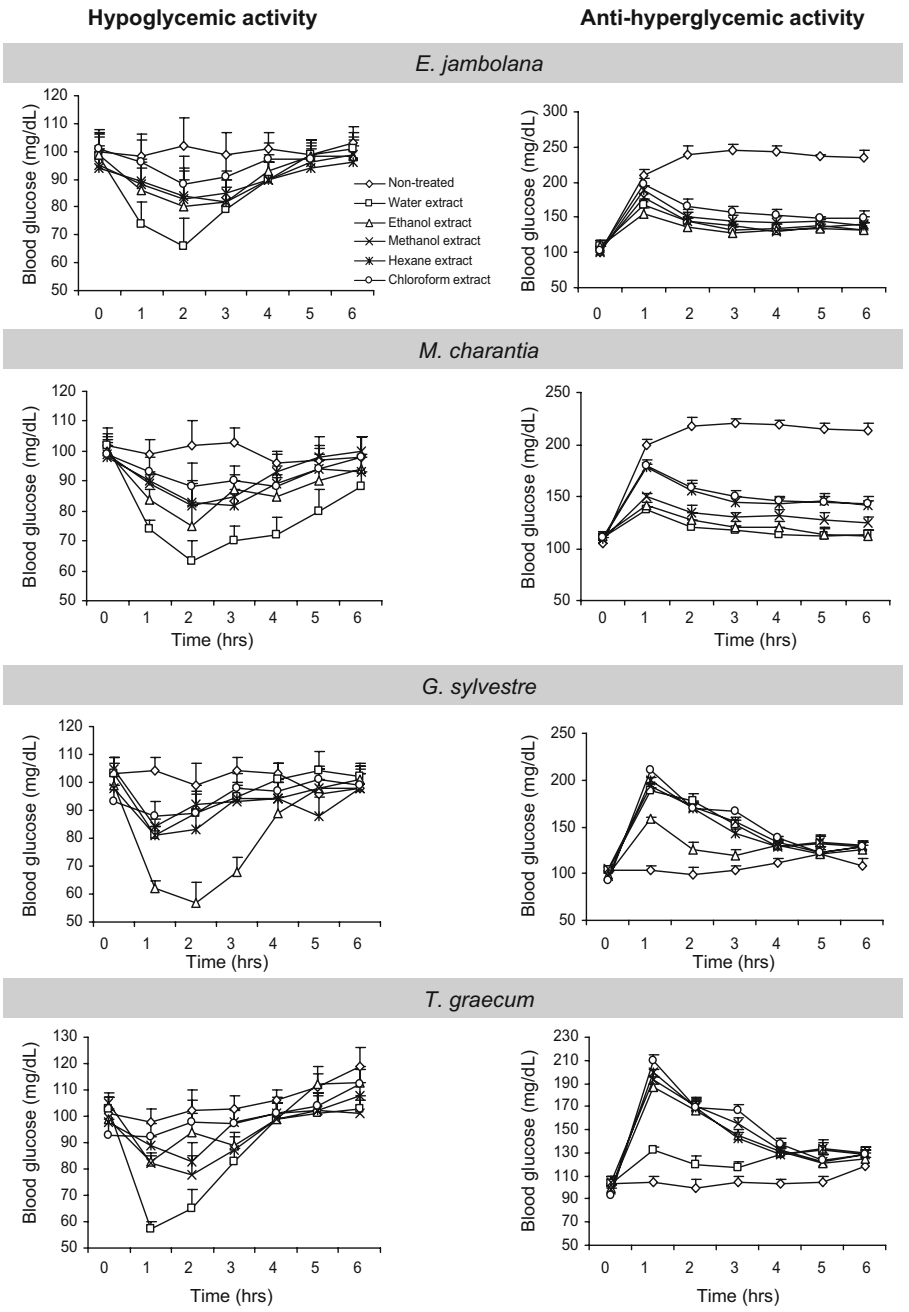

Fig. 1 Hypoglycemic and antihyperglycemic activities of various extracts

27% after 1 and 2 h, respectively, while methanol extracts moderately reduced (14% and 15% after 1 and 2 h, respectively) in the normal rats. No significant changes were observed in blood glucose values in animals ingested with hexane as well as chloroform extracts during whole 6-h screening period. Similarly, ethanol extract of *E. jambolana* inhibited

~40% increase in blood glucose levels in glucose-infused animals after 1 h and followed by the similar trend for other extracts as hypoglycemic potential (Fig. 1b).

