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Effects Of '*Citrullus colocynthis*' And '*Cucumis callosus*' On Oral Glucose Tolerance Test

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Abstract
The term diabetes was probably derived by Apollonius of Memphis around 250 BC. Diabetes is first recorded in English, in the form diabete. Diabetes mellitus is derived from the Greek word diabetes meaning siphon – (pass through) and the Latin word mellitus meaning honeyed or sweet. This is because in diabetes excess sugar is found in blood as well as the urine. The oral glucose tolerance test (OGTT) is vital for the characterization of metabolic syndrome, the natural evolution from prediabetes to Type-2 diabetes, and characterization of the metabolic actions of cardiovascular and metabolic drugs. Although the OGTT is rarely used as a diagnostic test for Type-2 diabetes it is widely used as a sensitive indicator of gestational diabetes. Phytochemical screening revealed that the fruits of C. colocynthis L. and C. callosus L. contain tertiary and quaternary alkaloids, glycoside, carbohydrates and saponin components. These results suggest that the ethanolic extract of fruits of C. colocynthis L. and C. callosus L. possesses oral hypoglycemic effect and its combination (C. colocynthis and C. callosus) produced synergistic action at same dose.
Keywords: Diabetes mellitus, Citrullus colocynthis L., Cucumis callosus L., Phytochemical screening, Oral hypoglycemic, Oral glucose tolerance test.

I. INTRODUCTION

Diabetes mellitus is metabolic disorder characterized by increasing blood glucose caused by a relative or absolute deficiency of insulin. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Insulin deficiency causes hyperglycemia [1]. Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, heart disease, and stroke, including sexual dysfunction [2].

Table 1: Diabetes mellitus has divided into four categories:

Type I (Insulin Dependent Diabetes Mellitus)	It is characterized by an absolute deficiency of insulin caused by massive beta cells lesions or necrosis [3].
Type II (Non-Insulin Dependent Diabetes Mellitus)	Type-2 diabetes is frequently accompanied by target organ insulin resistance that limits responsiveness to both endogenous and exogenous insulin [4].
Type III (Drug Induced Diabetes)	A number of drugs, corticosteroids, thiazids diuretics, beta blockers, antipsychotics, statin and protease inhibitors have been linked with increased risk development of diabetes [5].
Type IV (Gestational Diabetes)	In this type of diabetes blood sugar levels are elevated usually in second or last trimester of pregnancy and usually resolved during the postpartum period [6].

II. SYMPTOMS OF DIABETES

Table 2: Symptoms of Diabetes mellitus.

Heart and Blood Vessel Damage	Diabetes increases risk of cardiovascular disease, including coronary artery disease, heart attack, atherosclerosis and hypertension.
Neuropathy	Excess sugar, injure the wall of capillaries, this can cause tingling, numbness, burning that usually begins at the tip of the toes and gradually spread upward.
Nephropathy	Diabetes can damage tiny blood vessels of kidney that filter waste from blood by this severe damage can lead to kidney failure or irreversible end-stage kidney disease, which requires dialysis or a kidney transplant
Eye Damage	Diabetes can damage the blood vessels of the retina which potentially leading to blindness. Diabetes also increases the risk of cataracts and glaucoma.
Osteoporosis	Diabetes lowers the normal bone mineral density, increasing risk of osteoporosis [7].
Alzheimer's Disease	Type-2 diabetes may increase the risk of Alzheimer's disease and vascular dementia by blocking blood flow to the brain or causing strokes.
Miscarriage and Stillbirth	If blood sugar level is uncontrolled during the early stages of pregnancy, there is also an increased risk of the baby developing a birth defect [8,9].
Preeclampsia	Gestational diabetes raises risk of high blood pressure, as well as, preeclampsia — a serious complication of pregnancy that causes hypertension and other symptoms that can pressurize the lives of both mother and baby [10].

III. PLANT USED IN STUDY

- ***Citrullus colocynthis* L. (Indrayan, Bitter apple):**

Citrullus colocynthis L. is a desert viny plant that grows in sandy arid soils. It is native to the Mediterranean Basin and Asia and is distributed among the west coast of northern Africa, eastward through the Sahara, Egypt until India [11].

