IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ANTIDIABETIC EFFECTS OF COMBINATION OF HERBAL DRUG IN STRPTOZOTOCIN DIABETIC RATS

Raghavendra Pai*[1], [2]sanil Kumar R And [3]sanil Kumar

*[1&2]Department of Pharmacy, Annamalai University, Annamalai Nagar-608002, India.

[3]Accenture, Bangalore, India.

Abstract

To investigate the evaluation of various extract of antidiabetic activities of pet.ether, ethyl acetate and ethanolic extract combination of herbal drug. The shade dried herbal drug *Trigonella foenum graecum* and *Withania somnifera* powder was extracted with pet.ether, ethyl acetate and ethanol by continuous hot percolation method using Soxhlet apparatus. There was a recognize increment in the body weight in STZ fed group ($p<0.001$), which was reduced by administration of higher dose of ethyl acetate and ethanolic extract of *Trigonella foenum graecum* and *Withania somnifera* (200 mg/kg) than lower dose of ethyl acetate and ethanolic extract of *Trigonella foenum graecum* and *Withania somnifera* (100 mg/kg). Evaluate the plasma lipid profile and plasma lipoprotein such as HDL, VDL, VLDL and total cholesterol. The effect of tissue lipid content was free cholesterol, ester cholesterol, phospholipid, triglyceride and free fatty acid. This finding provides some biochemical basis for the use of various extract of dried herbal drug *Trigonella foenum graecum* and *Withania somnifera* as hypolipidemic agent having preventive and therapeutic effect against anti-diabetics. Thus, the results of the present study indicate that extracts of highdose, especially the ethanolic extract, showed a significant effect by attenuating the above lipid peroxidation in streptozotocin diabetes.

Keywords: **Antidiabetic, Herbal combination, Lipid profile, *Trigonella foenum graecum, Withania somnifera*.**

I. INTRODUCTION

Diabetes is one of the major causes of premature death worldwide. Every ten second a person dies from diabetes related causes mainly from cardiovascular complications. In 2007, diabetes caused 3.5 million deaths globally. Diabetes affects mainly the developing countries like India. Indeed, India presently has the largest number of diabetic patients in the world and has been infamously dubbed as the 'diabetic capital of the world. Diabetes mellitus is epidemic in India as a result of societal influence and changing lifestyles. Diabetes has been known in India for centuries as 'a disease of rich man' but now spread among all masses. According to International Diabetes Federation (IDF), the number of individuals with diabetes and its complication in 2019 crossed 366 million, with an estimated 4.6 million deaths every year.

For people with type 1 diabetes, daily insulin injections are essential to maintain health, so eat properly, keep blood glucose levels from going too low or too high, and monitor blood sugar levels. In America, pramlintide, marketed as Amylin, is used in addition to insulin by some people with type 1 diabetes to further help control their diabetes. Amylin is not currently prescribed in the UK. For people with type 2 diabetes, diet and exercise may be enough to control blood glucose levels in some. However, when diet and exercise is no longer efficient, anti-diabetic drugs may be prescribed. Medication will either be taken orally in the form of tablets (oral hypoglycemics), or be injected (insulin and GLP-1 receptor agonists).

The herbal seed of *Trigonella foenum-graecum* L. (fenugreek) is widely used for its medicinal properties all over the world and it is a very important spice in Indian culture. Around 260 species of *Trigonella* are diffused worldwide (*Abozeid, Zaki Turki, 2017;* Ahmad Sulaeman, 2019; Fohad Mabood Husain, 2015; Gausiya Bashri Sheo, 2016; Jasim B, 2017; Mehrnaz Riasat, 2015). The genus name *Trigonella* means 'tri-angled', maybe because of triangular shape of its flowers, whereas the species name *foenum-graecum* means 'Greek hay'. It is an annual crop and dicotyledonous plant belonging to the subfamily *Papilionaceae*, family *Fabaceae* (Mohamed A Farag, 2016; Naourez Ktari, 2017; Spandan Chaudhary, 2018; Srinivasan K, 2006; Subhapriya S, 2018). Another herbal root of *Withania somnifera* Linn commonly known as Ashwagandha, Indian ginseng, winter cherry is an important medicinal plant in the *solanaceae* (Akash Saggam, 2020; Anju Thakur, 2015; Aradhana Mishra, 2018; Bakhtiar Choudhary, 2015; Bipradut Sil, 2015;) family that has been used in ayurvedic and indigenous medicine for more than 3,000 years. Ashwagandha in Sanskrit means "horse's smell" probably originated from the odour of its root, which resembles that of sweaty horse (Ramesh B , 2005; Mohamed M Abdel-Daim, 2015; Nadia Alam, Monzur Hossain, 2012). The species name *somnifera* means "sleep-making" in Latin, attributed to sedating properties (Uma Chandran, 2013; Nathiya S, 2014).

