

# HYPOGLYCEMIC, HYPOLIPIDEMIC AND ANTIOXIDANT POTENTIALS OF SPECIALLY FORMULATE POLYHERBAL, FORMULATION IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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## Abstract

**Objective:** To investigate the antihyperglycemic, hypolipidemic and antioxidant properties of the specially formulate polyherbal formulation in Streptozotocin induced diabetic rats.

**Methods:** Multifactorial metabolic diseases, for instance diabetes develop several complications like hyperlipidemia, hepatic toxicity, immunodeficiency etc., Hence, instead of mono-drug therapy the management of the disease requires the combination of herbs. Marketed herbal drugs comprise of irrational combinations which makes their quality control more difficult. Phytoconstituents, despite having excellent bioactivity *in vitro* demonstrate less or no *in vivo* actions due to their poor lipid solubility, resulting in high therapeutic dose regimen. In the present study, polyherbal formulation of aqueous extracts of *Azadirachta indica*, *Camellia sinensis* and ethanol extract *Asparagus racemosus* F1 (N:G:S=2:2:1) 200 mg/kg 2:2:1, named F1, optimized based on STZ induced diabetic rats found to be significant decrease ( $P < 0.01$ ) in blood glucose level as compared to the diabetic control group of animals.

**Results:** Diabetes was induced in Albino rats by administration of streptozotocin (45mg/kg, I.P). The F1 (N:G:S=2:2:1) 200 mg/kg body weight was administered to diabetes induced rats for a period of 28 days, which possess better effect than F2 (N:G:S=2:1:2) 200 mg/kg and F3 (N:G:S=2:1:1) 200 mg/kg. Other biochemical parameters such as serum cholesterol, triglycerides, HDL-cholesterol, VLDL-cholesterol, LDL-cholesterol, urea, creatinin and proteins levels were also measured at the end of study. After checking the antidiabetic activity the F1 (N:G:S=2:2:1) 200

mg/kg on blood glucose was observed the best one, in order to justify it oxidative parameter , i.e.- superoxide dismutase (SOD), reduced glutathione (GSH) , serum thiobarbituric (TBAR) and LPO were also tested. These enzymes indicates its oxidative stress.

**Conclusion:**From the above results it is concluded that the formulation F1 (N:G:S=2:2:1) 200 mg/kg on blood glucose possesses significant antidiabetic,hypolipidemic and antioxidant effects in streptozotocin induced diabetic rats .

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**Keywords:** Azadirachta indica, Camellia sinensis , Asparagus racemosus, Streptozotocin

### Introduction

Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein, and lipid metabolism and by complications like retinopathy, microangiopathy, and nephropathy. According to WHO, diabetes mellitus will be the single largest non-communicable disease worldwide by the year 2025 with the largest diabetic population in India. ( American Diabetes, 2010) Current pharmacotherapeutics/medicines are not able to reverse hyperglycemia completely, have limited tolerability, and induce side effects.( Eurich et al., 2007) Therefore, a need for alternative therapy especially the natural plant sources having minimal side effects has arisen. About two hundreds of polyherbal formulations are available in the market (Data un-published) for the management of diabetes, majority of them contain eight or more drugs in the combination, quite complex to standardize; therefore, quality of the product cannot be assured. Since, FDA recommends the number of ingredients to be three or less than three in any combination;( Rao et al., 2007) in the present study the polyherbal formulation comprising of lyophilized hydro-alcoholic extracts of only three drugs combination was optimized.

Neem (*Azadirachta indica* A.uss.), Family: Meliaceae, Subfamily: Melioideae, Order: Meliales is an evergreen tree native to the Indian subcontinent. It also grows widely in several other countries of Asia, Australia, Africa and Central and South America. More recent studies have focused on animals, including one report which indicates that Neem's hypoglycemic effect is comparable to the prescription drug glibenclamide and noted that it may be beneficial in preventing or delaying the onset of disease (World journal vol-2).

