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Original Research Article

Hypoglycaemic Activity of *Acalypha fruticosa* Forssk Extracts in Normal Rabbits

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Abstract

Purpose: *To investigate the hypoglycaemic activity of various extracts of the aerial part of Acalypha fruticosa in normal rabbits.*
Methods: *The rabbits were randomly assigned to 8 groups (n = 3) and treated with a single oral dose. Group 1 served as normal control and received 2 % dimethyl sulfoxide (DMSO, 3 ml/kg); group 2 served as standard treatment and received metformin (300 mg/kg); groups 3 - 8 received different extracts of Acalypha fruticosa at a dose of (600 mg/kg) as follows: group 3, total methanol extract; group 4, Petroleum ether (PET) extract; group 5, chloroform extract; group 6, ethyl acetate extract; group 7, n-butanol extract; and group 8, aqueous extract. Serum glucose (fasting blood glucose, FBG) was determined using a diagnostic kit on a glucose analyzer.*
Results: *A single dose of the aqueous extract of Acalypha fruticosa significantly lowered ($p < 0.5$) FBG at 1, 2, 4, and 6 h by 16, 29.5, 19.8, and 23.9 %, respectively, compared with normal control group, whereas standard treatment (metformin) did not affect FBG at all of these time-points measured. Furthermore, the aqueous extract (AEA) lowered FBG at 1, 2, 4, and 6 h by 24, 34.4, 25.9, and 17 %, respectively ($p < 0.5$), compared with metformin treatment. Chloroform extract also significantly lowered FBG at 1, 2, and 4 h by 17.3, 24.8, and 14.7 %, respectively, compared to metformin treatment ($p < 0.5$).*
Conclusion: *The results of this study show that the aqueous extract of the aerial parts of Acalypha fruticosa possess potential hypoglycaemic activity but further studies are required to elucidate the exact mechanisms of action.*

Keywords: *Acalypha fruticosa, Hypoglycaemic activity, Fasting blood glucose, Metformin, Acute toxicity*

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease with life threatening complications, characterized by hyperglycaemia and alterations in carbohydrate, lipid and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. The International Diabetes Federation (IDF) estimates that 285 million people, 6.4 % of the world population, suffered from diabetes in 2010 and this prevalence will increase to 439 million people, 7.7 % of the world population by 2030 [1].

The treatment of hyperglycaemia in diabetic patients is directed towards achieving euglycemia and minimizes chronic complications

by administering oral hypoglycaemic agents. Over 400 plants as well as 700 recipes and compounds have been scientifically evaluated for Type 2 DM treatment [2]. Metformin was developed based on a biguanide compound from the anti-diabetic herb, French lilac, and is now a first line drug for Type 2 DM [3]. Thus Searching for new anti-diabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus [4,5]. Medicinal herbs contain diverse bioactive compounds (glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc.) and can have multiple actions on insulin action, insulin production, or both.

Acalypha fruticosa Forssk belongs to the family Euphorbiaceae, commonly known as 'Birch – leaved Acalypha' and it occurs widely in East and southern Africa, tropical Arabia, southern India, Sri Lanka and Myanmar [6]. It is a strong aromatic bushy shrub traditionally used, in Ayurvedic and African folk medicine, to treat cholera, sexually transmitted diseases, dyspepsia, stomach ache, skin diseases, wounds and poisonous bites [7-10]. In Yemen, leaf and stem have been used to treat skin diseases, malaria and wound [11]. In North Kenya, the root and the leaves are used to treat liver disorders [6]. Several pharmacological studies have revealed its antiepileptic [12], antioxidant [13,14], anti-bacterial [15], anti-inflammatory [16], anti-tumor [17], wound healing [18] and cytotoxic properties [13]. Qualitative analysis of phytochemicals of the various extracts of the aerial parts of *Acalypha fruticosa* indicated the presence of triterpenoids, steroids, saponins, tannins, phenols, flavonoids, alkaloids, anthraquinones and sugars. Quantitative estimation of these phyto-constituents showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and steroids [19].