II. MATERIALS AND METHODS

Collection and authentication of plant material

The herbal drug was collected from Madurai, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamil Nadu, India. The herbal drug powdered materials were put away in a hermetically sealed holder. The herbal drug were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Preparation of plant extract

The equal amount of seed of *Trigonella foenum graecum* and root of *Withania somnifera* herbal drug were extracted with pet.ether, ethyl acetate and ethanol at temperature between 60-70ºC by using soxhlet extractor. The solvent was evaporated by rotavapor to obtained viscous semi solid masses.

III. EXPERIMENTAL DESIGN

Study protocol for dose fixation

The animals were randomly divided into 7 groups of six animals each. Feeding was started by 9.30 a.m. and various extracts and glibenclamide (dissolved in water) were administered post orally using intragastric tube at 10.30 a.m. The duration of treatment was 21 days.

Group I	Normal Control
Group II	Diabetic control
Group III	Diabetic + Ethyl acetate extract 200 mg
Group IV	Diabetic + Ethyl acetate extract 400 mg
Group V	Diabetic + Ethanolic extract 200 mg
Group VI	Diabetic + Ethanolic extract 400 mg
Group VII	Diabetic + Glibenclamide (600 µg/kg b.wt.)

After 45 days of treatment, the animals were fasted for 12 h, sacrificed by cervical dislocation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of plasma glucose and ethylenediamine tetra acetic acid (EDTA) for the estimation of various biochemical parameters. Tissue (liver, kidney, heart and brain) were surgically removed, washed with cold physiological saline, cleared off adherent lipids and immediately transferred to ice-cold containers. Erythrocytes were also prepared for the estimation of various biochemical preparations.

Processing of blood and tissue samples

Serum preparation

Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min.

Plasma preparation

The blood, collected in a heparinized centrifuge tube, was centrifuged at 2000 rpm for 10 min and the plasma was separated by aspiration.

Erythrocyte preparation

After the separation of plasma, the buffy coat, enriched in white cells, was removed and the remaining erythrocytes were washed three times with physiological saline (19). A known volume of erythrocyte was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2500 rpm for 10 min and the supernatant was used for the estimation of enzymic antioxidants.

Biochemical determinations

Estimation of blood glucose

Glucose was estimated by the method of Trinder using reagent (Suresh N *et al.*, 2001).

Estimation of plasma insulin

Plasma insulin was assayed by the solid phase system amplified sensitivity immunoassay using reagent kits obtained from Medgenix-INS-ELISA, Biosource, Europe S.A., Belgium (Murray RR, 2003).

Estimation of haemoglobin

Haemoglobin in the blood was estimated by the method of John MC Murdy *et al.*, 2009(Helen Yeni Komshian, 2000).

Estimation of glycosylated haemoglobin (HbA_{1c})

Glycosylated haemoglobin in the blood was estimated by the method (Davidson MB,2012).

Analysis of lipid profile (Kedlaya R, 2004; Nakagami T, 2011; Nichol A, 2011)

Estimation of total cholesterol

Total cholesterol in the plasma, erythrocytes and tissues was estimated by the enzymic method (Jose Castro Perez,2019).

Estimation of HDL-cholesterol

HDL-cholesterol was estimated using the diagnostic kit based on the enzymic method (Gordon D, 1989).

Estimation of free fatty acids

Free fatty acids in the plasma and tissues were estimated by the method of Halkes CJM *et al*., (Mooks S, 2004).

Estimation of triacylglycerol

Triacylglycerol in the plasma and tissues were estimated using the diagnostic kit based on the enzymic method described by Gavin Hsmilton *et al*., (Gavin Hamilton, 2017).