Hypoglycemic and Antihyperglycemic Potential of *M. charantia* Extracts

Results in Fig. 1c, d show that water extract of *M. charantia* fruits exhibited highest hypoglycemic and antihyperglycemic effects in normal rats among all five extracts. It has been observed that water extract of *M. charantia* fruits reduced 39% and 40%, respectively, blood glucose in normal rats after 1 and 2 h, and also it inhibited 38% and 41% increased blood glucose levels in glucose-infused animals. Other extracts of *M. charantia* showed moderate and least hypoglycemic and antihyperglycemic activities in normal and glucose-infused rats.

Hypoglycemic and Antihyperglycemic Potential of *G. sylvestre* Extracts

Ethanol extract of *G. sylvestre* exhibited highest hypoglycemic and antihyperglycemic potential, and it has shown that this extract reduced 46% and 36% blood glucose levels in normal and glucose-infused rats, respectively, after 2 h of oral administration (Fig. 1e, f). Water extract also reduced 26% blood glucose levels in normal rats after 2 h, while methanol extract moderately reduced it (12%) in normal rats. No significant changes were observed in blood glucose values of animals ingested with hexane as well as chloroform extracts during whole experimental period.

Hypoglycemic and Antihyperglycemic Potential of *T. graecum* Extracts

Water extract of *T. graecum* exhibited hypoglycemic and antihyperglycemic potential in normal and glucose-infused rats (Fig. 1g, h). Results indicate that the oral administration of water extract of *T. graecum* seeds reduced 41% and 37% blood glucose in normal and glucose-infused rats, respectively, at 2 h. Ethanol extract of *T. graecum* seeds also reduced blood glucose by 25% after 2 h in the normal rats, while methanol extracts was reduced 13% at 2 h of screening period. Similar trend followed for antihyperglycemic potential of *T. graecum* extracts in glucose-infused rats.

Complementary Hypoglycemic and Antihyperglycemic Activity of Most Active Extracts from Different Plants with Glimepiride

Hypoglycemic and antihyperglycemic potential of most active extracts from all four plants (ethanol extract from *E. jambolana*, water extract from *M. charantia*, ethanol extract of *G. sylvestre*, and water extract of *T. graecum*) were tested for dose dependency (50–200 mg/kg body weight). It can be clearly see in Tables 2, 3, 4, and 5 that the highest dose (200 mg/kg body weight) of all the extracts was most effective to hypoglycemic and antihyperglycemic potential in normal and glucose-infused (hyperglycemic) rats. When we tested higher dose of these most active extracts with and without three different doses of glimepiride (5,10, and 20 mg/kg body weight), we found that combination of these most active extracts significantly reduced the dose of glimepiride for its hypoglycemic and antihyperglycemic potential. Results show that combination of 200 mg/kg body weight of most active extracts and 10 and 20 mg/kg body weight of glimepiride produced severe hypoglycemia in normal mice, while combination of 200 mg/kg body weight of most active extracts along with 5 mg/kg body weight of glimepiride exhibited very potential hypoglycemia and antihyperglycemic activities without producing hypoglycemic state.

Table 2 Complementary hypoglycemic and antihyperglycemic potential of ethanol extract of *E. jambolana* seeds and glimepiride in normal and glucose-infused rats