An earlier study showed that the chloroform and n-hexane extracts of *Acalypha indica*, at different concentrations, possessed dose dependent α-amylase inhibition against porcine pancreatic amylase [20]. Moreover, the ethanolic extract of *Acalypha indica* was reported to lower blood glucose level in STZ-Nicotinamide induced diabetic rats when compared to standard glibenclamide [21]. Therefore, the present study was aimed to investigate the potential hypoglycaemic activity of different polarity fractions obtained by extracting the aerial part of *Acalypha fruticosa* as well as its acute toxicity. To the best of our knowledge, this is the first scientific study conducted to assess the *in vivo* hypoglycaemic activity of the entitled plant.

EXPERIMENTAL

Plant material

The aerial part of *Acalypha fruticosa* was collected from Al-Mahweet area, Yemen (November - December 2012). The taxonomy and identification of the plant was confirmed by Dr. A. Al-Ajami, Department of Botany, Faculty of Science, Dhamar University, Yemen. A voucher specimen was deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University (voucher no. 115).

Extraction and fractionation of *Acalypha fruticosa*

Following collection, the aerial parts of the plant were left to dry outside under sunlight. The air dried powdered plant (1 kg) was extracted with 85 % methanol by cold maceration, till exhaustion. The combined methanolic extract was dried using rotary evaporator to give a dark crude residue (130 g) (MEA), from which 120 g were suspended in water and subsequently extracted with petroleum ether, chloroform, ethyl acetate and n-butanol. Each fraction was dried over anhydrous sodium sulphate and evaporated to dryness to yield fractions PEA (petroleum ether, 19 g) CEA (chloroform, 30 g), fraction EEA (ethyl acetate, 9.6 g), fraction BEA (n-butanol, 19 g) and fraction AEA (remaining aqueous mother liquor, 41 g).

Preliminary phytochemical screening

A preliminary phytochemical analysis of the *Acalypha fruticosa* extracts was carried out using thin layer chromatography (TLC) plates coated with silica gel 60 F254 for TLC. The mobile phase ethyl acetate: methanol: water (30:5:4) was used. Micro-drops of the concentrated solutions of the fractions obtained (methanol, petroleum ether, chloroform, ethyl acetate, n-butanol and aqueous extracts) were spotted on pre-coated Silica gel plates. The chromatogram after complete development was air dried and visualized with different chemical reagents to detect the presence of flavonoids, phenols, tannins, alkaloids, steroids, amino acids, saponins, triterpenes glycosides, and anthraquinones.

Experimental animals

Rabbits with an average weight of 1000 g were used for the hypoglycaemic activity study and Swiss Wistar albino mice with an average weight of 25 g were used for the acute toxicity study. All animals were fed with standard animal feed and

water. Animals were acclimatized to the laboratory conditions for 3 weeks prior to experimentation. All experiments carried out were approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana`a University (790-15/02/2013) and were conducted according to the standard guideline for the use of laboratory animals [22].

Acute toxicity study

Six mice were used and randomly assigned to control or treatment group (n = 3). The animals were deprived of food but given water 16 h prior to dosing. The methanol extract (5 g/kg) were then given orally to test group, while the control group received water at the same volume. Body weight, signs of toxicity (changes in skin and fur, eyes and mucus membrane, behavioral pattern including: tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma) and mortality were observed after administration at the third hour on the first day, and throughout the following 48 h and then daily thereafter for 14 days.

Determination of hypoglcaemic activity

For determination of the hypoglycaemic activity of *Acalypha fruticosa*, 24 rabbits (600 - 1000 g) were used and randomly assigned to 8 groups (3 animals per group). All rabbits were treated orally with a single dose. Group 1 served as normal control group and received distilled water and 2 % DMSO; group 2 served as standard treated with metformin (300 mg/kg) suspended in DMSO; groups 3 - 8 received different extracts of *Acalypha fruticosa* at a dose of (600 mg/kg) suspended in DMSO as follows: group 3 received methanolic extract; group 4 received n-butanol extract; group 5 received petroleum ether extract; group 6 received ethyl acetate extract; group 7 received chloroform extract; and group 8 received aqueous extract. Blood samples were collected, from the ear vein just prior to (0 hour) and at 1, 2, 4, 6 and 12 hours after dosing for acute study, into plain dry tubes and centrifuged at 3,000 rpm for 10 min and glucose was estimated.