Statistical analysis

All data were expressed as mean ± S.D of number of experiments (n = 6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 7.5 and the individual comparison were obtained by Duncan's Multiple Range Test (DMRT). A value of $p<0.05$ was considered to indicate a significant difference between groups.

IV. RESULTS AND DISCUSSION

Table 1 shows the level of blood glucose and plasma insulin in normal and experimental groups. The level of blood glucose was significantly increased whereas the level of plasma insulin was significantly decreased in diabetic rats. Oral administration of combination of herbal drug high dose of ethyl acetate, ethanolic extract and glibenclamide to diabetic animals significantly reversed all these changes to near normal levels.

Biochemical determinations

Table No.1: Effect of herbal drug on plasma insulin in normal and STZ-diabetic rats.

Name of the group	Plasma insulin (µU/mL)
Group I	17.45 ± 1.04^{a}
Group II	6.08 ± 0.78^{b}
Group III	12.10 ± 1.18^{c}
Group IV	12.63 ± 1.10^{c}
Group V	12.24 ± 0.98^{c}
Group VI	12.72 ± 0.82^{c}
Group VII	12.65 ± 0.86^{c}

Values are means ± S.D for six rats.

Values not sharing a common superscript differ significantly at $P< 0.05$ (DMRT).

Table 2 & 3 shows the body weight, level of blood glucose and plasma insulin in normal and experimental groups. The level of blood glucose was significantly increased whereas the body weight and level of plasma insulin was significantly decreased in diabetic rats. Oral administration of combination of herbal drug high dose of ethyl acetate, ethanolic extract and glibenclamide to diabetic animals significantly reversed all these changes to near normal levels.

Table No. 2: Effect of various extracts of herbal drug on body weight, blood glucose & urine sugar

Name of the group	Body weight (g)			Blood glucose (mg/dL)		
	0 day	45th day	Change (%)	0 day	45th day	Change (%)
Group I	182.45 ± 3.10	194.14 ± 3.06^{a}	7.08 (+)	75.33 ± 2.32	81.55 ± 5.92^{a}	6.75 (+)
Group II	184.18 ± 4.32	156.80 ± 4.33^{c}	15.26 (-)	245.60 ±2.95	290.36 ± 3.58^{c}	18.70 (+)
Group III	183.98 ± 3.32	$197.34 \pm 3.10^{a,b}$	6.34 (+)	246.35 ±1.92	148.22 ± 4.438^{b}	35.65 (-)
Group IV	183.48 ± 3.39	194.32 ± 2.76^{b}	5.82 (+)	248.54 ±2.36	117.32 ± 5.06^{d}	53.68 (-)
Group V	180.25 ± 3.02	$194.86 \pm 3.22^{a,b}$	6.04 (+)	245.58 ±1.68	130.84 ± 5.34^{b}	41.12 (-)
Group VI	184.48 ± 3.30	195.74 ± 2.64^{b}	5.78 (+)	247.85 ±2.55	118.10 ± 4.05^{d}	53.85 (-)
Group VII	182.43 ± 3.28	$194.04 \pm 3.20^{a,b}$	5.79 (+)	250.32 ±4.25	116.42 ± 4.34^{d}	53.80 (-)

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table No.3: Changes in blood glucose and plasma insulin levels

Groups	Fasting Blood Glucose (mg/dL)	Plasma insulin (µU mL)
Group I	83.08 ± 2.10^{a}	12.14 ± 0.74^{a}
Group II	256.12 ± 24.78^{b}	3.42 ± 0.58^{c}
Group III	146.65 ± 14.22^{d}	5.10 ± 0.28^{d}
Group IV	110.72 ± 12.96^{d}	6.54 ± 0.36^{d}
Group V	126.04 ± 6.72^{e}	5.84 ± 0.42^{e}
Group VI	106.26 ± 5.18^{e}	6.66 ± 0.35^{e}
Group VII	109.86 ± 7.96^{e}	6.52 ± 0.58^{de}

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

TBARS and hydroperoxides (Table 4) from brain homogenate were significantly decreased with combination of herbal drug high dose of ethyl acetate, ethanolic extract treatment whereas, diabetic control rats showed significantly increased levels of lipid peroxidation products.

Table No. 4: Effect of various extracts of herbal drug on lipid peroxidation markers in the erythrocytes.

