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ANTIDIABETIC ACTIVITY OF THE COMBINATION OF EUGENIA ZAMBOLLANA AND BITTER GUARD ON STREPTOZOCINE INDUCED DIABETES IN ALBINO RATS

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ABSTRACT
Eugenia Zambollana collected from the Botanical garden of R.K. Pharmacy College Kashipur Surai Sathiyaon Azamgarh, Utter Pradesh 500 gm seeds dried in sun light yielded 450 gm of seed powder. Then powders are macerated in ethanol 96% (one liter per day) for three days extraction. Subsequently, it was filtered and concentrated to yield an ethanol extract, and then diluted in distilled water for being administered in the rats. (*S.B.Sridhar et. Al 2005*). Bitter guard collected from the Botanical garden of R.K. Pharmacy College Kashipur Surai Sathiyaon Azamgarh .In the month of June then dried and ground. The fruit flesh power macerated in ethanol 95% one liter per day for three day extraction. Subsequently it was filtered and concentrated to yield an ethanolic extract and then dilute in distilled water for being administration in rat. (*Mo. Zahrul et.al 2012l*)

KEYWORDS: Eugenia Zambollana, Biter Guard, Streptozocine Induced, Diabetes.

INTRODUCTION
Generally Diabetes mellitus (DM) affects more than100 million people worldwide (6% of the population) and in the next 10 years it may affect about five times more people than it does now(Aditi Chaturvedi et.al 2009). The prevalence rate of diabetes is estimated to be 1–5% in India. Diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia).(MediLexicon International).

	Normal	Diabetes
Normal Diabetes	80-99 mg/dl	126 mg/dl and above
Fasting blood sugar	80-193 mg/dl	200 mg/dl and above
Random blood suger	80-193 mg/dl	200 mg/dl and above

TYPES OF DIABETES
- Type 1 diabetes occurs most frequently in children and young adults, although it can occur at any age. Type 1 diabetes accounts for 5-10% of all diabetes in the United States. There does appear to be a genetic component to Type 1 diabetes, but the cause has yet to be identified.
- Type 2 diabetes is much more common and accounts for 90-95% of all diabetes. Type 2 diabetes primarily affects adults, however recently Type 2 has begun developing in children. There is a strong correlation between Type 2 diabetes, physical inactivity and obesity.

SYMPTOMS
- Blurred vision
- Unusual thirst
- Frequent urination
- Slow-healing cuts
- Unexplained tiredness
- Rapid weight loss (Type 1 diabetes)
- Erectile dysfunction
- Numbness or tingling in hands or feet

CAUSES

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhan in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin. It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

"Brittle" diabetes, also known as unstable diabetes or labile diabetes, is a term that was traditionally used to describe the dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin - dependent diabetes. This term, however, has no biologic basis and should not be used. Still, type 1 diabetes can be accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis, and sometimes with serious hypoglycemia. Other complications include an impaired counter regulatory response to hypoglycemia, infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes.

Type 1 diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet. There is some evidence that suggests an association between type 1 diabetes and Coxsackie B4 virus. Unlike type 2 diabetes, the onset of type 1 diabetes is unrelated to lifestyle.

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type.

In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

Type 2 diabetes is due primarily to lifestyle factors and genetics. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet, stress, and urbanization. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders. Those who are not obese often have a high waist–hip ratio.

Dietary factors also influence the risk of developing type 2 diabetes. Consumption of sugar-sweetened drinks in excess is associated with an increased risk. The type of fats in the diet is also important, with saturated fats and trans fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk. Eating lots of white rice appears to also play a role in increasing risk. A lack of exercise is believed to cause 7% of cases.

Infection

Diabetics are highly susceptible to infection, especially by bacteria and fungi, possibly because phagocyte activity is depressed by insufficient intracellular glucose.

Infection may cause

- Complications in areas affected by peripheral neuropathy and changes in blood vessels, e.g. in the feet when sensation and blood supply are impaired
- Boils and carbuncles
- Vaginal candidiasis (thrush)
- Pyelonephritis.