Biochemical analysis

The serum samples obtained were transferred into Eppendorf tubes and analyzed by Cobas C 311 analyzer (Roche Diagnostics - GmbH, D-68298 Mannheim, Germany) for serum glucose using a commercially available kit (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Statistical analysis was performed using Social Package of Social Sciences (SPSS) version 11.5 (SPSS Inc, Chicago, IL, USA). The results are presented as mean ± standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test to compare the groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical examination of the different fractions (Table 1) revealed the presence of phenolic compounds in all the extracts, whereas flavonoids and amino acids were detected in methanol, chloroform, ethyl acetate, n-butanol and aqueous extracts of *Acalypha fruticosa*. Alkaloids were detected in the methanol and n-butanol extracts, while triterpenes glycosides were found in all extracts except the aqueous extract. Persistent foaming was observed for all the extracts, suggesting the presence of saponins.

Table 1: Phytochemical profile of aerial parts of *Acalypha fruticosa*

Phyto-constituent	Test	Extract					
		MEA	***PEA***	***CEA***	***EEA***	***BEA***	***AEA***
Flavonoids	KOH	+	-	+	+	+	+
	$AlCl_3$	+	-	+	+	+	+
Phenols	$FeCl_3$	+	+	+	+	+	+
+ tannins	NH_3 Vapour	+	+	+	+	-	-
Alkaloids	Dragendorff's Reagent	+	-	-	-	+	-
Amino acids+proteins	Xanthoproteic test	+	-	+	+	+	+
Steroids and Triterpene glycosides	Liebermann - Burchard's test	+	+	+	+	+	-

MEA, methanol; PEA, petroleum ether; CEA, Chloroform; EEA, ethyl acetate; BEA, n-butanol; AEA, aqueous

There were no toxic symptoms or mortality observed in any animals, which lived up to 14 days, following the administration of the methanol extract of the aerial parts of *Acalypha fruticosa* at a single dose level of 5 g/kg body weight. Moreover, the observed behavioral changes and toxicological signs (Table 2) showed no obvious differences between the treated and control animals. The general appearance of animals was normal (skin & fur, eyes and mucous membrane). The behavioral patterns of animals as well as breathing were also normal with no disturbance in food intake, water consumption or sleep.

Table 3 shows the hypoglycaemic activity of a single dose of the various extracts of *Acalypha fruticosa*. On comparing the level of FBG of the animals treated with either metformin (300 mg/kg) or the various extracts of Acalypha fruticosa (600 mg/kg) with that of the normal control animals showed that metformin did not affect FBG levels at all the time-points measured. On the other hand, the aqueous extract significantly lowered ($p < 0.5$) FBG at 1, 2, 4, and 6 hours by 16, 29.5, 19.8, and 23.9 %, respectively. However, chloroform extract significantly lowered ($p < 0.5$) FBG at 1 and 4 hours by 8.5 and 7.8 %. In contrast, ethyl acetate extract raised FBG significantly ($p < 0.5$) by 21.6 % at 1 h and no significant effect at other time points. On the other hand, on comparing the level of FBG of the animals treated with the various extracts of *Acalypha fruticosa* with that of the animals treated with metformin showed that the aqueous extract lowered FBG at 1, 2, 4, and 6 h by 24, 34.4, 25.9 and 17 %, respectively. Chloroform extract significantly lowered ($p < 0.5$) FBG at 1, 2 and 4 h by 17.3, 24.8, and 14.7 %, respectively, whereas the methanol extract significantly lowered ($p < 0.5$) FBG at 1 and 4 h by 13.2 and 16.8 %. In contrast, petroleum ether extract significantly lowered FBG by 17.2 % at 4 h, but raised FBG by 20 % at 6 h.

DISCUSSION

Traditional plant medicines are used throughout the world for a range of diabetic presentations. In light of this we made an attempt for the first time to study the effect of *Acalypha fruticosa* in normoglycaemic rabbits.