Tea is the most consumed drink in the world after water. Green tea (*Camellia sinensis*) is a 'non-fermented' tea and contains more catechins than black tea or oolong tea. Catechins are *in vitro* and *in vivo* strong antioxidants There is also epidemiological evidence that drinking green tea

(but not black tea or oolong tea) may help prevent diabetes. (Takatoshi et al., 2006)

*Asparagus racemosus*, traditionally known as shatavari means "who possesses a hundred husbands or acceptable to many". Asparagaceae is an ayurvedic plant with medical importance of tropical and subtropical India. Its medical usage has been reported in Indian & British Pharmacopeias and in traditional system of medicine Ethanol extract of *A. racemosus* causes lowering the blood sugar level(Gogte et al., 2000)

Symptoms of Diabetes mellitus are polyuria, polydipsia, losing weight without trying, blurry vision, Frequent infections- including skin infections, thrush, gingivitis, urinary tract infections and yeast infections, Slow healing of sores, Skin problems, such as itchiness or acanthosis Nigricans, Dizziness or fainting(Samreen et al., 2009). Common side effects of Synthetic drugs are - Hypoglycaemia, Nausea, Vomiting, Diarrhoea, Constipation, Headache, Anorexia. While our herbal drugs are also stand for the treatment of Diabetes mellitus with less or no unwanted effects as compare to synthetic diabetic medicines. *Azadirachta indica*, *Camellia sinensis* and *Asparagus racemosus* were used in traditional system of medicines for the treatment of diabetes mellitus so hypoglycemic agent have focused on plants which are used in traditional system of medicines because they may be better treatment than synthetic drugs which are available in market(Hevener et al., 2007). The present research article deals with the aim of evaluating the combined effects of Formulation of *Azadirachta indica*, *Camellia sinensis* and *Asparagus racemosus* leaves extract on blood glucose level on streptozocin diabetic rats.

## **Materials and methods**

### **Plant material**

Leaves of *Azadirachta indica*, *Camellia sinensis* and *Asparagus racemosus* were collected in the month of November 2011 from botanical garden of V.B.S.P university campus,Jaunpur, Uttarpradesh India. The plant was authenticated by Dr.A.K.Singh Head of the Department of Botany, T.D.P.G. College, Jaunpur, Uttarpradesh,India The leaves were cleaned and dried under the shade to avoid degradation of volatile oil.

### **Preparation of plant extract for Phytochemical Screening and Antidiabetic Studies**

The *Azadirachta indica*, *Camellia sinensis* and *Asparagus racemosus* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Azadirachta indica*, *Camellia sinensis* leaves was packed in a Soxhlet apparatus and extracted with water. Hundred grams of powdered *Asparagus racemosus*

leaves was packed in a Soxhlet apparatus and extracted with ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Anonymous et al., 1990).

The concentrated extract were used for antidiabetic studies. The phytochemical constituents like Alkaloids Carbohydrates, tannins, flavonoids, terpinoids were found..

### **Polyherbal formulation**

FDA recommends the number of ingredients to be three or less than three in any combination; hence in the present study a protocol for the preparation of 3 formulations was developed by taking three different best extract from three different plants *Azadirachta indica*, *Camellia sinensis* and ethanol extract *Asparagus racemosus*. Three different formulation F1 (N:G:S=2:2:1), F2 (N:G:S=2:1:2), F3 (N:G:S=2:1:1) were taken, which further goes through pharmacological investigation and the best formulation obtained was carried out for tablet formulation, evaluation and stability study.

### **Animals**

Normal healthy male Wistar albino rats  $200 \pm 25$  gm were used for present investigation. Animals were housed under standard environmental conditions at temperature ( $25 \pm 2^\circ\text{C}$ ), light and dark (12:12 h). Rats were feed standard pellet diet ((golden feed, New Delhi and water regularly) .

### **Acute Toxicity Study**

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD guidelines). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight and observed for 14 days. If mortality was observed in two out of three animals, then the dose and measurement were done on day 0, 7, 14, 21 and 28 of the study.

