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COMPARATIVE EVALUATION OF HYPOGLYCEMIC ACTIVITY, TRACE MINERALS AND PHYTOCHEMICAL CONTENTS OF SOME POTENTIAL MEDICINAL PLANT EXTRACTS

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ABSTRACT

Hypoglycemic activity of four selected plants extracts including seeds of *Citrus limon*, *Citrus paradisi*, *Avena fatua* and fruits of *Withania coagulans* were investigated in normal albino rabbits. The phytochemicals and minerals content of extracts were also analyzed. The experimental rabbits in fasting state were divided into different groups (6 animals/ group) including control (water 1ml/kg b.w.), negative control (0.05% dimethylsulphoxide 1ml/kg b.w.), positive control (glibenclamide 5 mg/kg b.w.) and test groups (200, 400 and 600 mg/kg b.w.) according to their organic and aqueous extracts treatments for oral glucose tolerance testing (OGTT). All the selected seeds and fruits extracts showed maximum presence of phytochemicals like alkaloids, flavonoids, phenols, saponins etc. along with trace minerals including Fe^{++}, Zn^{++}, Cu^{++}, Na etc. OGTT showed the significant (p<0.05) hypoglycemic activities of seeds extract of *C. paradisi* 400 and 600 mg (ethanolic) after 0 to 120 and 60 minutes respectively. Whereas its aqueous seeds extracts 400 mg produced significant (p < 0.05) hypoglycemia after 30 minutes as compared to positive control. Fruits extracts of *W. coagulans* 200 mg (methanolic and aqueous) significantly (p<0.05) decreased blood glucose after 60 and 120 minutes respectively, likewise aqueous extracts 400 and 600 mg significantly (p<0.05) reduced blood glucose after 60 to 120 and 0 minutes respectively. Seeds extracts of *C. limon* (ethanolic) 600 mg produced hypoglycemia (p<0.05) after 0 minute as compared to positive control. Therefore, out of four plants *C. limon, C. paradisi* (ethanolic seeds extracts) and *W. coagulans* (fruits extract) were found best in reducing blood glucose tolerance.

Key words: *C. limon*, *C. paradisi*, *W. coagulans*, OGTT, minerals.

INTRODUCTION

Type 2 diabetes is considered as a constant endocrine threat that enhances insensitivity of target organs towards insulin entry and in a long run it also manifests cardiovascular related casualties (Liu *et al*., 2010). Asian population has higher rate of insulin resistance than other regions and right now 25% people are diabetic worldwide (Maiti *et al*., 2004), that would be predicted up to 439 million in 2030 (Shaw *et al*., 2010; Olokoba *et al*., 2012).

Natural herbs are the vital for their hypoglycemic constituents. The use of the synthetic drugs is being restricted due to their side effects (Valiathan, 1998). World Health Organization (WHO) also endorses the medicinal value of herbal extracts and its practice for curing diabetes according to the relevant customs and cultures (Biesalski, 2004). Better clinical experiences of herbal medicines (Hui, 2009) also provide an alternative method of treatment, as they are featuring apparent effectiveness, low cost, safety, affordability and minimal side effects (Jouad *et al*., 2001). Minerals are also the vital source for maintaining good health (Siddiqui *et al*., 2014). Mostly they are essential components of enzymes like cytochromes (Matsumura *et al*., 2000) and regulate many cellular/ humoral immunities. Trace minerals reduces the risk of insulin resistance by influencing sugar breakdown pathways, like co-factors, opening of insulin receptors, and increasing insulin sensitivity (Akhuemokhan *et al*., 2013).

Avena fatua (family: Poaceae) is indigenous to mostly all continents including Asia. This plant is used as a nerve tonic and stimulant and its seeds are diuretic, emollient and refrigerant in nature. *Avena sativa* (common oats) though belongs to the same family also has reported to have various pharmacological activities (Khare, 2007) such as antioxidant and anti-inflammatory (Andersson *et al.*, 2013; Al-Snafi, 2015).