Name of the group	Erythrocytes	
	TBARS (nmol/mg protein)	Lipid hydroperoxides (µmol/mg protein)
Group I	1.89 ± 0.18^{a}	1.05 ± 0.08^{a}
Group II	4.88 ± 0.32^{b}	1.39 ± 0.06^{b}
Group III	3.04 ± 0.15^{a}	1.31 ± 0.05^{a}
Group IV	2.32 ± 0.22^{c}	1.25 ± 0.09^{c}
Group V	2.79 ± 0.18^{a}	1.28 ± 0.07^{a}
Group VI	2.17 ± 0.24^{c}	1.09 ± 0.08^{c}
Group VII	2.30 ± 0.20^{d}	1.22 ± 0.10^{c}

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Plasma insulin, blood haemoglobin and glycosylated haemoglobin (Table 5) were significantly increased with combination of herbal drug high dose of ethyl acetate, ethanolic extract treatment whereas, diabetic control rats showed significantly decreased levels of plasma insulin, blood haemoglobin and glycosylated haemoglobin.

Table No. 5: Effect of various extracts of herbal drug on plasma insulin, blood haemoglobin and glycosylated haemoglobin.

Name of the group	Insulin (µU/mL)	Haemoglobin (g/dL)	Glycosylated haemoglobin (mg/g of Hb)
Group I	17.56 ± 0.72^{a}	14.10 ± 0.55^{a}	0.43 ± 0.04^{a}
Group II	5.54 ± 0.54^{c}	6.21 ± 0.52^{b}	1.20 ± 0.10^{b}
Group III	12.88 ± 0.76^{b}	9.88± 0.74^{a}	0.69 ± 0.08^{a}
Group IV	16.21 ± 0.48^{d}	12.70 ± 0.78^{c}	0.52 ± 0.06^{c}
Group V	13.22 ± 0.54^{b}	10.72± 0.60^{a}	0.62 ± 0.04^{a}
Group VI	16.35 ± 0.38^{d}	13.15 ± 0.68^{c}	0.50 ± 0.05^{c}
Group VII	16.28 ± 0.52^{d}	12.86 ± 0.75^{d}	0.51 ± 0.03^{c}

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 6 shows the levels of LDL-C, VLDL-C, and HDL-C in the plasma of diabetic rats. The diabetic rats had elevated levels of plasma LDL-C, and VLDL-C and decreased level of HDL-C as compared with normal control rats. Diabetic rats treated with combination of herbal drug high dose of ethyl acetate, ethanolic extract treatment whereas, glibenclamide reversed serum lipid profiles to near normal levels.

Table No. 6: Effect of various extracts of herbal drug on HDL, LDL and VLDL - cholesterol in the plasma.

Name of the group	Plasma		
	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	VLDL-cholesterol (mg/dL)
Group I	48.78 ± 3.34^{ab}	24.86 ± 1.56^{a}	10.84 ± 1.12^{a}
Group II	25.98 ± 2.14^{c}	43.74 ± 8.22^{b}	26.10 ± 1.56^{b}
Group III	35.46 ± 3.10^{b}	49.88 ± 1.23^{a}	17.12 ± 0.68^{a}
Group IV	44.90 ± 2.24^{d}	32.25 ± 3.66^{c}	13.54 ± 1.10^{c}
Group V	40.64 ± 2.92^{b}	41.86 ± 1.24^{a}	14.10 ± 0.85^{a}
Group VI	46.52 ± 2.34^{d}	30.05 ± 3.82^{c}	12.95 ± 1.46^{c}
Group VII	45.76 ± 3.68^{a}	32.15 ± 2.26^{d}	13.23 ± 1.25^{d}

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 6 shows the levels of LDL-C, VLDL-C, and HDL-C in the plasma of diabetic rats. The diabetic rats had elevated levels of plasma LDL-C, and VLDL-C and decreased level of HDL-C as compared with normal control rats. Diabetic rats treated with combination of herbal drug high dose of ethyl acetate, ethanolic extract treatment whereas, glibenclamide reversed serum lipid profiles to near normal levels.

Table 7 shows the levels cholesterol, free fatty acids, triglycerides and phospholipids in liver of diabetic rats. The diabetic rats had elevated levels of cholesterol, free fatty acids, triglycerides and phospholipids in liver as compared with normal control rats. Diabetic rats treated with combination of herbal drug high dose of ethyl acetate, ethanolic extract treatment whereas, glibenclamide reversed serum lipid profiles to near normal levels.