Renal Failure

This is due to changes affecting the walls of small blood vessels and infection, and is a common cause of death indiabetics (Anne Waugh et al 2003)

Current Treatment of Diabetes

Through Allopathic Drugs:

- Insulin
- Oral hypoglycemic

I. Sulfonylurea

- Tolbutamide
- Glibenclamide

I. **Biguanide**

- Metformin

II. Thiazolidinedione

- Rosiglitazone

Through Herbal Drugs

- Acacia
- Aloe
- Brassica
- Cassia
- Gymnema
- Rauwalfia
- Tinospora

Tinospora cordifolia (Willd.) Miers, which is known by the common names guduchi and giloy, is a herbaceous vine of the family Menispermaceae indigenous to the

tropical areas of India, Myanmar and Sri Lanka and has a long history of use in Ayurvedic medicine (the traditional medicine of India) as an antidiabetic, anticancer,immune stimulating, cholesterollowering, and liver protectant.T. cordifolia is sold by herbalists in open market for this purpose. The stem extract of T. cordifolia has an antidiabetic effect. However, these have not yet been incorporated into pharmaceutical dosage forms. Identity of bioactive compounds and study of their mechanisms of action are very recent. Therefore, the present study was carried out to identify primary compounds present in the leaves of T. cordifolia and to provide proof for the antidiabetic nature of plant extract and its component magnoflorine through investigation of their ARI activity in streptozotocin (STZ)-induced diabetic rats.

OBJECTIVE AND PLAN OF WORK

Aim & Objective

The seed of Eugenia Jambolana, And Biter Guard is being used traditionally from centuries India for the treatment of diabetes. Antidiabetic Activity of the Combination of Eugenia Zambollana and Bitter Guard on Streptozocine Induced Diabetes in Albino Rats.

Plan of Work

- Collected the Eugenia Zambollana and Bitter guard seed from Botanical garden R.K. Pharmacy College Kashipur Surai Sathiyaon Azamgarh Uttar Pradesh.
- Plant Authentifection.
- Selected the group of Albino rat.
- Streptozocine Induced Diabetes In Albino Rats
- Perform the combinational Study of Eugenia Zambollana and Bitter guard seed compare to standard drug Metformin.
- Conclusion.

MATERIALS AND METHODS

Eugenia Zambollana collected from the Botanical garden of R.K. Pharmacy College Kashipur Surai Sathiyaon Azamgarh , Utter Pradesh 500 gm seeds dried in sun light yielded 450 gm of seed powder. Then powders are macerated in ethanol 96% (one liter per day) for three days extraction. Subsequently, it was filtered and concentrated to yield an ethanol extract, and then diluted in distilled water for being administered in the rats. (S.B.Sridhar et. Al 2005).

Bitter guard collected from the Botanical garden of R.K. Pharmacy College Kashipur Surai Sathiyaon Azamgarh .In the month of June then dried and ground. The fruit flesh power macerated in ethanol 95% one liter per day for three day extraction. Subsequently it was fitered and concentrated to yield an ethanolic extract and then dilute in distilled water for being administration in rat. (Mo. Zahrul et.al 2012l).

ANIMAL

A laboratory rat is a rat of the species Rattus norvegicus(brown rat) which is bred and kept for scientific research. Laboratory rats have served as an important animal model for research in psychology, medicine, and other fields.

Fig. 3: Wister Albino Rat.

The first time one of these albino rat mutants was brought into a laboratory for a study was in 1828, in an experiment on fasting. Over the next 30 years rats were used for several more experiments and eventually the laboratory rat became the first animal domesticated for purely scientific reasons. Streptozotocin drug use in albino rat for enhances the diabetic activity in my experimental project. Healthy Wistar albino rats (150 – 250 gm body weight).

Experimental Design

- Group 1 – The rats received oral saline 10 ml/kg BW (control group).
- Group 2 – The rats received a single dose of the combination of bitter gourd (60 mg/200 g BW) and Eugenia jambolana (50 mg/200 g BW) with a ratio of 50:50 w/w percentage, orally.
- Group 3 – The rats received a single dose of the combination of bitter gourd (60 mg/200 g BW) and Eugenia jambolana (50 mg/200 g BW) with a ratio of 75:25 w/w percentage, orally.
- Group 4 – The rats received a single dose of the combination of bitter gourd (60 mg/200 g BW) and Eugenia jambolana (50 mg/200 g BW) with a ratio of 25:75 w/w percentages orally.
- Group 5 – The rats received metformin dose 9. 05 mg/kgBW, orally.