Withania coagulans (genus of shrubs) widely distributed in the Eastern Mediterranean region and South Asia. Its different parts including flowers are effective in reducing hyperglycemia, inflammation, free radicals and tumor production, also improves cardiac and liver function (Maurya *et al*., 2008). Similarly many *Citrus* plants provides essential oils and valuable compounds for instance hypericin alkaloids, lactons, polyacetylene, acyclic sesquiterpenes, etc. (Sah *et al*., 2011; Hindhi and Chabuck, 2013). *Citrus limon* (family Rutaceae) is a rich source of polyphenolic compounds that highlighted lemons for over weight problems, dislipidemia, hyperglycemia and insulin resistance (Naim *et al*., 2012). Other flavonoids like naringin and hesperidin also reported to have antidiabetic activity (Pari and Suman, 2010). Grapefruit (*Citrus paradisi*) is another member of citrus family and excellent source of phytochemicals. It is a good source of vitamin C, pectin fiber and antioxidant lycopene (Fellers *et al*., 1990). Studies reported that grapefruit lowers cholesterol and there seeds and peels are rich in phenolic compounds (Oboh and Ademosun, 2006). Oral glucose tolerance test (OGTT) is a prognostic tool to identify impaired glycemia or insulin insensitivity along with other sugar related metabolic condition (Kim *et al*., 2014).

The presence of medicinal components provides the base to investigate their potential hypoglycemic activity using oral glucose tolerance test in experimental rabbits. Moreover *in-vitro* phytochemicals and trace minerals examination of seeds extracts of *Avena fatua, Citrus limon, Citrus paradisi* and fruit extracts of *Withania coagulans* were also done.

MATERIALS AND METHODS

Experimental rabbits

Rabbits (male albino, body weight of 1.5-2.0 kg) were kept in animal house of Biochemistry department (University of Karachi) after purchased from Dow University of Health Sciences (DUHS), Karachi. Standard laboratory diet and water *ad libitum* were provided to all experimental rabbits.

Extract Preparation

The fruits of *Citrus limon, Citrus paradisi* were bought and their seeds were separated, washed and then dried. It was kept as (KU/BCH/SAQ/04). Dried seeds of *Avena fatua* and fruits of *Withania coagulans* were purchased from Hamdard Dawakana, Sadar, Karachi and authenticated as (KU/BCH/SAQ/06). The selected seeds and fruits were coarsely powdered then 40 g of powder of each plant was taken in 1L water and concentrated to one half at 100 ºC then cooled at room temperature and filtered (Whatman no 42, 125mm). The filtrate then concentrated till dryness in lyphilizer (Freeze Dryer) in order to obtained brown residue and kept in refrigerator. The same method was repeated to prepare organic extracts where we used ethanol/methanol (1L; 95%) in which seed and fruit were overnight soaked at room temperature and then filtered separately and concentrated at 40ºC till dryness in a rotary vacuum evaporator till brown residue obtained (Mudassir and Qureshi, 2015) and stored.

Qualitative and quantitative phytochemical screening

Qualitative evaluation of the selected plant extracts was made by methods described by (Lateef and Qureshi, 2014), whereas quantitatively alkaloids, flavonoids, saponins and total phenols were analyzed by the methods described earlier (Harborne, 1973; Boham and Kocipai-Abyazan, 1974: Obadoni and Ochuko, 2002; Slinkard and Singleton, 1977).

Trace minerals analysis

The samples of selected plant extracts were analyzed by atomic absorption spectrophotometer (PG990) for its mineral content.

Animal groups for oral glucose tolerance test

Overnight fasted experimental rabbits were divided into control and test groups (Fig. 1). Each test group had further three different groups for both aqueous and organic extract (200, 400 and 600 mg/kg) (Fig. 1). Rabbits in each group had their relevant oral treatment first then they were given 2 g of glucose /kg body weight immediately with the help of feeding tube. Glucometer was used to monitor blood glucose (from ear vein) at 0, 30, 60, and 120 minutes. Glibenclamide (5 mg) was used for positive control and 0.05% DMSO (dimethyl sulphoxide) was used as vehicle for organic extracts.

Determination of percent glycemic change

Change in percentage of glucose between control and test groups was calculated as described by (Mudassir and Qureshi, 2015) with the given formula

$$\% \text{ Glucose reduction} = \frac{[(G_x - G_o)]}{G_o} \times 100$$

Where G_o = mean blood glucose level of control group at different time gap

G_x= mean blood glucose levels of each test group and positive control at different time gap respective to control

Statistical analysis

Results are presented as mean ± SEM (Standard Error Mean) through one way analysis of variance (ANOVA) followed by LSD (Least Significant Difference) test (SPSS, version 17.0). The differences were rated significant at $p < 0.05$.