On day 28, All animals were sacrificed by under mild ether anesthesia .The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution, and immediately processed by the paraffin technique. Sections of 5 micron thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

### **Antidiabetic potential assessment**

After overnight fasting (deprived of food for 16 h had been allowed free access to water), diabetes was induced in rats by I.P. injection of STZ dissolved in 0.1M cold sodium citrate buffer (pH 4.5) at a dose of 55 mg/kg body weight). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. After a 72 hrs for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats. Fasting blood glucose estimation and measurement were done on day 0, 7, 14, 21 and 28 of the study.

### **Experimental Design**

All animals were divided randomly into six groups with six animal in each group(David et al., 2012). Group I received normal control (vehicle), Group II diabetic control, Group III diabetic control + standard, Group IV diabetic control + F1 (N:G:S=2:2:1) 200 mg/kg, Group V diabetic control + F2 (N:G:S=2:1:2) 200 mg/kg and Group VI diabetic control + F3 (N:G:S=2:1:1) 200 mg/kg. All dosing of test samples was done p.o. throughout the experimentation.

### **Statistical analysis**

All data were analyzed by One way ANOVA followed by Bonferroni test.  $p < 0.05$  was considered as level of significance. All data are presented in mean $\pm$ SD.

### **Biochemical Parameters**

The blood samples were collected on day 0, 7, 14, 21 and 28 of the study by puncturing the retro orbital plexus under mild anesthesia. On 28<sup>st</sup> day, 18 h fasted animals were sacrificed by cervical dislocation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 3000 rpm for 10 min for the estimation of glucose, total cholesterol,( Roeschlau et al.,1974) total triglycerides, low density lipoprotein (LDL), and high density lipoprotein (HDL) levels.( Rajasekaran et al., 2006) The liver of the sacrificed animals was dissected out, weighed, washed with saline, blotted dry and homogenate using tris and phosphate buffers for the purpose. The homogenate was analyzed for total glycogen content. ( Kits et al., 1955)

### **Results**

Table.1 depicts the hypoglycemic effect of a single dose of F1 (N:G:S=2:2:1) 200 mg/kg body weight was administered to diabetes induced

rats for a period of 28 days, which possess better effect than F2 (N:G:S=2:1:2) 200 mg/kg and F3 (N:G:S=2:1:1) 200 mg/kg.

Table 1: BLOOD GLUCOSE LEVEL (mg/dl)

Group No.	Treatment	Blood glucose level on:*				
		0 day	7 day	14 day	21 Day	28 Day
1	Normal Control (Vehicle)	92.6±1.374	94.6±1.448	95.7±1.436	96.1±1.754	96.5±1.266
2	Diabetic Control	285.2±4.094 <sup>a</sup>	290.8±5.153 <sub>a</sub>	295.6±4.927 <sub>a</sub>	302.0±6.811 <sub>a</sub>	304.8±7.049 <sub>a</sub>
3	Diabetic Control + Standard	289.7±3.962 <sub>a</sub>	208.2±8.295 <sub>a,b</sub>	151.3±7.088 <sub>a,b</sub>	134.1±6.291 <sub>a,b</sub>	113.0±8.555 <sub>a,b</sub>
4	Diabetic Control + F1(N:G:S=2:2:1) 200 mg/kg	290.1±4.648 <sub>a</sub>	216.3±4.095 <sub>a,b</sub>	160.3±5.853 <sub>a,b</sub>	151.6±5.750 <sub>a,b</sub>	139.2±4.167 <sub>a,b</sub>
5	DIABETIC CONTROL + F2 (N:G:S=2:1:2) 200 mg/kg	287.6±5.715 <sub>a</sub>	223.7±4.452 <sub>a,b</sub>	174.7±3.416 <sub>a,b</sub>	163.8±5.075 <sub>a,b</sub>	149.7±3.431 <sub>a,b</sub>
6	DIABETIC CONTROL + F3 (N:G:S=2:1:1) 200 mg/kg	292.6±4.018 <sub>a</sub>	227.4±4.185 <sub>a,b</sub>	181.7±3.651 <sub>a,b</sub>	169.3±4.675 <sub>a,b</sub>	154.9±3.639 <sub>a,b</sub>