Table 2: General appearance and behavioral observations on control and treated groups following various treatments

Observation	Control group at all times	0.5 h	24 h	48 h	2 weeks
Skin & Fur	N	N	N	N	N
Eyes	N	N	N	N	N
Mucous membrane	N	N	N	N	N
Behavioral pattern	N	N	N	N	N
Breathing	N	N	N	N	N
Tremors	N.O[a]	N.O[a]	N.O[a]	N.O[a]	N.O[a]
Salivation	N.O[a]	N	N	N	N
Diarrhea	N.O[a]	N.O[a]	N.O[a]	N.O[a]	N.O[a]
Lethargy	N.O[a]	N.O[a]	N.O[a]	N.O[a]	N.O[a]
Sleep	N.O[a]	N	N	N	N
Coma	N.O[a]	N.O[a]	N.O[a]	N.O[a]	N.O[a]

N = normal; [a] not observed

Table 3: Effect of a single dose of *Acalypha fruticosa* extracts on fasting blood glucose of normal rabbits

Groups	Fasting blood glucose (mg/dl)					
	0	1 h	2 h	4 h	6 h	12 h
Control	100 ± 3.4[bc]	94 ± 2.3[edc]	94 ± 1.7[bac]	99 ± 1.7[ba]	96.3 ± 0.3[bac]	106 ± 12.7[a]
Metformin	111.3 ± 2[ba]	104 ± 2.8[bac]	101 ± 4.6[ba]	107 ± 4[a]	88.3 ± 2.6[bc]	90.3 ± 2[a]
MEA	123.3 ± 2.6[a]	90.3 ± 0.8[edf]	90.3 ± 7.2[bc]	89 ± 2.3[bc]	86 ± 2.8[c]	89..3 ± 0.3[a]
PEA	102.3 ± 4.3[bc]	101.6 ± 3.2[bdc]	97 ± 7.7[ba]	88.6 ± 12.7[bc]	106 ± 2[a]	94.6 ± 7.6[a]
CEA	105.3 ± 8.3[bc]	86 ± 0.5[ef]	76 ± 2.3[dc]	91.3 ± 4.9[dc]	99 ± 2.8[ba]	98.3 ± 3.1[a]
EEA	108.3 ± 6[b]	114.3 ± 2[a]	95 ± 1.7[bac]	92 ± 4.3[bac]	100.3 ± 0.3[ba]	94 ± 6.3[a]
BEA	112 ± 4.5[a]	106 ± 2.8[ba]	110.3 ± 3.4[a]	94.6 ± 2[bac]	93.6 ± 3.3[bac]	97 ± 4.7[a]
AEA	93.3 ± 2[c]	79 ± 8[f]	66.3 ± 11.8[d]	79.3 ± 6.6[c]	73.3 ± 8.9[d]	95.3 ± 4.3[a]

Values with same letter are not significant, whereas values with different letters are significant p < 0.5; MEA, methanol; PEA, petroleum ether; CEA, Chloroform; EEA, ethyl acetate; BEA, n-butanol; AEA, aqueous

In this study, a single dose of the aqueous extract (600 mg/kg) of *Acalypha fruticosa* significantly lowered the FBG with respect to both the control (16 - 24 %) and the metformin treated group (17 - 34 %). This observed potential hypoglycaemic activity might be due to the reported occurrence of flavonoids in *Acalypha fruticosa* [19]. Previous investigations have demonstrated a promising role for flavonoids as anti-diabetic agents [23,24]. Flavonoids such as kampferol and quercetin have been reported to affect the pancreatic β-cells leading to their proliferation and secretion of more insulin [25,26]. The long-term consumption of quercetin appears to control blood glucose levels in streptozotocin (STZ)-induced diabetic animals [27,28]; and has also been suggested that quercetin protects the pancreas against oxidative stress in STZ-treated animals, improving hyperglycemia [29,30]. A recent study revealed that the blood glucose lowering activity of flavonoid compounds: boswellic acid, ellagic acid, quercetin, and rutin may be by stimulating β-cells to release more insulin [31]; and suggested that the pronounced activity of these compounds could be because of enhanced peripheral glucose utilization by skeletal muscle in addition to that of β-cell stimulation. In addition, quercetin has been reported to inhibit α-glucosidase activity *in vitro* [32,33]; and its use on glucose absorption in obesity, and obesity with type 2 diabetes patients on oral glucose tolerance test has been investigated [34].

Additionally, the observed hypoglycaemic effect might be the result of the presence of minerals in *Acalypha fruticosa*. Quantitative estimation of minerals in the dried powder of its aerial part showed the concentration of macro-elements (K, Na, Ca, Mg and S) to range from 0.01 - 4.23 %, with calcium being present in high amount followed by magnesium and potassium, and that of the micro-elements (Zn, Cu, Fe, Mn, Bo and Mo) from 0.02 - 87.62 ppm with iron and manganese being present in higher concentrations [19]. Microelements as cofactors of enzymes play a vital role in improving glucose metabolism and increasing insulin sensitivity [35]. Manganese has been reported to improve glucose metabolism, and its deficiency may interrupt the insulin secretion process and increases insulin resistance. Potassium has also been reported to improve insulin sensitivity.