RESULTS

Trace minerals and phytochemical analysis

Iron, potassium, zinc, cobalt, chromium, and sodium were found in high quantity in *W. coagulans* extracts as compared to *C. limon* and *C. paradisi* (Table 3).

Phytochemical Analysis

Qualitative and quantitative analysis showed most of the phytochemicals in all selected plant extracts (Table I). Quantitatively the maximum content of total phenol was found only in aqueous *C. limon* extracts as 325 mg/g and its ethanolic extracts showed 190 mg/g, whereas 410 mg of alkaloids and 100 mg of flavonoid were found per gram of dried plant of *C. limon* (Table 2). The total phenolic content in aqueous *C. paradisi* was 177 mg/g and its ethanolic extract showed 80 mg/g (Table 2).

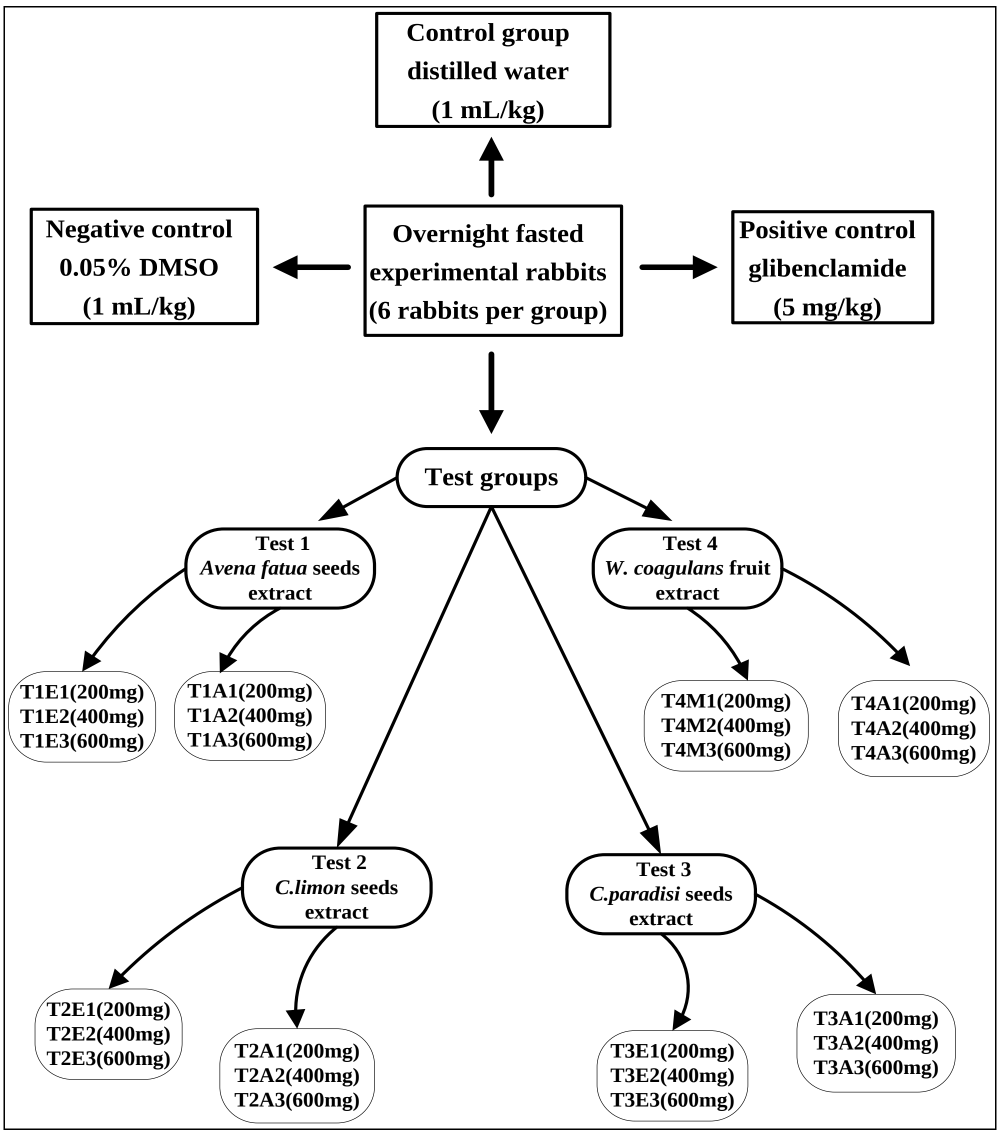

Fig. 1. Animal grouping for OGTT with their treatments. (E= ethanolic, A= aqueous, M= methanolic).