Table No. 7: Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in liver.

Groups	Cholesterol (mg/100g wet tissue)	Free fatty acids (mg/100g wet tissue)	Triglycerides (mg/100g wet tissue)	Phospholipids (mg/100g wet tissue)
Group I	326.54±26.32^{a}	604.68 ±22.84^{a}	355.18 ±28.06^{a}	1627.65±25.23^{a}
Group II	531.20±15.22^{b}	927.46 ±40.34^{b}	625.87±17.65^{b}	1856.32±22.23^{b}
Group III	486.65±10.14^{a}	845.28 ±22.35^{a}	598.10 ±21.64^{a}	1812.25±24.82^{a}
Group IV	449.76±21.32^{c}	812.35± 15.82^{c}	538.45 ±18.54^{c}	1766.98±19.22^{c}

Group V	452.23±811.12^{a}	818.25 ±22.36^{a}	550.76 ±21.42^{a}	1780.10±24.50^{a}
Group VI	431.86 ± 17.22^{c}	792.62± 16.44^{c}	496.45 ±16.65^{c}	1738.64±15.18^{c}
Group VII	448.25 ± 13.36^{c}	809.23 ± 44.12^{c}	536.40±35.54^{d}	1766.42±21.10^{c}

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Table 8 shows the levels cholesterol, free fatty acids, triglycerides and phospholipids in kidney of diabetic rats. The diabetic rats had elevated levels of cholesterol, free fatty acids, triglycerides and phospholipids in kidney as compared with normal control rats. Diabetic rats treated with combination of herbal drug high dose of ethyl acetate, ethanolic extract treatment whereas, glibenclamide reversed serum lipid profiles to near normal levels.

Table No. 8: Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in kidney.

Groups	Cholesterol (mg/100g wet tissue)	Free fatty acids (mg/100g wet tissue)	Triglycerides (mg/100g wet tissue)	Phospholipids (mg/100g wet tissue)
Group I	368.44 ± 12.33^{a}	440.10 ± 13.23^{a}	293.23 ± 15.30^{a}	1410.64 ± 27.95^{a}
Group II	549.45 ± 20.35^{b}	752.56 ± 21.45^{b}	515.68 ± 25.24^{b}	2035.12 ± 32.18^{b}
Group III	493.12 ± 14.33^{a}	654.26 ± 16.28^{a}	459.16± 23.25^{a}	1864.98 ± 28.33^{a}
Group IV	451.83 ± 19.25^{c}	601.65 ± 34.32^{c}	430.84 ± 14.38^{c}	1828.86 ± 25.87^{c}
Group V	472.25 ± 10.76^{a}	635.43 ± 15.21^{a}	442.54± 17.42^{a}	1848.62 ± 36.30^{a}
Group VI	440.54 ± 10.33^{c}	582.18 ± 28.25^{c}	411.63 ± 16.26^{c}	1776.84 ± 24.45^{c}
Group VII	445.60 ± 10.12^{c}	598.23 ± 28.42^{d}	427.16 ± 30.24^{d}	1825.18 ± 32.28^{d}

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

V. CONCLUSION

The result of present study revealed that the ethyl acetate and ethanolic extract of dried herbal drug *Trigonella foenum graecum* and *Withania somnifera* significantly reduced the plasma lipid and lipoprotein profile. It also significantly reduced the tissues free cholesterol, ester cholesterol and triglycerides. This finding provides some biochemical basis for the use of ethyl acetate and ethanolic extract of dried herbal drug *Trigonella foenum graecum* and *Withania somnifera* as antihyperlipidemic agent having preventive and curative effect against hyperlipidemia. Since the study of induction of the antioxidant enzymes is considered to be a reliable marker for evaluating the antiperoxidative efficacy of the medicinal plant, these findings are suggestions of possible antiperoxidative role played by *Trigonella foenum graecum* and *Withania somnifera* extract in addition to its antidiabetic effect.

VI. REFERENCES

[1] Abozeid, Zaki Turki, FathiEl-Shayeb AND Zhonghua Tang.(2017). Embryo and seedling morphology of some Trigonella L. species (Fabaceae) and their taxonomic importance. ELSEVIER, 2(30): 57-65.