The combination of bitter gourd and Eugenia jambolana, metformin or glibenclamide was per orally administered at the day 16. One hour after administration, the the blood samples were collected from retro-orbital plexus, and centrifuged at 1,000 rpm for 10 min. Serum was removed for determination of glucose by GOD-PAP Reagent Kit (DiaSys).(Muhammad Zahrul Mujahid et al 2013)

PLANT PROFILE

- Eugenia jambolana

CLASSIFICATION
Kingdom: Plantae

Syn. Syzigium cumini (L)
Division: Magnoliophyta

Fig-2.

Class: Magnoliosida
Family: Myrtaceae
Genus: *Eugenia*
Species: *jambolana Lam.*

Synonyms
- English: Black plum, Jaman
- Hindi: Jamun
- Marathi: Jambhul
- Kannad: Ama-Phala, Jambunerale, Nayinerale
- Sanskrit: Brahaspati, Jambavam
- Telugu: Goyya-Pandu, Jam-Pandu

Botanical Description
It has been valued in Ayurveda and Unani system of medication used. It is a large evergreen tree up to 30 m high. Bark pale brown, slightly rough on old stems.Leaves opposite, simple, entire, elliptic to broadly oblong, smooth, glossy, somewhat leathery, 7.5-15 cm long, short pointed at tips. Flowers white 7.5-13 mm across in branched clusters at known as stem tips, calyx cuplike; 4 petals, fused into a cap; many stamens. Fruit variable in size up to 2.5 cm long, ellipsoid or oblong, crowned with truncate calyx-limb, black with pink juicy pulp. It is widely distributed throughout all over india.

Nutritional value per 100 g (3.5 oz)		
Energy	251 kJ (60 kcal)	
Carbohydrates **Dietary fiber**	14g 0.6g	
Fat	0.23g	
Protein	0.995g	
Vitamins **Thiamine (B1)** **Riboflavin (B2)** **Niacin (B3)** **Vitamin B6** **Vitamin C**	 0.019mg 0.009mg 0.245mg 0.038mg 11.15mg	 2% 1% 2% 3% 14%
Water	87.75 g	

USE
- The seed powder of *S.* cumini *(*Eugenia jambolana) is used by the diabetic patients to control the blood sugar level (B. *CHAUDHARY et al Jan-March 2012).*
- It is much pharmacological activity of anti-inflammatory action and lowering blood cholesterol (Shweta Sharma et al. IRJP 2012, 3).
- The fruit is acrid, sweet, cooling and astringent to the bowels and removes bad smell form mouth, biliousness, stomachic, astringent, diuretic and antidiabetic.
- The bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and used for the treatment of sore throat, bronchitis, asthma, thirst, biliousness, dysentery and ulcers. It is also a good blood purifier. (Muniappan Ayyanar et al 2012).
- Bitter gaurd consists of fresh fruit of Momordica charantia Linn. (Fam. Cucurbitaceae); a monoecious climber found throughout the country often undercultivation, upto an altitude of 1500 m. (Ayurvedic Pharmacopoeia of India, Part-I, Second Volume Page n.89).

MATERIAL AND METHOD FOR E.JAMBOLLANA
Habitat of Plant
Syzygium cumini, known as jambul, jambolan, jamblang or jamun, is an evergreen tropical tree in the flowering plant family Myrtaceae also called Indian black berry. It grow india, Pakistan, Sri Lanka, Africa, Mexico, Kenya, Nepal, Malaysia, Australia.

Soil Condition For Cultivation
Mostly soil is growing for favorable condition. All kinds of soils are fine. Well drained fertile soil with good organic matter content is best. However Eugenia jambolana grow well in moist soil having good drainage soil ph 6 -7.5 is fine.

Syzygium cumini trees start flowering from March to April. The flowers are fragrant and small, about 5 mm in diameter. The fruits develop by May or June and resemble large berries; the fruit of Syzygium species is described as "drupaceous". The fruit is oblong, ovoid. Unripe fruit looks green. As it matures its color changes to pink, then to shining crimson red and finally to black color.

Collection of Plant Material
Fresh matured seed of E. Jambolana were collected from botanical garden of our institute R. K. Pharmacy college Kashipur Surai Sathiaon Azamgarh garden and were identified by Pharmacognosy expert. At the time of collection standard herbarium record sheets were completed with the name of the collector, collection no, date locality and local name.

Authentication
Prof.N.K Dubey department of Botany, Banasas Hind University, Varanasi was authenticated Eugenia jambolana Lam .syn Syzygium cumini (Linn.)Skeels.Voucher spacimen Number Myr/2015/2 Myrtaceae.