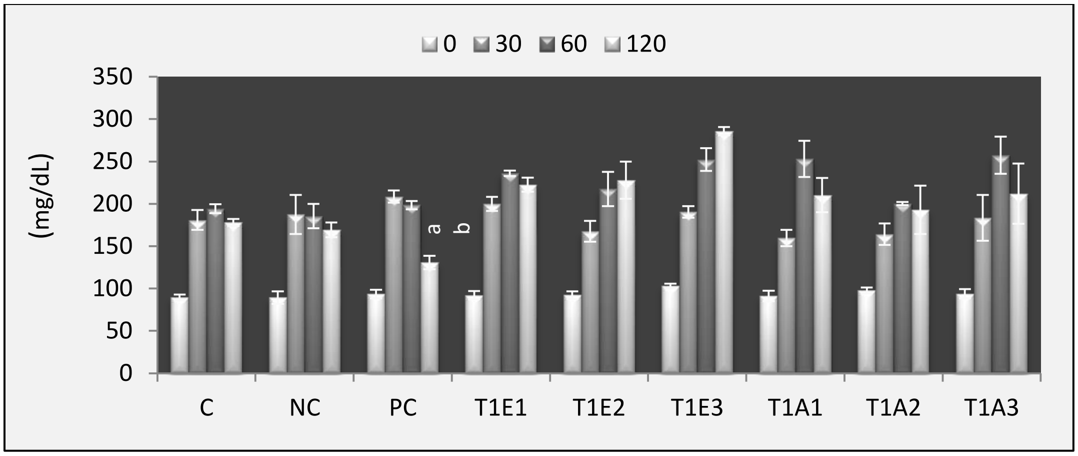

Fig. 2. Effect of Aqueous and Ethanolic seeds extracts of *A. fatua* during glucose tolerance test in rabbits.
Each bar represented as ±SEM mean (n=6). [a] $p<0.05$, when compared with control, [b] $p<0.05$, when compared with negative control.

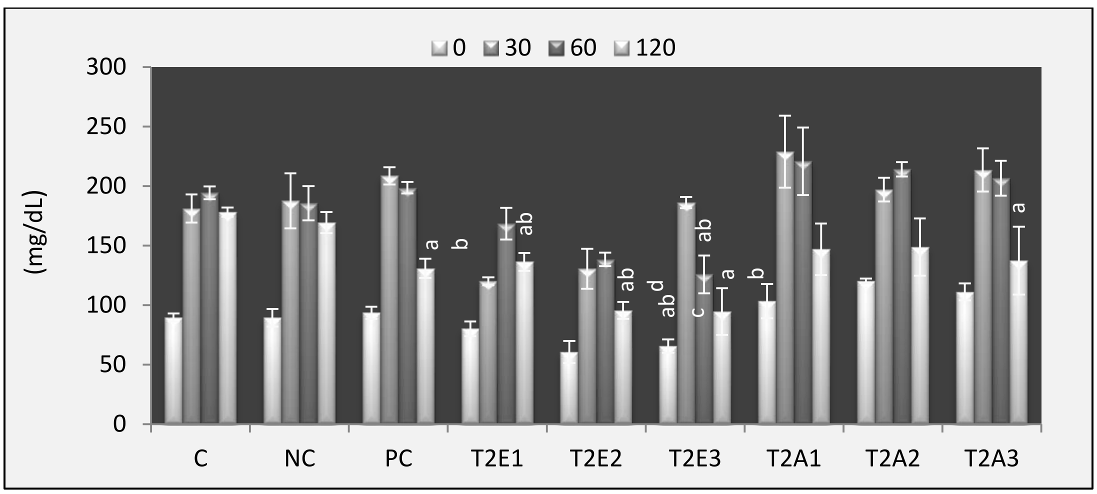

Fig. 3. Effect of Aqueous and Ethanolic seeds extracts of *C. limon* during glucose tolerance test in rabbits.
Each bar represented as ±SEM mean (n=6). [a] $p<0.05$, when compared with control, [b] $p<0.05$, when compared with negative control, [c] $p<0.05$, when compared with positive control, [d] $p<0.05$ when compared between test groups.