\* All data presented in Mean ± SD (n=6)

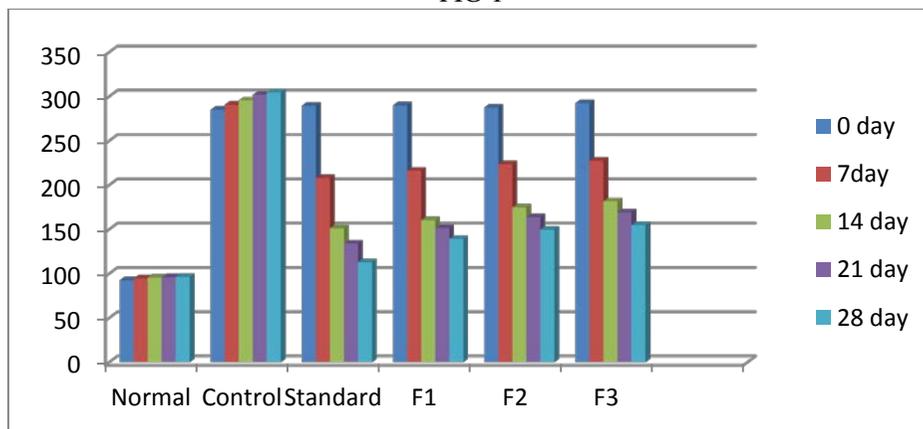
a - P<0.05 as compared to vehicle treated animals (group -1)

b- P<0.05 as compared to Diabetic control animals (group -2)

*The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). Power of performed test with alpha = 0.050: 1.000All Pairwise Multiple Comparison Procedures (Bonferroni t-test).*

Fig-1 represent the graphically representation of various group starting from normal to F3. In graphical representation I observed that F1 possess maximum anti diabetic activity comparison to F2 & F3.

FIG-1



The hypoglycemic extract of *polyherbal formulation F1,F2,F3* was found to be decrease the blood glucose level. Streptozotocin induced diabetic rats showed significant decreased ( $p<0.01$ ) blood glucose level compared with normal rats.. The levels of serum lipid profile TC,TG,HDL and LDL of control and streptozotocin induced diabetic rats were presented in **Table 2**.

TABLE 2: Effect of F1,F2,F3 on the serum lipid profile of normal, diabetic induced and drug treated rats

Group	Treatment	Lipid Profile (mg/dL)*			
		Total Cholesterol	Triglycerides	LDL	HDL
I	Normal Control (Vehicle)	81.8±3.855	51.3±5.526	42.5±5.998	37.2±5.300
II	Diabetic Control	148.9±6.221 <sup>a</sup>	98.9±6.370 <sup>a</sup>	82.1±5.667 <sup>a</sup>	17.6±4.526 <sup>a</sup>
III	Diabetic Control + Standard	94.6±3.345 <sup>b</sup>	56.3±4.150 <sup>b</sup>	44.8±4.308 <sup>b</sup>	35.1±6.646 <sup>b</sup>
IV	Diabetic Control + F1 (N:G:S=2:2:1) 200 mg/kg	109.0±4.238 <sup>b</sup>	66.5±2.484 <sup>b</sup>	53.7±3.925 <sup>b</sup>	30.4±6.977 <sup>b</sup>
V	DIABETIC CONTROL + F2 (N:G:S=2:1:2) 200 mg/kg	117.0±4.151 <sup>b</sup>	73.5±2.613 <sup>b</sup>	61.7±2.136 <sup>b</sup>	25.7±1.699 <sub>b</sub>
VI	DIABETIC CONTROL + F3 (N:G:S=2:1:1) 200 mg/kg	119.2±4.166 <sup>b</sup>	75.5±2.720 <sup>b</sup>	63.7±2.173 <sup>b</sup>	24.4±1.673 <sub>b</sub>