Extraction of Plant Material
Eugenia Jambolana collected from the Botanical garden of of R.K. Pharmacy College Kashipur Surai Sathiaon, Azamgarh Utter Pradesh. 500 gm seeds dried in sun light yielded 450 gm of seed powder. Then powders are macerated in ethanol 96% (one liter per day) for three days extraction. Subsequently, it was filtered and concentrated to yield an ethanol extract, and then diluted in distilled water for being administered in the rats.

Phytochemaical Examination of Drug
The seed extracts of Syzygium cumini were analysed for the presence of alkaloids, glycosides, tri terpenoids, steroids, saponins, flavonoids, tannins and carbohydrates according to standard methods.

- **Test for Alkaloids**

2 ml of dilute hydrochloric acid was added to the 5 ml of extract then treated with Dragondroff's reagent, appearance of an orange brown precipitate showed the presence of alkaloids.

- **Test for Glycosides**

The extract was hydrolysed with dilute hydrochloric acid for few hours on a water bath.1 ml of pyridine and a few drops of sodium nitroprusside solution were added. Then 2-3 drops of dilute NaOH was mixed. Pink colour produced which turn into red indicated presence of glycosides.

- **Test for Triterpenoids**

About 5 ml of extract was mixed in 2 ml of chloroform; 2 ml of acetic anhydride and a few drops of conc. H2SO4 was added. Reddish violet colour indicated the presence of triterpenoids.

- **Test for Steroids**

10ml of chloroform was mixed with 2ml of extracts and conc. H2SO4 was added to form lower layer. A reddish yellow colour at the interface was an indicative of the presence of steroidal ring.

- **Test for Saponins**

15 ml of distilled water was added to the extract and shaken vigorously until formation of a stable persistent froth which indicates presence of saponins.

- **Test for Flavonoids**

Few drops of dilute NaOH was mixed with 2 ml of extract. A yellow solution that turns colourless showed the presence of flavonoids.

- **Test for Tannins**

In a test tube containing little quantity of extract few drops of 1 % lead acetate were added.Yellow precipitate appeared it showed the presence of tannins.

Photochemical test of seed extract of Syzygium cumini

Type of extract			
S.N	**Phytoconstituents**	**Ethyl acetate extract**	**Methanol extract**
1	Alkaloids	+	+
2	Glycoside	+	+
3	Triterpinoids	+	+
4	Steroid	+	+
5	Saponins	++	++
6	Flavonoids	++	++
7	Tannins	+	+
8	Carbohydrates	_	_

+ indicates presence of the Phytoconstituents.
++ indicates present in more quantity of the Phytoconstituents.
– indicates absence of the Phytoconstituents.

MATERIAL AND METHOD FOR
Bitter guard (Momordica charantia)
Habitat of plant

- Momordica charantia, known as bitter melon, bitter gourd, bitter squash, or balsam-pear.Bitter gourd tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit.

Soil Condition For Cultivation
Soil should be fertile, but well-drained, with a pH of 5.5 to 6.7. Adding composted manure or compost to enrich soil results in good yields. Tropical and subtropical regions. Warm and dry weather with 300c-350c temperature is optimum. The seed is sown from January to March for summer season crop, June-July for rainy season crop in the plains and March to June in the hills.

The Bitter gourd fruits are harvested when they are immature; the fruits are harvested when they reach marketable size. Although the fruit is harvested before it fully ripens, it should be allowed to attain a good size and colour. They should be firm, and the outside color glossy green. Its surface should not lose its bright and glossy appearance. At harvesting, the calyx and stem-end are left attached to the fruit. Large, round varieties should be handled with care. Over mature fruits are spongy and seedy.

Authentication
Prof.N.K Dubey department of Boteny, Banasas Hind University, Varanasi was authenticated Momordica charintia Linn(Voucher spacimen Number Cucurbit/2015/1.

Extraction of Plant Material

Bitter guard collected from the Botanical garden of R.K. Pharmacy College Kashipur Surai Sathiyaon Azamgarh .In the month of June then dried and ground. The fruit flesh power macerated in ethanol 95% one liter per day for three day extraction. Subsequently it was fitered and concentrated to yield an ethanolic extract and then dilute in distilled water for being administration in rat.

Phytochemaical Examination of Drug

- **Test for identification of Alkaloids**

About 0.5 gm of methanol extract was taken in a test tube and was diluted and homogenized with 10 ml distilled water, dissolved in 20 ml dilute HCl solution and clarified by filtration. The filtrate was tested with Drangendroff's and Mayer's reagent. The treated solution was observed for precipitation of white or creamy colour.