Groups	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Normal rats							
Ethanol extract							
200 mg/kg bw	98 ± 8.5^{aA}	78 ± 12.5^{bA}	82 ± 12.4^{bA}	88 ± 13.3^{bA}	90 ± 12.3^{bA}	94 ± 14.5^{aA}	95 ± 13.2^{aA}
100 mg/kg bw	93 ± 7.9^{aA}	77 ± 13.4^{bB}	85 ± 16.2^{bB}	87 ± 14.5^{bB}	92 ± 11.8^{aA}	91 ± 14.5^{aA}	94 ± 10.8^{aA}
50 mg/kg bw	99 ± 14.3^{aA}	90 ± 10.5^{bB}	86 ± 11.1^{bB}	89 ± 13.8^{bB}	91 ± 10.9^{aA}	98 ± 12.2^{aA}	96 ± 14.5^{aA}
Glimepiride							
20 mg/kg bw	97 ± 10.5^{aA}	78 ± 10.5^{bA}	86 ± 12.4^{bA}	91 ± 10.7^{bA}	95 ± 10.0^{aA}	94 ± 9.8^{aA}	93 ± 15.4^{aA}
10 mg/kg bw	95 ± 11.7^{aA}	83 ± 10.3^{bA}	89 ± 12.2^{bB}	94 ± 10.9^{aA}	90 ± 14.1^{aA}	95 ± 11.3^{aA}	96 ± 15.2^{aA}
5 mg/kg bw	96 ± 12.3^{aA}	89 ± 14.5^{bB}	90 ± 11.4^{bB}	92 ± 10.5^{bA}	94 ± 12.2^{aA}	95 ± 10.4^{aA}	94 ± 13.3^{aA}
Ethanol extract + glimepiride							
200+20 mg/kg bw	95 ± 11.8^{aA}	57 ± 15.2^{bA}	30 ± 13.5^{cA}	ND	ND	ND	ND
200+10 mg/kg bw	92 ± 8.7^{aA}	65 ± 12.9^{bA}	33 ± 13.9^{cA}	ND	ND	ND	ND
200+5 mg/kg bw	98 ± 9.8^{aA}	76 ± 17.9^{bA}	38 ± 15.9^{cA}	42 ± 14.5^{c}	53 ± 16.5^{c}	84 ± 10.2^{b}	99 ± 12.4^{aA}
Hyperglycemic rats							
Ethanol extract							
200 mg/kg bw	108 ± 12.4^{aA}	158 ± 17.5^{bA}	132 ± 10.2^{bA}	133 ± 12.3^{bA}	138 ± 11.9^{cA}	130 ± 9.3^{dA}	131 ± 15.6^{aA}
100 mg/kg bw	110 ± 12.8^{aA}	165 ± 15.4^{bB}	135 ± 12.8^{bB}	136 ± 15.9^{bB}	133 ± 12.9^{cA}	135 ± 15.2^{aA}	133 ± 11.9^{aA}
50 mg/kg bw	103 ± 10.7^{aA}	170 ± 11.9^{bC}	140 ± 15.2^{bB}	13714.3^{cC}	135 ± 13.0^{aB}	132 ± 15.1^{aA}	130 ± 12.2^{aA}
Glimepiride							
20 mg/kg bw	100 ± 12.8^{aA}	188 ± 10.2^{bA}	139 ± 11.3^{cA}	138 ± 11.9^{bA}	127 ± 9.3^{dA}	138 ± 15.8^{cA}	139 ± 12.0^{aA}
10 mg/kg bw	106 ± 15.8^{aA}	198 ± 9.9^{bB}	148 ± 9.2^{bB}	145 ± 20.7^{cB}	135 ± 19.9^{dA}	130 ± 18.4^{aA}	330 ± 19.4^{aA}
5 mg/kg bw	105 ± 20.9^{aA}	225 ± 18.8^{bC}	167 ± 15.8^{cC}	160 ± 14.8^{cC}	150 ± 15.4^{cA}	145 ± 12.2^{aB}	135 ± 20.9^{aA}
Ethanol extract + glimepiride							
200 +20 mg/kg bw	105 ± 10.9^{aA}	155 ± 12.8^{bA}	120 ± 12.6^{bA}	125 ± 18.8^{bA}	135 ± 13.2^{cA}	130 ± 14.9^{dA}	132 ± 9.8^{aA}
200 +10 mg/kg bw	108 ± 9.9^{aA}	165 ± 10.8^{bA}	130 ± 18.9^{bA}	123 ± 15.8^{bA}	120 ± 16.9^{cB}	136 ± 15.2^{aA}	131 ± 16.3^{aA}
200+5 mg/kg bw	100 ± 18.4^{aA}	185 ± 14.8^{bA}	133 ± 18.3^{bB}	124 ± 12.4^{cB}	123 ± 18.8^{dC}	132 ± 18.9^{aA}	130 ± 13.7^{aA}