[2] Ahmad Sulaeman and Mahani Mahani. (2019). Trigona Propolis and Its Potency for Health and Healing Process. Science Direct journal and books, 5(23): 1-8.

[3] Fohad Mabood Husain, Iqbal Ahmad, Mohd Shahnawaz Khan, and Nasser Abdulatif Al-Shabib. (2015). *Trigonella foenum-graceum* (Seed) Extract Interferes with Quorum Sensing Regulated Traits and Biofilm Formation in the Strains of Pseudomonas aeruginosa and Aeromonas hydrophila. Journals/ecam, 5(3): 25-30.

[4] Gausiya Bashri Sheo and Mohan Prasad. (2016). Exogenous IAA differentially affects growth, oxidative stress and antioxidants system in Cd stressed *Trigonella foenum-graecum* L. seedlings: Toxicity alleviation by up-regulation of ascorbate-glutathione cycle. Ecotoxicology and Environmental Safety, 13(2): 329-338.

[5] Fohad Mabood Husain, Iqbal Ahmad, Mohd Shahnawaz Khan, and Nasser Abdulatif Al-Shabib. (2015). *Trigonella foenum-graceum* (Seed) Extract Interferes with Quorum Sensing Regulated Traits and Biofilm Formation in the Strains of Pseudomonas aeruginosa and Aeromonas hydrophila. Journals/ecam, 5(3): 25-30.

[6] Jasim B, Roshmi Thomas, Jyothis Mathew, E.K. Radhakrishnan. (2017). Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonella foenum-graecum* L.). Saudi Pharmaceutical Journal, 2(5): 443–447.

[7] Mehrnaz Riasat. (2015). A Cytogenetic Study on Some Perennial Trigonella (Fenugreek) in Iran. Research Journal of Recent Sciences, 4(5): 10-13.

[8] Mohamed A Farag, Dalia M Rasheed, Matthias Kropf and Andreas G Heiss. (2016). Metabolite profiling in Trigonella seeds via UPLC-MS and GC-MS analyzed using multivariate data analyses. Springer link, 7(5): 453-461.

[9] Naourez Ktari, ImenTrabelsi, SanaBardaa, MehdiTriki, Intidhar Bkhairia, Rabab Ben Slama-Ben Salem, Moncef Nasri and Riadh Ben Salah. (2017). Antioxidant and hemolytic activities, and effects in rat cutaneous wound healing of a novel polysaccharide from fenugreek (*Trigonella foenum-graecum*) seeds. International Journal of Biological Macromolecules, 95(7): 625-64.

[10] Spandan Chaudhary, Pooja Shah Chaudhary,Surendra K Chikara and Mahesh C Sharma. (2018). Review on Fenugreek (*Trigonella foenum-graecum* L.) and its Important Secondary Metabolite Diosgenin, Chaudhary S et al / Not Bot Horti Agrobo, 46(1): 22-31.

[11] Srinivasan K. (2006). Fenugreek (*Trigonella foenum-graecum*): A Review of Health Beneficial Physiological Effects. Food Reviews International, 5(22): 203-224.

[12] Subhapriya S and Gomathipriya P. (2018). Green synthesis of titanium dioxide (TiO_2) nanoparticles by *Trigonella foenum-graecum* extract and its antimicrobial properties. Microbial Pathogenesis, 11(6): 215-220.

[13] Akash Saggam, Girish Tillu, Santosh Dixit, Preeti Chavan-Gautam, Swapnil Borse, Kalpana Joshi and Bhushan Patwardhan. (2020). *Withania somnifera* (L.) Dunal: A potential therapeutic adjuvant in cancer. Journal of Ethnopharmacology, 25(5): 11-27.

[14] Anju Thakur & Himangini. (2015). Revival of germination and vigour of aged seeds of *Withania somnifera* by seed invigoration treatments. Indian Journal of Plant Physiology, 20(5): 391-395.

[15] Aradhana Mishra, Satyendra Pratap Singh, Sahil Mahfooz, Arpita Bhattacharya, Nishtha Mishra, Pramod Arvind Shirke and Nautiyal CS. (2018). Bacterial endophytes modulates the withanolide biosynthetic pathway and physiological performance in *Withania somnifera* under biotic stress. Microbiological Research, 12(5): 17-28.