Table 3: Botanical Classification of *C. colocynthis L*.

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyte
Division	Magnoliophyta
Class	Magnoliopsida
Order	Violales
Family	Cucurbitaceae
Genus	Citrullus
Species	Colocynthis

The main chemical contain of fruit pulp is colocynthin (the bitter principle up to 14 %), colocynthein (resin), colocynthetin, quercetin, flavonoids, pectin gum and triterpen glycosides. Seed contain a fixed oil (17 %), albuminiods (6 %), vitamin B1, B2 and niacin [12, 13, 14].

Medicinal Uses:

1. Antimycobacterial Activity:
Citrullus colocynthis L. traditionally used natural remedy for tuberculosis. It has antitubercular activity against drug sensitive and drug resistant Mycobacterium tuberculosis and Mycobacterium other than tuberculosis (MOTT) bacilli [15].

2. Anti-inflammatory Activity:
C. colocynthis L. fruit and seed at immature state for anti-inflammatory activity using the carrageenan induced paw edema assay in rats. Therefore, *C. colocynthis* could be a potential useful for further evaluation for inflammatory diseases [16].

3. Hypolipidemic:
A daily intake of 300 mg day of powdered seeds of *C. colocynthis* can lower the triglyceride and cholesterol concentration significantly in non diabetic hyperlipidemic patients [17].

4. Anti – Alopecia:
C. colocynthis has positive activity in androgen-induced alopecia. Petroleum ether extract of *C. colocynthis* was applied topically for its hair growth-promoting activity [18].

5. Antioxidant and Free Radical Scavenging:
Methanolic fruit extract of *C. colocynthis* L. was screened to evaluate its free radical scavenging effect. The highest antioxidant and free radical scavenging ability of the fruit extract was observed at a concentration of 2500 microg ml (-1) [19].

- ***Cucumis callosus L*. (Wild melon):**

Native to dry areas of India being common throughout the south America, areas of Thailand, Egypt and Africa, eastward through Iran to India and other parts of tropical Asia [20].

Table 4: Botanical classification of *Cucumis callosus L*.

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyte
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Cucumis
Species	Callosus

The main chemical contents of fruit pulp is alkaloids, carbohydrates, amino acids, glycosides, fixed oils and fat, tannins, phytosterols, flavonoids and saponins [21].

Medicinal Uses:

1. **Analgesic Activity:**

Analgesic activity was assessed by acetic acid induced writhing test and tail immersion method.

2. **Anti-Inflammatory Activity:**

Methanolic extract of *C. callosus* shows anti-inflammatory activity in carrageenan induced rat paw edema.

3. **Antioxidant Activity**:

Alcoholic extract of seeds of *Cucumis callosus* Cogn possesses the significant antioxidant activity. Antioxidant activity of plant extract showed radical potent scavenging activity and also antioxidant activity help to reduce pain [22].

IV. COLLECTION AND AUTHENTICATION OF PLANTS

The fruits of *C. colocynthis* L. and *C. callosus* L. were purchased from local herb store of Sonebhadra and authenticated from Obra Degree College, Sonebhadra, U.P. The fruits were cleaned, washed, chopped to small pieces and then dried at room temperature. The dried fruit was powdered and passed from 40-50 mesh size sieves.

EXTRACTION OF PLANTS

100 gm of fruits powder were taken in soxhlet apparatus and extracted into 800 ml ethanol (90%) for 48 hours. It was shaken frequently during the first 6 hours and allowed to stand for 18 hours. There after it was filtered rapidly and then filtrate was evaporated with the help of vacuum evaporator at the temperature of 450C.

PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACT

The extracts were subjected to preliminary qualitative test for the presence of carbohydrates, amino acid, proteins, steroids, glycosides, alkaloids, tannins, phenolic compound and flavonoids [23].