CONCLUSION

The results presented in this study show that the aqueous extract of the aerial part of *Acalypha fruticosa* possesses hypoglycaemic activity in normal rabbits, and thus has potentials for development into therapeutic agents for the treatment of diabetes mellitus. However, further studies are required for the isolation and structural elucidation of the active component(s) of the plant material.

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REFERENCES

1. *Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Practice 2010; 87(1): 4-14.*
2. *Singh J, Cumming E, Manoharan G, Kalasz H, Adeghate E. Medicinal chemistry of the anti-diabetic effects of momordica charantia: Active constituents and modes of actions. Open Med Chem J 2011; 5(2): 70-77.*
3. *Oubr´e AY, Carlson TJ, King SR, Reaven GM. From plant to patient: An ethnomedical approach to the identification of new drugs for the treatment of NIDDM. Diabetologia 1997; 40(5): 614-617.*
4. *Nicasio P, Aguilar-Santamarı´a L, Aranda E, Ortiz S, Gonza´lez M. Hypoglycemic effect and chlorogenic acid content in two Cecropia species. Phytotherapy Res 2005; 19: 661-664.*
5. *Suman BS, Afreena N, Krishna MP, Pothapragada SM. Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus. J Ethnopharmacol 2006; 104: 367-373.*
6. *Schmelzer GH. Acalypha fruticosa Forssk. In: Medicinal Plants 1. Plant Resource of Tropical Africa. 2008; pp 21-23.*
7. *Muthukumarasamy S, Mohan VR, Kumaresan S, Chelladurai V. Herbal remedies of Palliyar tribe of Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, Western Ghats, Tamil Nadu for poisonous bites. J Econ Taxonomy and Botany 2003; 27: 761-764.*
8. *Ignacimuthu S, Ayyanar M, Sankara Sivaraman K. Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). J Ethnobiol Ethnomed 2006; 2: 25-.*
9. *Senthilkumar M, Gurumoorthi P, Janardhanan K. Some medicinal plants used by Irular, the tribal people of Marudhamalai hills, Coimbatore, Tamil Nadu. Nat prod radiance 2006; 5(5): 382-388.*
10. *Anandakumar AM, Paulsamy S, Sathish kumar P, Senthilkumar P. Preliminary phytochemical studies for the quantification of secondary metabolites of*

medicinal importance in the plant Acalypha fruticosa Forssk. J Appl Natural Sci 2009; 1: 41-43.