[16] Bakhtiar Choudhary, Shetty A, and Deepak G Langade. (2015). Efficacy of Ashwagandha (*Withania somnifera* [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. Ayu., 36(1): 63–68.

[17] Bipradut Sil, Chiranjit Mukherjee, Sumita Jha and Adinpunya Mitra. (2015). Metabolic shift from withasteroid formation to phenylpropanoid accumulation in cryptogein-cotransformed hairy roots of *Withania somnifera* (L.) Dunal. Protoplasma, 25(2): 1097–1110.

[18] Ramesh B and Pugalendi KV (2005). Impact of umbelliferone on erythrocyte redox status in STZ-diabetic rats. Yale J Biol Med, 78:133-44.

[19] Murray RR, Granner DK, Mayes PA, and Rodwell VW. (2003). Harper's Biochemistry. Gluconeogenesis and the Control of Blood Glucose, 26th edition. Stamford, Connecticut. Appleton and Lange, 5(7): 153-62.

[20] Helen Yeni Komshian, Marcello Carantoni, Fahim Abbasi and Gerald M Reaven. (2000). Relationship between several surrogate estimate of insulin resistance & quantification of insulin mediated glucose disposal in 490 healthy non diabetic volunteers. Diabetes Care, 23(2): 171-175.

[21] Davidson MB, Navar MD, Echeverry D, Duran P. (2012). U-500 regular insulin, Clinical Diabetes, 30 (2): 76-86.

[22] Jose Castro Perez, Stephen F Previs, David G Mc Laren, Vinit Shah and Gowri Bhat. (2019). Invivo DZO labelling to quantity static & dynamic changes in cholesterol & cholesterol esters by high resolution LC/MS. Journal of lipid research, 7(5): 12-25.

[23] Siedel J, Hagele EO, Ziegenhorn J, and Wahlefeld AW. (1983). Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem, 2(7):1075.

[24] Gordon D and Rifkind B. (1989). Current concepts: High density lipoproteins: the clinical implications of recent studies. N Engl J Med, 3(21):1-5.

[25] Mooks S, Halkes CJM. Bilesen s & castro cabeza s M. (2004). In-vivo regulation of plasma free fatty acids in insulin resistance. Metabolism Science direct., 53(9), 12-17.

[26] Gavin Hamilton, Alexandra N Schlein and Michael S. (2017). In-vivo triglyceride composition of abdominal adipose tissues measured by H MRS at 3T. Jouranal of magnetic resonance imaging, 45(5): 1455-1463.

[27] Nadia Alam, Monzur Hossain, Md Abdul Mottalib, Siti Amrah Sulaiman, Siew Hua Gan and Md Ibrahim Khalil. (2012). Methanolic extracts of *Withania somnifera* leaves, fruits and roots possess antioxidant properties and antibacterial activities. BMC Complementary and Alternative Medicine, 12(7): 79-95.

[28] Uma Chandran and Bhushan Patwardhan. (2013). Network ethnopharmacological evaluation of the immunomodulatory activity of *Withania somnifera*, Journal of Ethnopharmacology, 19(7): 250-256.

[29] Nathiya S, Durga M and Devasena T. (2014). Therapeutic role of *Trigonella foenum-graecum* [Fenugreek] – A Review. International Journal of Pharmaceutical Sciences Review and Research, 27(2): 74-80.

[30] Mohamed M Abdel-Daim, Mabrouk A Abd Eldaim, Abeer GA Hassan. (2015). *Trigonella foenum-graecum* ameliorates acrylamide-induced toxicity in rats: Roles of oxidative stress, proinflammatory cytokines, and DNA damage. Biochemistry and Cell Biology, 93(3): 192-198.

[31] Kedlaya R, Vasudeval DM. (2004). Inhibitiol of lipid peroxidation by botanical studies. Life sciences, 76(1): 21-28.

[32] Nakagami T, Yamamoto Y, Fukushima S, Oya J, Iwamoto Y. (2011). Assessment of cholesterol absorption and synthesis in japanese patients with type-2 diabetes and lipid-lowering effect of ezetimibe. J Diabetes Metab, 2(3): 132- 139.

[33] Nichol A, Chandra Sekar M. (2011). Successful management of extremely insulin-resistant obese diabetic patient with insulin glargine, U-500 regular insulin and pramlintide. J Diabetes Metab, 2(3): 139-143.