\* All data presented in Mean ± SD (n=6)

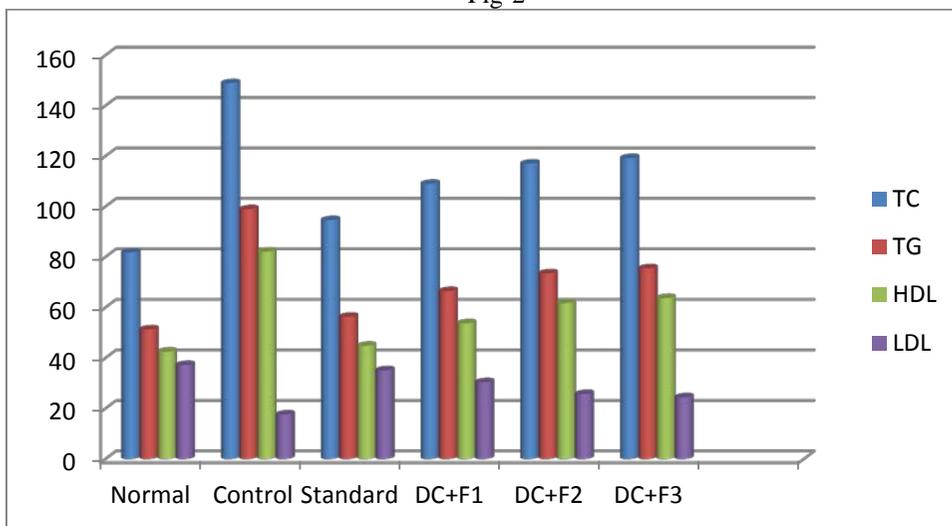
a -  $P<0.05$  as compared to vehicle treated animals (group -1)

b-  $P<0.05$  as compared to Diabetic control animals (group -2)

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ( $P = <0.001$ ). Power of performed test with alpha = 0.050: 1.000All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

**Fig-2** represent the graphically representation of various group starting from normal to F3.In graphical representation we observed that F1 possess maximum lipidemic activity comparison to F2 &F3.

Fig-2



**Effect of best formulation F1 (N:G:S=2:2:1) 200 mg/kg on in- vivo antioxidant parameters**

The superoxide dismutase activity was found to be reduced in erythrocytes of animals treated with streptozotocin . GSH is a major non-protein thiol in living organisms, which plays a central role in co-ordinating the body’s antioxidant defense processes. (Latha et al., 2003) Perturbation of GSH status of a biological system can lead to serious consequences. SOD, CAT,GSH and TBAR constitute a mutually supportive team of defense against reactive oxygen species (ROS) The results (**Table 3**) showed decreased oxidant enzymes of streptozotocin induced diabetic rats.. These indicate that, plant extract inhibit oxidative damage due to the antiperoxidative effect of ingrediants present in F1 (N:G:S=2:2:1) 200 mg/kg.