ANIMALS

Experimental rats were processed in accordance to Committee for Purpose of Supervision and Control of Experimental Animal (CPCSEA registration no. 1044/c/07/CPCSEA). Albino wistar male rats weighing 150-200 g was used for the study. They were maintained in the animal house for experimental purpose. The animals were maintained under controlled conditions of temperature (22 ± 3°C), humidity (30 to 70 %) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress.

EXPERIMENTAL DESIGN

Oral Glucose Tolerance Test (OGTT):

Overnight fasted (18 h) normal rats are divided into five groups each group containing 6 animals (n= 6).
Group I - Normal Control (Saline)
Group II - *Citrullus colocynthis* L. ethanolic extract (300 mg/kg, p.o) [24]
Group III - *Cucumis callosus* L. ethanolic extract (500 mg/kg, p.o) [25]
Group IV - *Citrullus colocynthis* L. + *Cucumis callosus* L. ethanolic extract (300 + 500 mg/kg, p.o)

Group V – Glibenclamide (10 mg/kg, p.o) [26].
After 30 min. of respective administration, the rats of all groups orally treated with 2 g/kg of glucose. Blood samples were collected from retro orbital sinus just prior to glucose administration and at 30, 60, 90 and 120 min. after glucose loading. Blood glucose levels were measured immediately by using glucometer. [27]

STATISTICAL ANALYSIS
Blood glucose levels were expressed in mg/dl as mean ± SD. The data were statistically analyzed using ANOVA followed by Dunnet's test.

V. RESULT

Preliminary Phytochemical Analysis:

The preliminary phytochemical screening of extract of *Citrullus colocynthis* L. gave positive tests for carbohydrates, resins, saponin, anthraquinone, flavonoids, steroids and alkaloids and extract of *Cucumis callosus* L. gave positive tests for carbohydrate, alkaloid, protein, saponin, flavonoids, tannin and glycosides.

Table 5: Phytochemical screening of *Citrullus colocynthis* L. and *Cucumis callosus* L.

fruits extract Tested For	**Tests**	***C. colocynthis* L. extract**	***C. callosus* L. extract**
Carbohydrate	Molisch	(+)	(+)
Alkaloid	Wagner's	(-)	(+)
Protein	Biuret	(-)	(+)
Steroids	Salkowski	(+)	(-)
Glycosides	Sodium hydroxide	(+)	(+)
Saponins	Foam	(+)	(+)
Tannins	Ferric chloride	(-)	(+)
Flavonoids	Ferric chloride	(+)	(+)
Resin	Ferric chloride	(+)	(-)

(+) = Present, (-) = Absent

Oral Glucose Tolerance Test:

In the Oral Glucose Tolerance Test the effect of ethanolic fruit extract of *Citrullus colocynthis* L., *Cucumis callosus* L. and their combination therapy at half dose is shown in below graph.