Values are means±SD of six animals in each group. Values with different superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$. Values with different superscripts (uppercase letters) in a column (dose-dependent for a particular group) are significantly different at the level of $p<0.05$

ND not determined

Discussion

Hyperglycemia (increased blood glucose levels more than normal levels) is a chronic state of diabetic condition; in fact, chronic hyperglycemia is the defining characteristic of the disease. The pathophysiology of hyperglycemia in diabetic state is very complicated and affects by many daily activities such as food intake, exercise, etc. Long-term hyperglycemia causes several microvascular (i.e., nephropathy, neuropathy, and retinopathy) and macrovascular (i.e., cardiovascular diseases) complications of diabetes [19, 20].Therefore, the control of hyperglycemia needs special attention in diabetic conditions. Although, there are various oral hypoglycemic regimens are available in market, but conventional therapies for diabetes have many shortcomings like side effects and high rate of secondary failure. On the other hand, herbal extracts are expected to have similar efficacy without side effects as that of conventional drugs. Hence, in present study, we evaluated comparative

Table 3 Complementary hypoglycemic and antihyperglycemic potential of water extract of *M charantia* seeds and Glimepiride in normal and glucose infused rats

Groups	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Normal rats							
Water extract							
200 mg/kg bw	98 ± 9.5^{aA}	80 ± 10.5^{bA}	84 ± 10.4^{bA}	92 ± 11.3^{bA}	94 ± 10.3^{bA}	94 ± 10.5^{aA}	95 ± 10.2^{aA}
100 mg/kg bw	93 ± 7.9^{aA}	83 ± 10.4^{bB}	85 ± 15.2^{bB}	88 ± 12.5^{bB}	98 ± 9.8^{aA}	91 ± 10.5^{aA}	94 ± 8.8^{aA}
50 mg/kg bw	97 ± 11.3^{aA}	90 ± 9.5^{bB}	88 ± 10.1^{bB}	89 ± 11.8^{bB}	91 ± 10.9^{aA}	98 ± 10.2^{aA}	97 ± 11.5^{aA}
Glimepiride							
20 mg/kg bw	99 ± 10.5^{aA}	78 ± 9.5^{bA}	88 ± 9.4^{bA}	93 ± 10.7^{bA}	98 ± 12.0^{aA}	95 ± 10.8^{aA}	93 ± 12.4^{aA}
10 mg/kg bw	98 ± 11.7^{aA}	89 ± 10.3^{bA}	97 ± 10.2^{bB}	99 ± 9.9^{aA}	93 ± 14.1^{aA}	94 ± 10.3^{aA}	97 ± 10.2^{aA}
5 mg/kg bw	96 ± 12.3^{aA}	90 ± 11.5^{bB}	93 ± 10.4^{bB}	95 ± 9.5^{bA}	97 ± 11.2^{aA}	95 ± 9.4^{aA}	96 ± 10.3^{aA}
Water extract + glimepiride							
200+20 mg/kg bw	95 ± 11.8^{aA}	58 ± 12.5^{bA}	33 ± 10.2^{cA}	ND	ND	ND	ND
200+10 mg/kg bw	92 ± 9.7^{aA}	68 ± 10.1^{bA}	36 ± 13.9^{cA}	ND	ND	ND	ND
200+5 mg/kg bw	98 ± 9.8^{aA}	76 ± 17.9^{bA}	38 ± 16.4^{cA}	44 ± 13.2^{c}	53 ± 15.8^{c}	84 ± 10.3^{b}	98 ± 14.4^{aA}
Hyperglycemic rats							
Water extract							
200 mg/kg bw	102 ± 12.4^{aA}	185 ± 19.5^{bA}	155 ± 17.2^{bA}	156 ± 20.3^{bA}	160 ± 17.7^{cA}	154 ± 20.3^{dA}	146 ± 15.6^{aA}
100 mg/kg bw	101 ± 11.8^{aA}	189 ± 17.4^{bB}	160 ± 14.8^{bB}	154 ± 18.9^{bB}	152 ± 1.9^{cA}	155 ± 17.2^{aA}	149 ± 20.9^{aA}
50 mg/kg bw	103 ± 12.7^{aA}	200 ± 16.9^{bC}	170 ± 15.2^{bB}	166 ± 14.3^{cC}	165 ± 14.0^{aB}	163 ± 16.1^{aA}	154 ± 18.2^{aA}
Glimepiride							
20 mg/kg bw	105 ± 19.5^{aA}	190 ± 18.2^{bA}	160 ± 18.3^{cA}	158 ± 16.9^{bA}	155 ± 17.3^{dA}	160 ± 11.8^{cA}	152 ± 20.8^{aA}
10 mg/kg bw	106 ± 17.2^{aA}	210 ± 15.9^{bB}	168 ± 16.2^{bB}	165 ± 14.7^{cB}	160 ± 18.1^{dA}	164 ± 13.4^{aA}	153 ± 16.4^{aA}
5 mg/kg bw	103 ± 12.4^{aA}	228 ± 12.8^{bC}	179 ± 16.8^{cC}	173 ± 12.8^{cC}	174 ± 16.4^{cA}	175 ± 19.2^{aB}	163 ± 18.9^{aA}
Water extract + glimepiride							
200 +20 mg/kg bw	102 ± 12.9^{aA}	150 ± 15.8^{bA}	130 ± 18.6^{bA}	132 ± 16.8^{bA}	140 ± 19.2^{cA}	139 ± 14.4^{dA}	130 ± 15.4^{aA}
200 +10 mg/kg bw	109 ± 17.9^{aA}	158 ± 19.1^{bA}	140 ± 14.9^{bA}	150 ± 14.2^{bA}	155 ± 15.4^{cB}	153 ± 16.2^{aA}	150 ± 18.3^{aA}
200+5 mg/kg bw	108 ± 14.4^{aA}	168 ± 18.8^{bA}	150 ± 15.3^{bB}	152 ± 18.5^{cB}	158 ± 14.8^{dC}	152 ± 16.9^{aA}	145 ± 17.7^{aA}