Table-3

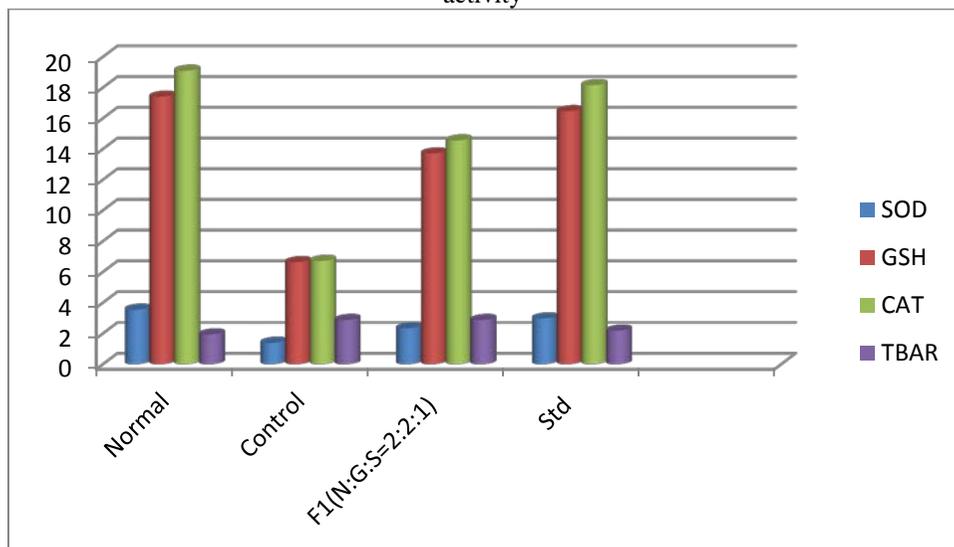
Treatment Group	Enzymes involved in oxidative stress*			
	SOD	GSH	CAT	TBAR
Vehicle treated	3.54±0.09	17.40±0.05	19.09±0.01	1.93±0.006
Diabetic control	1.39±0.08 <sup>a</sup>	6.63±0.15 <sup>a</sup>	6.71±0.10 <sup>a</sup>	2.87±0.004 <sup>a</sup>
F1(AZ:CS:AR=2:2:1) 200 mg/kg	2.33±0.04 <sup>b</sup>	13.7±0.07 <sup>b</sup>	14.55±1.13 <sup>b</sup>	2.87±0.055
Std group	2.97±0.02 <sup>b</sup>	16.46±0.08 <sup>b</sup>	18.15±0.09 <sup>b</sup>	2.18±0.008 <sup>b</sup>

\*All data presented in Mean±SD

a - P<0.05 as compared to vehicle treated group

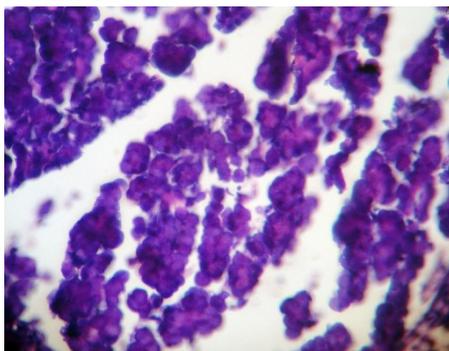
b- P<0.05 as compared to diabetic control group