- **Test for identification of Flavonoids**

About 0.5 gm of extract was introduced into 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered. About 4 ml of the filtrate was shake]-\n with 1 ml 1% aluminium chloride solution and incubated for 10 min. Formation of yellow colour in the presence of1 ml dilute ammonia solution indicated the presence of flavonoids.

- **Test for identification of Phenols**

About 0.5 gm of extract was taken in a test tube, mixed with 100ml distilled water and heated gently. To this, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour.

- **Test for identification of Saponins**

About 0.5 gm of methanol extract was taken in a test tube and 5 ml distilled water was added to it. The solution was shaken vigorously and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

- **Test for identification of Steroids**

About 0.5 gm of methanol extract was taken in a test tube and 2 ml of acetic anhydride was added to it and 2 ml of sulphuric acid was added by the sides of the test tube and observed for the colour change to violet or blue green.

- **Test for identification of Tannins**

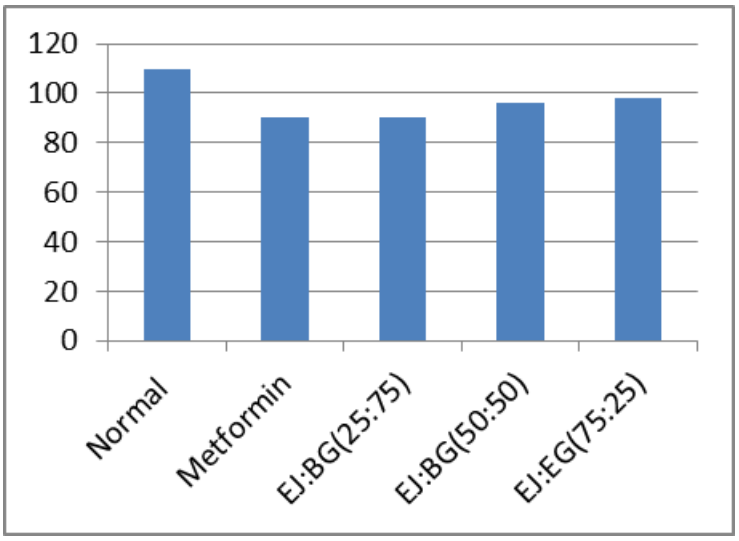
Five grams of the ground powder was extracted with 10 ml ammonical chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M sulphuric acid. Creamish white precipitate was observed for the presence of tannins.

RESULT AND DISCUSSION

Effect of Drug Of Eugenia Jambolana & Bitter Guard On Wister Rat-

Drug	Drug	N. of rat	Quantity Dose	Blood Glucose Level (mg/dl)
Group I	Normal Sline	5	10ml/kg	110±3
Group II	Metformin	5	9.05mg	80±3
Group III	EJ:BG 25:75	5	62.5+225 287mg/kg	90±3
Group IV	EJ:BG 50:50	5	125+150 275mg/kg	96±3
Group V	EJ:BG 75:25	5	187.5+75 262.5mg/kg	98±3

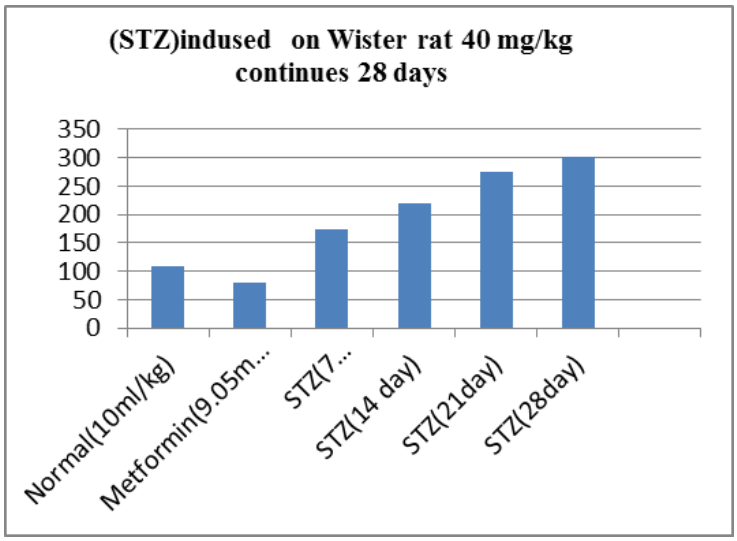
Note – Eugenia jambolana 250mg/kg and Biter guard 300mg/kg



INDUCE STRPTOZOCIN

Streptozocin (STZ) on Wister rat 40 mg/kg continues 28 days for destruction of beta β cell of pancreas then cause of hyperglycemia.