Values are means ± SD of six animals in each group. Values with different superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$. Values with different superscripts (uppercase letters) in a column (dose-dependent for a particular group) are significantly different at the level of $p<0.05$

ND not determined

hypoglycemic and antihyperglycemic potential of four medicinal plants, i.e., *E. jambolana*, *M. charantia*, *G. sylvestre*, and *T. graecum*. After a thorough reviewing the literature on antidiabetic effects of *E. jambolana*, *M. charantia*, *G. sylvestre*, and *T. graecum*, it has been found that various studies reported various combinations/extracts of different part of these plants in different diabetic models [8–17, 21], but it has not been cleared which is the best and safer extract to be used for human consumption. Thereby, present study was conducted to find out the safer and best hypoglycemic extract of all plants (*E. jambolana* seeds, *M. charantia* fruits, *G. sylvestre* leaves, and *T. graecum* seeds). Interestingly, we found that ethanol extracts of *E. jambolana* and *G. sylvestre* and water extracts of *M. charantia* and *T. graecum* were most active for hypoglycemic and antihyperglycemic potential among five extracts (ethanol, methanol, hexane, and chloroform). The reason for most active hypoglycemic and antihyperglycemic activities of these plant extracts might be due to their phytochemical composition. Therefore, we analyzed the phytoconstituents content in

Table 4 Complementary hypoglycemic and antihyperglycemic potential of ethanol extract of *G Sylvestre* leaves and Glimepiride in normal and glucose-infused rats