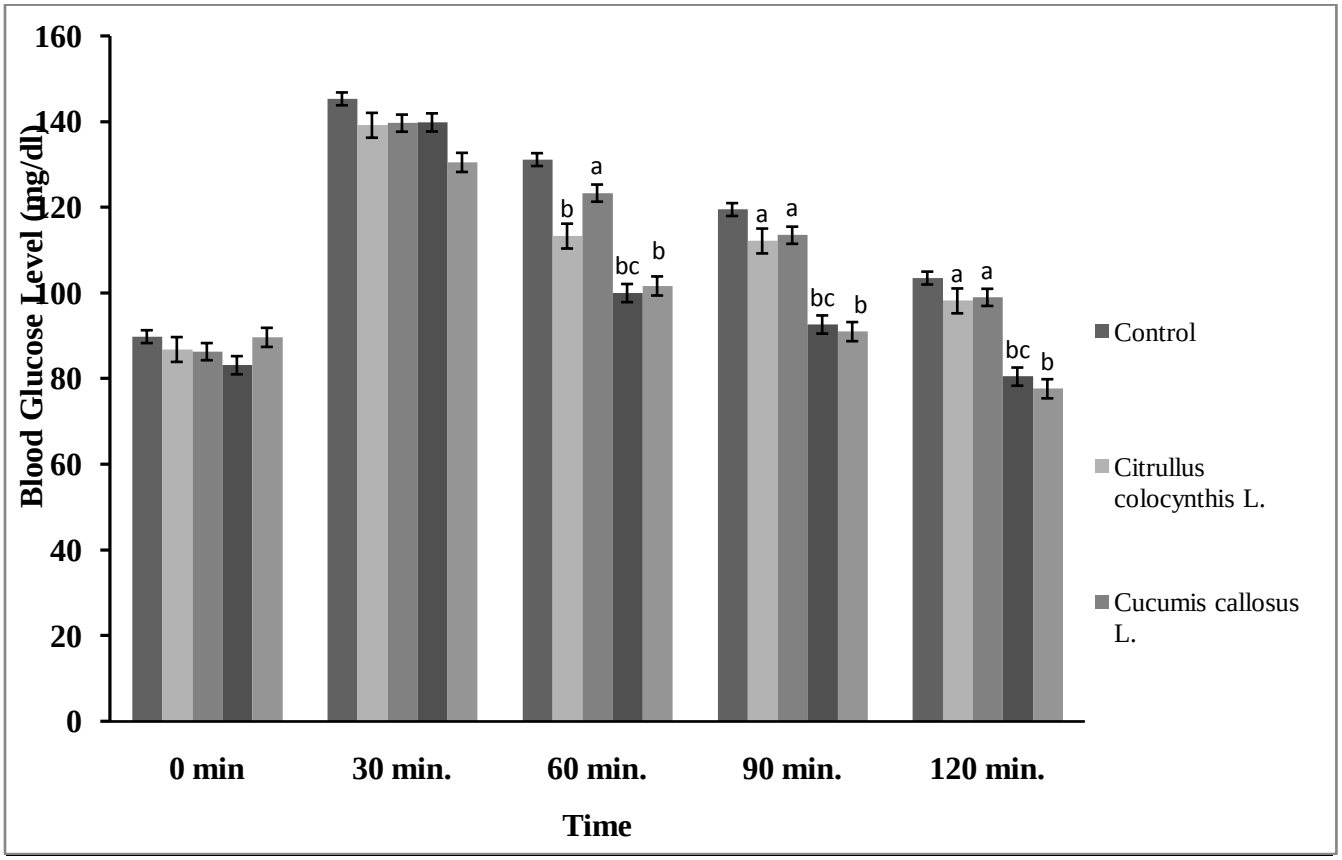

Figure 1: Effect of ethanolic fruit extracts on the glucose-induced hyperglycemia in normal rats. The results are expressed as means ± SEM. a=P<0.05, b= P<0.01, when experimental groups are compared with control group and c= not significant when combination group is compared with positive group, Glibenclamide. (ANOVA followed by Dunnet's test)

VI. DISCUSSION

Treatment employing with two or more herbs in combination has the advantage of producing maximum therapeutic efficacy than the single herb treatment. Combination therapy may provide synergistic, potentiative pharmacological properties within themselves because of presence of vast range of phytobioactive constituents [28].

The present work was focused to establish the therapeutic efficacy and probable benefit associated with the combination therapy at same doses of ethanolic fruit extracts of *Citrullus colocynthis* L. and *Cucumis callosus* L. in comparison to their individual treatments and standard drug, Glibenclamide.

Citrullus colocynthis L. and *Cucumis callosus* L. attributed to the vital phytoconstituents contained like – carbohydrate, glycosides, flavonoids and saponins. The anti-oxidant and free radical scavenging properties of flavonoids and other polyphenolic compounds of the both plants might be responsible for the antidiabetic activity in the combination therapy as well as in the individual treatments of both the plants.

In the present work showed that the ethanolic fruit extract of *Citrullus colocynthis* L. (300 mg/kg), *Cucumis callosus* L. (500 mg/kg) and the combination therapy of both plant extract at same dose (300+500 mg/kg) respectively reduced blood sugar slightly significant (P<0.05) and significantly (P<0.01) in normoglycemic rats at 60, 90 and 120 min.