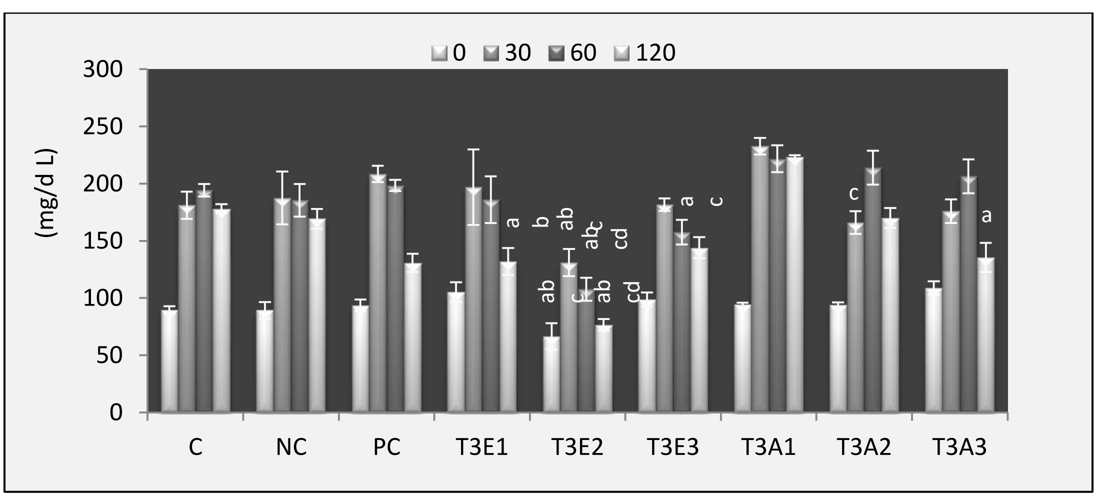

Fig. 4. Effect of Aqueous and Ethanolic seeds extracts of *C. paradisi* during glucose tolerance test in rabbits.
Each bar represented mean ±SEM (n=6). [a] $p<0.05$, when compared with control, [b] $p<0.05$, when compared with negative control, [c] $p<0.05$, when compared with positive control, [d] $p<0.05$ when compared between test groups.

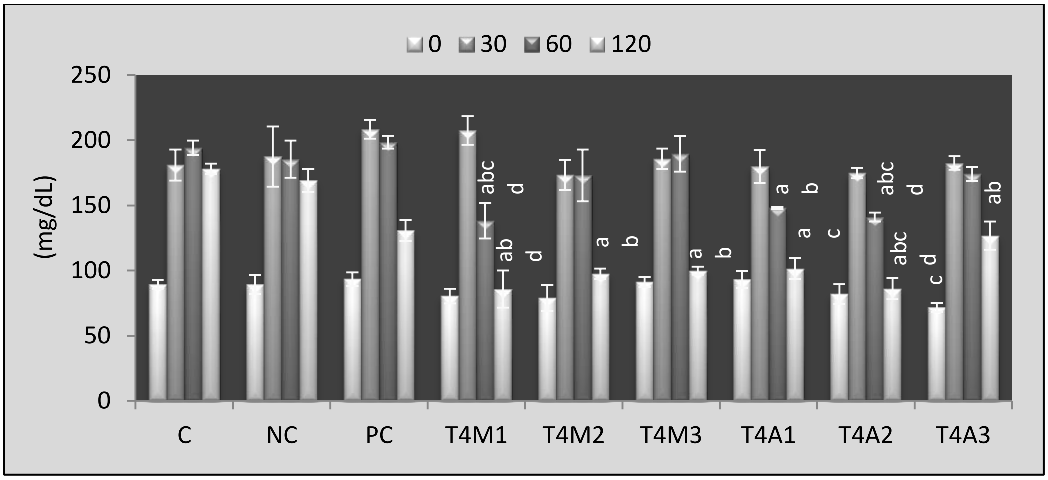

Fig. 5. Effect of Aqueous and Methanolic fruit extracts of *W. coagulans* during glucose tolerance test in rabbits.
Each bar represented as mean ±SEM (n=6). [a] $p<0.05$, when compared with control, [b] $p<0.05$, when compared with negative control, [c]$p<0.05$, when compared with positive control, [d] $p<0.05$ when compared between test groups.