Groups	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Normal rats							
Ethanol extract							
200 mg/kg bw	99 ± 8.9^{aA}	79 ± 10.2^{bA}	78 ± 10.4^{bA}	82 ± 11.5^{bA}	89 ± 12.3^{bA}	90 ± 11.5^{aA}	93 ± 10.2^{aA}
100 mg/kg bw	94 ± 9.9^{aA}	83 ± 11.4^{bB}	80 ± 12.2^{bB}	86 ± 10.5^{bB}	90 ± 9.8^{aA}	91 ± 10.5^{aA}	96 ± 8.8^{aA}
50 mg/kg bw	97 ± 11.3^{aA}	90 ± 9.5^{bB}	88 ± 10.1^{bB}	89 ± 11.8^{bB}	91 ± 10.9^{aA}	98 ± 10.2^{aA}	98 ± 11.5^{aA}
Glimepiride							
20 mg/kg bw	99 ± 9.5^{aA}	80 ± 10.5^{bA}	81 ± 11.4^{bA}	86 ± 10.5^{bA}	90 ± 12.0^{aA}	94 ± 10.8^{aA}	96 ± 12.0^{aA}
10 mg/kg bw	98 ± 11.7^{aA}	89 ± 10.3^{bA}	97 ± 13.2^{bB}	99 ± 10.9^{aA}	93 ± 14.1^{aA}	94 ± 10.3^{aA}	99 ± 10.0^{aA}
5 mg/kg bw	95 ± 12.3^{aA}	90 ± 11.7^{bB}	91 ± 10.5^{bB}	93 ± 12.5^{bA}	97 ± 11.2^{aA}	99 ± 12.4^{aA}	102 ± 10.3^{aA}
Ethanol extract + glimepiride							
200+20 mg/kg bw	98 ± 9.8^{aA}	60 ± 13.5^{bA}	32 ± 12.2^{cA}	ND	ND	ND	ND
200+10 mg/kg bw	94 ± 9.7^{aA}	70 ± 15.1^{bA}	35 ± 10.9^{cA}	ND	ND	ND	ND
200+5 mg/kg bw	99 ± 8.8^{aA}	78 ± 14.0^{bA}	40 ± 12.4^{cA}	41 ± 13.2^{c}	52 ± 14.8^{c}	78 ± 9.3^{b}	90 ± 12.4^{aA}
Hyperglycemic rats							
Ethanol extract							
200 mg/kg bw	102 ± 18.4^{aA}	182 ± 18.5^{bA}	156 ± 16.2^{bA}	153 ± 19.3^{bA}	154 ± 19.7^{cA}	160 ± 15.3^{dA}	150 ± 17.6^{aA}
100 mg/kg bw	108 ± 19.8^{aA}	200 ± 17.4^{bB}	190 ± 19.8^{bB}	180 ± 18.9^{bB}	183 ± 22.0^{cA}	188 ± 19.5^{aA}	180 ± 20.9^{aA}
50 mg/kg bw	107 ± 19.7^{aA}	220 ± 17.9^{bC}	210 ± 17.9^{bB}	212 ± 18.3^{cC}	198 ± 18.8^{aB}	195 ± 18.8^{aA}	185 ± 19.2^{aA}
Glimepiride							
20 mg/kg bw	105 ± 22.6^{aA}	190 ± 19.2^{bA}	160 ± 19.3^{cA}	165 ± 19.9^{bA}	164 ± 23.3^{dA}	166 ± 15.8^{cA}	163 ± 23.8^{aA}
10 mg/kg bw	100 ± 19.2^{aA}	198 ± 18.9^{bB}	180 ± 16.2^{bB}	183 ± 15.7^{cB}	190 ± 18.1^{dA}	$195\pm20.9.4^{aA}$	186 ± 16.4^{aA}
5 mg/kg bw	106 ± 20.4^{aA}	225 ± 16.8^{bC}	210 ± 18.5^{cC}	213 ± 16.8^{cC}	214 ± 16.9^{cA}	218 ± 19.2^{aB}	209 ± 14.9^{aA}
Ethanol extract + glimepiride							
200 +20 mg/kg bw	106 ± 8.9^{aA}	145 ± 16.8^{bA}	128 ± 15.6^{bA}	130 ± 16.8^{bA}	132 ± 19.2^{cA}	137 ± 18.4^{dA}	131 ± 16.4^{aA}
200 +10 mg/kg bw	105 ± 8.9^{aA}	157 ± 17.1^{bA}	140 ± 17.9^{bA}	147 ± 16.2^{bA}	150 ± 18.4^{cB}	152 ± 15.2^{aA}	145 ± 19.3^{aA}
200+5 mg/kg bw	102 ± 8.4^{aA}	170 ± 19.8^{bA}	162 ± 12.3^{bB}	161 ± 18.4^{cB}	165 ± 14.8^{dC}	170 ± 19.9^{aA}	164 ± 19.7^{aA}