Group	Drug	N. Of Rat	Quantity Dose	Blood Glucose Level (mg/dl)
I	Normal	5	10ml/kg	110±3
II	Metformin	5	9.05mg/kg	80±3
All group III TO V	STZ(7 day)	5	40mg/kg	174±3
	STZ Continues (14day)	5	40mg/kg	220±3
	STZ Continues (21day)	5	40mg/kg	274±3
	STZ Continues (28day)	5	40mg/kg	302±3



Streptozocin (STZ) on Wister rat 40 mg/kg continues 28 days for destruction of beta β cell of pancreas then cause of hyperglycemia.

After 28 days induced STZ Blood glucose level 298±3 mg/dl.

After 28 Days Induced STZ Effect of Eugenia Jambolana: Biter Guard.

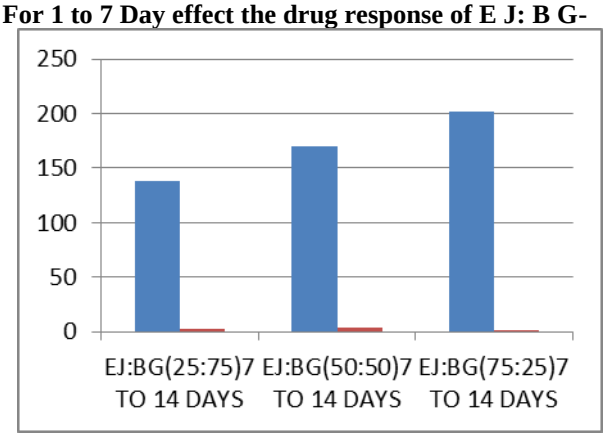
Group	Drug	N. Of Rat	Quantity Dose	Blood glucose (mg/dl)
I	Normal Sline	5	10ml/kg	110±3
II	Metformin	5	9.05mg	90±3
III	EJ:BG(7days) 25:75	5	62.5+225 287mg/kg	218±3
IV	EJ:BG(7days) 50:50	5	125+150 275mg/kg	234±3
V	EJ:BG(7days) 75:25	5	187.5+75 262.5mg/kg	250±3

For 7 to 14 Day effect the drug response of E J: B G

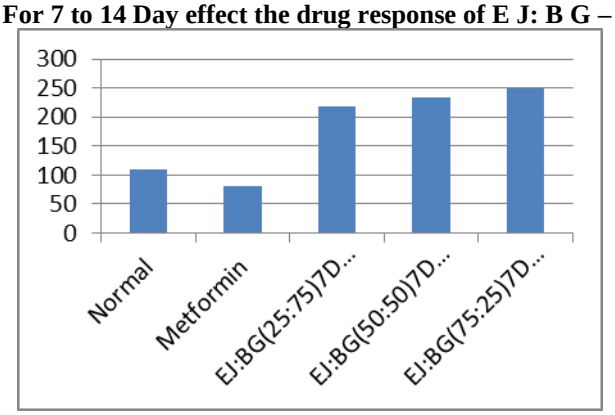
Group	Drug	N. Of Rat	Quantity Dose	Blood glucose (mg/dl)
III	EJ:BG(14days) 25:75	5	62.5+225 287mg/kg	138±3
IV	EJ:BG(14days) 50:50	5	125+150 275mg/kg	170±3
V	EJ:BG(14days) 75:25	5	187.5+75 262.5mg/kg	202±3

After 28 Days Induced STZ Effect Of Eugenia Jambolana: Biter Guard

For 1 to 7 Day effect the drug response of E J: B G-



For 7 to 14 Day effect the drug response of E J: B G –



CONCLUSION

To date, Eugenia Zambollana And Bitter Guard has been extensively studied worldwide for its medicinal properties to treat a number of diseases. It is described as a versatile plant worthy of treating almost any disease inflicted on mankind.The different compounds may act either separately or together to exert their medicinal effects. These different compounds seem to exert their beneficial effects via several mechanisms to control and treat diabetes mellitus.

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