Table 1. Qualitative Phytochemical Analysis of Seed and Fruits Extracts of Selected Plants.

Phytoconstituents	*C. limon* (seeds)		*C. paradise* (seeds)		*W. coagulans* (fruits)		*A. fatua* (seeds)	
	(E)	(A)	(E)	(A)	(M)	(A)	(E)	(A)
Alkaloids	+	+	-	-	+	+	+	+++
Flavonoids	+	+	-	-	+	+	+++	+
Resins	+	-	-	-	+	+	+++	-
Saponins	-	-	-	-	+	+	-	-
Steroids	+	++	+	+++	+	+	+	+
Cardiac glycosides	++	+	-	-	+	+	NA	NA
Carbohydrates	++	-	-	+	+	+	-	+++
Tanins	+	+	-	-	+	+	+++	++
Triterpenoids	+	+	-	-	+	+	+	-
Anthraquinone	+	+	-	+	NA	NA	+++	+++
Phlobatanins	NA	NA	NA	NA	+	+	+	-
Glycosides	-	+	NA	NA	+	+	NA	NA

A=aqueous, E=ethanol, M=methanol, (+) present, (-) absent; NA = not available

Oral glucose tolerance test and percent glycemic change

- **Effect of *Avena fatua* seeds extracts**

All test groups treated with *Avena fatua* seeds extract (T1E1to T1A3) did not reduce blood glucose level during OGTT (Fig. 2).

- **Effect of *Citrus limon* seeds extracts**

T2E1(200 mg/kg) showed a significant hypoglycemic activity $p<0.05$ after 30 & 120 minutes and $p<0.001$ after 60 minutes where as T2E2 and T2E3 (400 & 600 mg) significantly decreased blood glucose level $p<0.001$and $p<0.05$ respectively at 0, 30, 60 & 120 minutes as compared to control, positive and negative controls (Fig. 3). All doses of T2E1 reduced percent glycemia (–18 to −39%) after 0, 30 & 120 minutes, whereas after 60 minutes T2E2 and T2E3 (400 and 600 mg) showed glycemic change as −21 and −27% respectively.

- **Effect of *Citrus paradisi* seeds extracts**

T3E2 (400 mg) showed significant hypoglycemic activity $p<0.05$ at 0, 30 & 120 minutes after the oral treatment. At 30 & 60 minutes the same dose decrease blood glucose level $p<0.001$ significantly where as T3E3 (600 mg) showed a significant decrease in glucose level after 60 minutes, $p<0.05$ as compared to control, negative and positive controls (Fig. 4).

The percentage glucose reduction was found as −24, −23, −14, and −5% in T2E2 (400 mg) at 0, 30, 60 & 120 minutes.T3E3 (600 mg) showed −18% and −19% glucose reduction after 60 & 120 minutes than positive control group where −5 and −3% reduction in blood glucose level were observed at 0 & 120 minutes.

- **Effect of *Withania coagulans* fruits extracts**

T3A1 and T3A2 (200 & 400 mg) showed significant $p<0.05$ hypoglycemic activity after 30 min, $p<0.001$ after 60 &120 minutes, whereas T3A3 (600 mg) showed hypoglycemia significantly $p<0.05$ after 60 minutes as compared to control, negative and positive controls. All doses T3M1 to T3M3 (200-600 mg) showed significant hypoglycemic activity $p<0.05$and $p<0.001$ after 120 minutes as compared to control, negative and positive controls. T3M1 (200 mg) significantly decreased blood glucose $p<0.05$ after 0 & 30 minutes and $p<0.001$ after 60 minutes where as T3M2 (400 mg) showed a significant reduction in blood glucose $p<0.001$ after 60 min. as compared to control, negative and positive controls (Fig. 5). T3A2 (400 mg) decreased percent glycemia from 0 to 120 min. as −13, −9, −11 and −8%. T3A1 (200mg) reduced blood glucose level as −23, −18 and −24% respectively after 30, 60 & 120 minutes. Similarly T3A3 (600mg) also reduced blood glycemia as −10 and −1% after 0 & 60 minutes. T3M1 to M3 (200-600 mg) also showed percent glycemic reduction (−4 to −35%) from 0 to 120 minutes.