Values are means ± SD of six animals in each group. Values with different superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$. Values with different superscripts (uppercase letters) in a column (dose-dependent for a particular group) are significantly different at the level of $p<0.05$

ND not determined

these plant extracts, and we found that bioactive constituents were significantly higher in different extracts, for example, flavonoids were significantly higher in ethanol extract of *E. jambolana*, which convince with other studies suggested that the flavonoid-rich extract of *E. jambolana* has potent hypoglycemic and hypolipidemic effect [11, 22, 23]. For *M. charantia*, water extract was most active hypoglycemic and antihyperglycemic, which might be due higher availability of phytoconstituents, i.e., alkaloids, phenols, tannins, and saponins. It has also been reported earlier that chemical constituents of *M. charantia* named cucurbitane triterpenoids are precise active compounds responsible for the antidiabetic activity of this plant [24]. Ethanol extract of *G. sylvestre* also exhibited antidiabetic property, which might be due to increased saponin content in this extract of *G. sylvestre*. It has been known that *G. sylvestre* contains triterpene saponins belonging to oleanane and dammarene classes, which might be responsible for higher hypoglycemic and antihyperglycemic potential [25]. Similarly, water extract of *T. graecum* also showed potent

Table 5 Complementary hypoglycemic and antihyperglycemic potential of water extract of *T. graecum* seeds and glimepiride in normal and glucose-infused rats

Groups	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Normal rats							
Water extract							
200 mg/kg bw	106 ± 9.3^{aA}	81 ± 9.4^{bA}	74 ± 10.4^{bA}	82 ± 11.3^{bA}	92 ± 10.3^{bA}	99 ± 10.5^{aA}	108 ± 11.2^{aA}
100 mg/kg bw	102 ± 7.9^{aA}	86 ± 8.4^{bB}	79 ± 17.2^{bB}	84 ± 10.2^{bB}	98 ± 9.5^{aA}	101 ± 9.4^{aA}	104 ± 9.8^{aA}
50 mg/kg bw	101 ± 11.3^{aA}	89 ± 9.5^{bB}	80 ± 10.1^{bB}	88 ± 9.8^{bB}	101 ± 10.9^{aA}	108 ± 10.2^{aA}	107 ± 10.3^{aA}
Glimepiride							
20 mg/kg bw	104 ± 10.5^{aA}	78 ± 9.5^{bA}	88 ± 9.4^{bA}	103 ± 9.7^{bA}	108 ± 12.0^{aA}	111 ± 11.8^{aA}	109 ± 12.4^{aA}
10 mg/kg bw	108 ± 11.7^{aA}	89 ± 10.3^{bA}	97 ± 10.2^{bB}	109 ± 10.9^{aA}	103 ± 14.1^{aA}	109 ± 10.3^{aA}	107 ± 10.2^{aA}
5 mg/kg bw	107 ± 12.3^{aA}	103 ± 11.1^{bB}	113 ± 9.4^{bB}	115 ± 10.4^{bA}	102 ± 10.2^{aA}	101 ± 9.4^{aA}	106 ± 13.3^{aA}
Water extract + glimepiride							
200+20 mg/kg bw	105 ± 11.8^{aA}	65 ± 9.4^{bA}	34 ± 10.2^{cA}	ND	ND	ND	ND
200+10 mg/kg bw	101 ± 9.7^{aA}	72 ± 10.1^{bA}	35 ± 11.9^{cA}	ND	ND	ND	ND
200+5 mg/kg bw	108 ± 9.8^{aA}	78 ± 10.9^{bA}	41 ± 12.4^{cA}	45 ± 11.2^{c}	57 ± 10.8^{c}	89 ± 7.3^{b}	105 ± 10.4^{aA}
Hyperglycemic rats							
Water extract							
200 mg/kg bw	132 ± 13.7^{aA}	159 ± 16.3^{bA}	137 ± 17.2^{bA}	128 ± 8.6^{bA}	124 ± 6.9^{cA}	113 ± 7.8^{dA}	129 ± 10.8^{aA}
100 mg/kg bw	131 ± 12.1^{aA}	167 ± 7.4^{bB}	147 ± 11.4^{bB}	129 ± 9.9^{bB}	129 ± 8.3^{cA}	118 ± 10.2^{aA}	121 ± 11.3^{aA}
50 mg/kg bw	127 ± 8.7^{aA}	234 ± 9.7^{bC}	176 ± 12.2^{bB}	129 ± 11.8^{cC}	128 ± 8.9^{aB}	122 ± 9.9^{aA}	130 ± 7.1^{aA}
Glimepiride							
20 mg/kg bw	138 ± 8.5^{aA}	172 ± 6.2^{bA}	161 ± 6.3^{cA}	142 ± 6.9^{bA}	128 ± 7.3^{dA}	123 ± 11.8^{eA}	129 ± 19.8^{aA}
10 mg/kg bw	121 ± 9.2^{aA}	209 ± 9.7^{bB}	169 ± 9.2^{bB}	143 ± 8.7^{cB}	129 ± 18.1^{dA}	134 ± 13.4^{aA}	124 ± 15.4^{aA}
5 mg/kg bw	129 ± 13.4^{aA}	134 ± 9.7^{bC}	168 ± 6.8^{cC}	134 ± 6.8^{cC}	124 ± 6.4^{cA}	144 ± 8.2^{aB}	133 ± 8.5^{aA}
Water extract + glimepiride							
200 +20 mg/kg bw	119 ± 9.0^{aA}	101 ± 8.4^{bA}	111 ± 7.6^{bA}	123 ± 8.8^{bA}	124 ± 9.2^{cA}	118 ± 9.4^{dA}	132 ± 9.4^{aA}
200 +10 mg/kg bw	122 ± 7.9^{aA}	119 ± 7.9^{bA}	125 ± 10.9^{bA}	132 ± 8.2^{bA}	117 ± 8.4^{cB}	121 ± 6.2^{aA}	128 ± 8.3^{aA}
200+5 mg/kg bw	139 ± 6.4^{aA}	142 ± 19.8^{bA}	132 ± 12.3^{bB}	133 ± 6.4^{cB}	143 ± 14.8^{dC}	129 ± 15.9^{aA}	132 ± 7.7^{aA}