VII. CONCLUSION

In conclusion, the experimental evidence obtained in the present laboratory animal study indicate that ethanolic fruit extract of *Citrullus colocynthis* L. and *Cucumis callosus* L. possess anti-diabetic properties and combination of these plant extract at same dose shows synergistic action, due to presence of major phytochemical constituents such as alkaloids, carbohydrates, glycosides and phenolic, which suggest the presence of biologically active components which may be worth further investigation and elucidation.

The observed results proved that synergistic antihyperglycemic effect of the combination therapy of same dose of *Citrullus colocynthis* L. and *Cucumis callosus* L. This also gives an opportunity to reduce the dose of herbs in order to avoid the burden of herbal over dosing.

BIBLIOGRAPHY

[1] Tanko, Y, Yerima, M, Mahdi, M.A, Yaro, A.H, Musa, K.Y and Mohammed, A, 2008. Hypoglycemic activity of methanolic stem bark of *Adansonnia digitata* extract on blood glucose levels of Streptozocin-Induced diabetic wistar rats. *International Journal of Applied Research in Natural Products*, Vol. 1, Issue 2, pp. 32-36.

[2] Tripathi, V, Verma, J, 2014. Current updates of Indian antidiabetic medicinal plants. *International Journal of Research in Pharmacy and Chemistry*, Vol. 4, Issue 1, pp. 114-118.

[3] Wang, T.J, Larson, M.G, Vasan, R.S, Cheng, S, Rhee, E.P and Cabe, M.E, 2011. Metabolite Profiles and the Risk of developing diabetes. *Nature Medicine*, Vol. 17, Issue 4, pp. 448-453.

[4] Vionnet, N, Stoffel, M, Takeda, J, Yasuda, K, Bell, G.I and Zouali, H, 1992. Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature*, Vol. 356, pp. 721-722.

[5] Tripathi, V, Verma, J, 2014. Different models used to induce diabetes: A Comprehensive Review. *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 6, Issue 6, pp. 29-32.

[6] Strojek, K, 2003. Features of macrovascular complications in type-2 diabetic patients. *Acta Diabetologica*, Vol. 40, pp. 334-337.

[7] Casqueiro, J, Casqueiro, J and Alves, C, 2012. Infections in patients with diabetes mellitus: a review of pathogenesis. *Indian Journal of Endocrinology and Metabolism*, Vol. 16, Issue 1, pp. 27-36.

[8] Holt, G.I., 2004. Diagnosis, epidemiology and pathogenesis of diabetes mellitus an update for Psychiatrists. *British Journal of Psychiatry*, Vol. 184, pp. 55- 63.

[9] Bacha F, Lee S, Gungor N, Arslanian SA, 2010. From pre-diabetes to type 2 diabetes in obese youth: pathophysiological characteristics along the spectrum of glucose dysregulation. Diabetes care, 33(10), pp. 2225-31.

[10] Cheung, K.W and Wong, S.F, 2011. Gestational Diabetes Mellitus Update and Review of Literature. *Reproductive System and Sexual Disorder*, pp. 1-6.

[11] Borhade, P, Deshmukh, T, Patil, V and Khandelwal, K, 2013. Review on *Citrullus colocynthis*. *International Journal of Research in Pharmacy and Chemistry*, Vol. 3, Issue 1, pp. 46-53.

[12] Agarwal, V, Sharma, A.K, Upadhyay, A, Singh, G and Gupta, R., 2012. Hypoglycemic effects of *Citrullus colocynthis* roots. *Acta Poloniae Pharmaceutica* – Drug Research, Vol. 69, Issue 1, pp. 75-79.

[13] Gurudeeban, S, Satyavani, K and Ramanathan, T, 2010. Bitter Apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. *Asian Journal of Plant Science*, Vol.1, pp. 1-8.

[14] Yoshikawa, M, Morikawa, T, Kobayashi, H, Nakamura, A, Matsuhira, K and Nakamura, S, 2007. Bioactive saponins and glycoside. XXVII. Structure of new Cucurbitane – type triterpen glycoside and anti allergic constituents from *Citrullus colocynthis*. *Chemical and Pharmaceutical Bulletin*, Vol. 3, pp. 428-434.