11. *Fleurentin J, Pelt J-M. Repertory of drugs and medicinal plants of Yemen. J Ethnopharmacol 1982; 6: 85-108.*
12. *Govindu S, Adikay S. Evaluation of antiepileptic activity of chloroform extract of Acalypha fruticosa in mice. Pharmacol Res 2014; 6(2): 108-112.*
13. *Rajkumar V, Guha G, Ashok Kumar R. Therapeutic potential of Acalypha fruticosa. Food Chem Toxicol 2010; 48: 1709-1713.*
14. *Thambiraj J, Paulsamy S, Sevukaperumal R. Evaluation of In vitro antioxidant activity in the traditional medicinal shrub of western districts of Tamilnadu, India, Acalypha fruticosa Forssk. (Euphorbiaceae). Asian Pacif J Trop Biomed 2012; 2(1): S127-130.*
15. *Vinoth M. Antibacterial activity and the mode of action of alkaloid rich fraction from the leaves of Acalypha fruticosa. Int J Ethnomed Pharmacol Res 2013; 1(1), 1-6.*
16. *Gupta M, Mazumdar UK, Sivahkumar T, Vamis MLM, Karki S, Sambathkumar R, Manikandan L. Antioxidant and anti-inflammatory activities of Acalypha fruticosa. Nig J Natural Prod Med 2003; 7: 25-29.*
17. *Sivakumar T, Venkatachala Murthi MN, Kumutha P. Evaluation of anti-tumor and anti-oxidant activity of Acalypha fruticosa in Ehrlich's ascites carcinoma bearing Swiss Albino mice. Res J Pharmaceut Biol and Chem Sci 2010; 1(4): 191-199.*
18. *Gopalakrishnan S, Saroja K, Elizabeth JD. Wound healing activity of Acalypha fruticosa Forssk. J Pharmacy Res 2010a; 3(9): 2190-2191.*
19. *Gopalakrishnan S, Saroja K, Elizabeth JD. Chemical investigation of aerial parts of Acalypha fruticosa forssk. Der Pharma Chemica 2010b; 2(5): 383-389.*
20. *Nandhakumar M, Tamil Iniyan G, Senthilkumar M, Dinesh Kumar B, Mitra A. In vitro assay of alpha amylase inhibitory activity of Indian medicinal herb Acalypha indica. J Clin Diagnostic Res 2009; 3(2): 1475-1478.*
21. *Raghuram RA, Goverdhan RP, Venkateshwarlu E, Srinivas N, Ramya CH, Nirmala D. Anti-diabetic and hypolipidemic effect of acalypha indica in streptozotocin-nicotinamide induced type II diabetic rats. Int J Pharm Pharmaceut Sci 2012; 4: 205-212.*
22. *National Institute of Health, USA. Public Health Service Policy on Human Care and Use of Laboratory Animals. 2002.*
23. *Song Y, Manson JE, Buring JE, Sesso HD, Liu S. Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: A prospective study and cross-sectional analysis. J Am Coll Nutr 2005; 24: 376-384.*
24. *Jung M, Park M, Lee H-C, Kang Y-H, Kang ES, Kim SK. Antidiabetic agents from medicinal plants. Curr Med Chem 2006; 13: 1203-1218.*
25. *Adewole SO, Caxton-Martins EA, Ojewole JAO. Protective effect of quercetin on the morphology of pancreatic β-cells of streptozotocin-treated diabetic rats. Afr J Tradit Complem Alternat Med 2006; 4: 64-74.*
26. *Kobori M, Masumoto S, Akimoto Y, Takahashi Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Mol Nutr Food Res 2009; 53: 859-868.*
27. *Shetty AK, Rashmi R, Rajan MGR, Sambaiah K, Salimath PV. Antidiabetic influence of quercetin in streptozotocin-induced diabetic rats. Nutr Res 2004; 24: 373-381.*
28. *Anjaneyulu M, Chopra K. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol 2004; 31: 244-248.*
29. *Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Pharmacol Res 2005; 51: 117-123.*
30. *Youl E, Bardy G, Magous R, Cros G, Sejalon F, Virsolvy A, Richard S, Quignard JF, Gross R, Petit P, Bataille D, Oiry C. Quercetin potentiates insulin secretion and protects INS-1 pancreatic β-cells against oxidative damage via the ERK1/2 pathway. Br J Pharmacol 2010; 161: 799-814.*
31. *Jadhav R, Puchchakayala G. Hypoglycemic and antidiabetic activity of flavonoids: Boswellic acid, ellagic acid, quercetin on streptozotocin-nicotinamide induced type 2 diabetic rats. Int J Pharm Pharmaceut Sci 2012; 4(2): 251-256.*
32. *Ishikawa A, Yamashita H, Hiemori M, Inagaki E, Kimoto M, Okamoto M, et al. Characterization of inhibitors of postprandial hyperglycemia from the leaves of Nerium indicum. J Nutr Sci Vitaminology 2007; 53: 166-173.*
33. *Jo SH, Ka EH, Lee HS, Apostolidis E, Jang HD, Kwon YI. Comparison of antioxidant potential and rat intestinal α-glucosidases inhibitory activities of quercetin, rutin, andisoquercetin. Int J Applied Res Natural Prod 2009; 2: 52-60.*
34. *Mariana TP, Rolfy OA, Rafael Villalobos M, Narender S. A comparative study of flavonoid analogues on Steptozotocin nicotinamide induced diabetic rats: Quercetin as a potential antidiabetic agent acting via 11β – Hydroxysteroid dehydrogenase type 1 inhibition. Eur J Med Chem 2010; 45: 2606-2612.*
35. *Khan AR, Awan FR. Metals in the pathogenesis of type 2 diabetes. J Diabet Metabol Disorders 2014; 13: 16.*