Values are means ± SD of six animals in each group. Values with different superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$. Values with different superscripts (uppercase letters) in a column (dose-dependent for a particular group) are significantly different at the level of $p<0.05$

ND not determined

hypoglycemic and antihyperglycemic potential, and on the basis of the results of the present study, it might be speculated that this activity might be due to higher content of tannins in water extract than those of other extracts (Table 1). Tannins and saponins are water soluble components that have been reported for hypoglycemic potential [26, 27]. Although these results show that most active extracts of these plants might due to availability of higher content of bioactive constituents and might be used for treatment for regulation of increased blood glucose levels, before recommending these extracts for human consumption, it is necessary to check their toxicity effects in terms of long time consumption. Further study is warranted to isolate bioactive constituents for hypoglycemic and antihyperglycemic potential.

In addition, we also studied the combination of most active extracts of all four selected plants with standard drug; glimepiride was also investigated to find out how much dose of glimepiride can be reduced by combining most active extracts. This part of study shows very interesting results that the combination of most active extract significantly reduced the

dose of glimepiride (from 20 to 5 mg/kg bw) in glucose-overloaded animals, and it was safer (no hypoglycemic shock) in normal animals also, while combination with higher doses (20 and 10 mg/kg bw) was chronic to produce hypoglycemia in normal rats. The hypoglycemic and antihyperglycemic effects of these plant extracts might be either stimulating pancreatic B cells to secrete more insulin (insulin secretor) or increased insulin sensitivity in peripheral tissues, i.e., adipose tissue, muscle, and live to clear blood glucose at faster rate. The exact mechanism of action of these plant extracts is not known and needs further studies to find out the exact mechanism of action. But on the basis of these results, it may be speculated that most active extracts of different plants had similar activity as glimepiride as an insulin secretor in glucose-overloaded animals.

In conclusion, it may suggest that the combination of most active extracts with glimepiride of all four plants may play an important role to reduce the blood glucose levels in chronic diabetic conditions, but higher dose may cause hypoglycemic shock in normal or prediabetic state. Moreover, further study is required to isolation, purification, and characterization of active component(s) from most active extracts which might pave a good independent and/or complementary regimen for the treatment of diabetes mellitus.

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