Table 2. Quantitative Phytochemical Analysis of Seeds / Fruits Extracts of Selected Plants.

Plants	Extracts	Phytoconstituents (mg/g of plant material)			
		Total phenols	Alkaloids	Flavonoids	Saponins
C. limon	(aqueous)	32.5	410*	100*	NA
	(ethanolic)	190	-	-	NA
C. paradisi	(aqueous)	177	NA*	NA	NA
	(ethanolic)	80	-	NA	NA
W. coagulans	(methanolic)	125	15*	200*	15*

*mg/g of seeds or fruits powder of selected plant; NA = not available

Table 3. Trace mineral content (ppm) of selected plants.

Trace minerals	*C.paradisi* (ethanolic extract)	*C.limon* (ethanolic extract)	*W.coagulans* (methanolic extract)
Fe	0.697	0.829	4.562
Zn	3.774	1.456	2.372
Co	bdl	0.367	0.524
Cr	0.224	bdl	0.608
Na	357.98	245.54	486.095
K	46.754	43.489	63.77

bdl = below detection limit

DISCUSSION

The bioactivity of medicinal plants can be expressed in terms of their counteractive worth and negligible side effects (Kumar *et al*., 2014). Previously, many herbs have been investigated for having therapeutic compounds like alkaloid in *Rauwolfia serpentina* was involved to delay post prandial blood glucose in normal mice (Azmi and Qureshi, 2012a) as well as improved insulin response in type 1 diabetic mice (Azmi and Qureshi, 2013). Similarly, flavonoids of *Trichlia emetic* and *Opilia ametacea,* improved hyperglycemia, hyperlipidemia in type 2 diabetic animal models (Konate *et al*., 2014).

In the present work all the selected plants (*Avena fatua, Citrus limon, C. paradisi*, and *Withania coagulans*) showed significant percentage of medicinal compounds including alkaloids, carbohydrates, cardiac glycosides, flavonoids, glycosides, phlobatanins, resins, saponins, steroids, tannins and triterpenoids. The quantitative analysis showed highest total phenol content in *C. limon*, than *C. paradisi* and *W. coagulans.* Similarly, amount of flavonoids were found high in *W. coagulans* and *C. limon*. Alkaloids were found high in *C. limon* than in *W. coagulans*. Saponins were found only in *W. coagulans.*

Apart from *A. fatua* all selected seeds and fruits extracts showed hypoglycemic activities in experimental animals during glucose tolerance analysis in which ethanolic seeds extracts of *C. limon* and *C. paradisi* were found more effective in normalizing blood sugar level in experimental rabbits than its aqueous seed extracts, interestingly ethanolic seeds extract of *C. limon* is used first time in the present study and it also showed highest phenolic content. Methanolic and aqueous fruits extract of *W. coagulans* also found helpful in reducing hyperglycemia (Lateef and Qureshi, 2014). The enrichment of phenols, alkaloids, flavonoids and other compounds in these extracts might ameliorate glucose tolerance in glucose-induced hyperglycemic rabbits. Moreover the presence of cobalt (Co), chromium (Cr), iron (Fe), potassium (K), sodium (Na) and zinc (Zn) in the organic extracts of *C. limon, C. paradisi* and *W. coagulans* endorsed the role of minerals in improving sugar metabolism (Akhuemokhan *et al*., 2013) as minerals supplementation has become an integral part in the treatment of hyperglycemia and it was also reported that trace minerals complement the release of insulin in reducing the diabetic complications(Zheng *et al*., 2008).

Thus hypoglycemic activity of the extracts could be due to cations and medicinal compounds that improved blood glucose level either by sensitizing target cells for insulin or catalyzed the enzyme activity of glucose metabolism but their mode of actions is needed to be explored.

Conclusion

All the selected plant extracts are a rich source of electrolytic and medicinal compounds that can play an important role in insulin metabolism that showed glucose lowering activity. Further diabetic animal trial would be helpful to know about the mechanism of action and to look at other beneficial activities of these extracts in the treatment of hyperglycemia.

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