**Fig-3** represent the graphically representation of F1 which possess maximum antioxidant activity

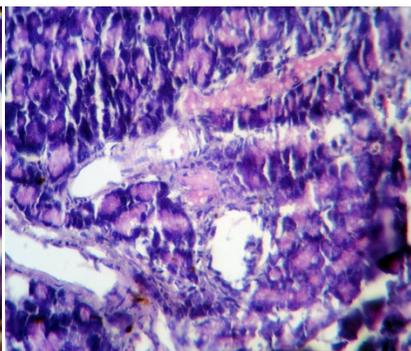


### Histopathology

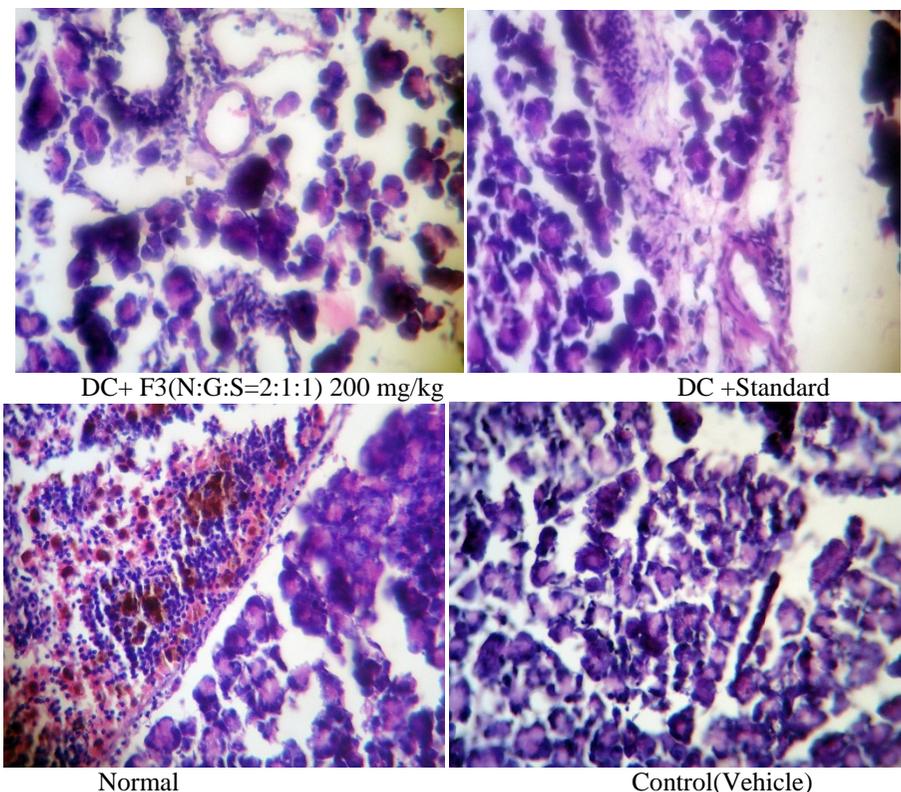
For histopathological study, On day 28, All animals were sacrificed by under mild ether anesthesia .The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution, and immediately processed by the paraffin technique. Sections of 5 micron thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination. Then the slides were examined by pathologist and the pictures were clicked with the help of a binocular microscope fixed with a camera.



DC+F1(N:G:S=2:2:1) 200 mg/kg



DC+F2(N:G:S=2:1:2) 200 mg/kg



## Discussion

Diabetes mellitus is a serious metabolic disorder with micro and macrovascular complications that results insignificant morbidity and mortality. Lipid peroxidation has been implicated in the pathogenesis of naturally occurring or induced diabetes . There is a wide range of antioxidant defenses which protects against the adverse effects of free radicals production *in vivo* .STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood glucose in animals (Kumar et al., 2011) .The present study indicate that various formulation F1,F2,F3 showed antidiabetic properties against STZ induced diabetic model and also it proved to have hypolipidemic activity. A significant reduction ( $P < 0.01$ ) was observed in F1(N:G:S=2:2:1) 200 mg/kg.. The reduction in the level of serum cholesterol, triglycerides, LDL and HDL cholesterol confirms the possibility that major functions of the extract are on the protection of vital tissues (kidney and liver) including the pancreas, thereby reducing the causation of diabetes in experimental animals. The portal tracts showed portal triad with portal vein, hepatic artery and bile duct, where as the diabetic mice liver tissue section showed marked

structural alterations in the liver as a result of absence of insulin. The results indicated a primary and secondary effect of diabetic state.

### **Conclusion**

In conclusion, the present study has shown that the out of three formulation formulation F1(N:G:S=2:2:1) 200 mg/kg has antidiabetic and antihyperlipidaemic effects. Since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, terpenoids, tannins, carbohydrate and phenols . Several authors reported that flavonoids, sterols,terpenoids, phenolic acids are known to be bioactive antidiabetic principles(Oliver et., 1986 & Rhemannetal., 1989 ). Flavonoids are known to regenerate the damaged beta cells in the streptozotocin diabetic rats (Chakravarthy et al., 1980). Phenolics are found to be effective antihyperglycemic agents (Manickam et al., 1997). In the present study, the phytochemical analysis of aqueous extracts of *Azadirachta indica*, *Camellia sinensis* and ethanol extract of *Asparagus racemosus* clearly points out the presence of above said active phytochemicals. The results obtained from the present study show that the Polyherbal Formulation of *Azadirachta indica*, *Camellia sinensis* and *Asparagus racemosus* had beneficial effect on blood glucose level in streptozocin induced diabetic rats .

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