[15] Rasool, K and Jahanbakhsh, T, 2011. Anti candidal screening and antibacterial of *Citrullus colocynthis* in South East of Iran. *Journal of Horticulture and Forestry*, Vol. 3, Issue 13, pp. 392–398.

[16] Saba, A.B and Oridupa, A.O, 2010. Search for a novel antioxidant, anti-inflammatory/ analgesic or anti-proliferative drug: Cucurbitacin hold the ace. *Journal of Medicinal Plants Research*, Vol. 4, Issue 25, pp. 2821-2826.

[17] Daradka, H, Almasad, M.M, El-Banna, N.M and Samara, O.H, 2007. Hypolipidemic effects of *Citrullus colocynthis* L. in rabbits. *Pakistan Journal of Biological Science*, Vol. 10, Issue 16, pp. 2768-2771.

[18] Dhanotia, R, Chauhan, N.S, Saraf, D.K and Dixit, V.K, 2011. Effect of *Citrullus colocynthis* Schrad fruits on testosterone-induced alopecia. *Natural Product of Research*, Vol. 25, Issue 15, pp. 1432- 1443.

[19] Kumar, S, Kumar, D, Saroha, K, Singh, N and Vashishta, B, 2008. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. Methanolic fruit extract. *Acta Pharmaceutica*, Vol. 58, Issue 2, pp. 215-220.

[20] Chand, T, Bhandari, A, Kumawat, B.K, Sharma, A, Bansal, V.K and Pareek, A, 2012. Phytochemical Investigation of seed of *Cucumis callosus* (Rottl.) Cogn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, Vol. 3, Issue 2, pp. 570-576.

[21] Arora, R, Kaur, M and Gill N.S, 2011. Antioxidant activity and Pharmacological evaluation of *Cucumis melo var. agrestis* Methanolic Seed Extract. *Research Journal of Phytochemistry*, Vol. 5, Issue 3, pp. 146-155.

[22] Chand, T, Bhandari, A, Bhupendra, K.K, Pawan, K, Sharma, S and Verma, R, 2012. In vitro antioxidant activity of alcoholic extract of seeds of *Cucumis callosus* (Rottl.) Cogn. *American Journal of Pharmatech Research*, Vol. 2, Issue 3, pp. 985-992.

[23] Turner, M.A, 1965. Screening methods in pharmacology. *Academic Press*, pp. 26.

[24] Agarwal, V, Sharma, A.K, Upadhyay, A, Singh, G and Gupta, R., 2012. Hypoglycemic effects of *Citrullus colocynthis* roots. *Acta Poloniae Pharmaceutica* – Drug Research, Vol. 69, Issue 1, pp. 75-79.

[25] Bidkar JS, Ghanwat DD, Bhujbal MD and Dama GY, 2012. Antihyperlipidemic activity of Cucumis melofruit peel extracts in high cholesterol diet induced hyperlipidemia in rats. *J Complement Inteqr Med*, Vol. 9, Issue 22, pp.1515-1553.

[26] Sharma, V.K, Kumar, S, Patel, H.J and Hugar, S, 2010. Hypoglycemic activity of *Ficus Glomerata* in alloxan induced diabetic rats. *International Journal of Pharmaceutical Sciences Review and Research*, Vol. 1, Issue 2, pp. 18-22.

[27] Heroor, S, Beknal, A and Mahurkar, N, 2013. Synergistic activity of bark extracts of *Pongamia glabra* and *Ficus Glomerata* in alloxan-induced diabetic rats. *World Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 2, Issue 6, pp. 6640-6652.

[28] Raghavendra, H.G, Sen, S, Sivarami, Y.R, Sridhar, C, Chakraborthi, R and Suchitra, B, 2011. Synergistic activity of *Tribulus terrestris* and *Annona squamosa* extracts against alloxan induced diabetes and hyperlipidemia in rats. *Pharma Science Monitor*, Vol. 2, Issue 2, pp. 12-22.

