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HYPOGLYCEMIC AND TISSUE-PROTECTIVE EVALUATION OF FICUS KRISHNAE IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Diabetes is the most common endocrine disorder associated with carbohydrate metabolism and is a major cause of disability and hospitalization. *Ficus krishnae* belongs to the family Moraceae has been used in ancient folklore medicine the parts of this plant such as Stem bark and leaves are used to treat diabetes. Hence the present study is aimed to investigate the anti-diabetic activity of methanol extract of *Ficus krishnae Linn* in alloxan induced diabetes albino rats. Alloxan was administered as a single dose (120 mg/kg, b.wt) to the normal rats to induce diabetes. Oral administration of methanol extracts from stem bark of *Ficus krishnae* at the dose of 200 & 400 mg/kg body weight/day for 14 days was treated to alloxan-induced diabetic rats. The fasting blood sugar levels, body weight, serum biochemical analysis and histopathology study were conducted in alloxan-induced diabetic rats. On the basis of our findings, it is confirmed that the F.k stem bark could be used as potential antidiabetic agent for better management of diabetes mellitus.

KEYWORDS: *Ficus krishnae*, Diabetes mellitus, Acute toxicity, Albino rats, Soxhlet extraction.

INTRODUCTION

Diabetes mellitus is the disorder characterized by chronic, progressive, systemic condition of impaired carbohydrate metabolism.[1] International diabetes federation has estimated that diabetes in 2010 has rised to 285 million, contributing 6.4% of the world adult population; also predicted that they are in 2030 the number of people with diabetes mellitus (DM) will rise to 438 million.[2] At present the DM is managed by insulin and oral administration of hypoglycemic drugs such as biguanides and sulfonylureas. However, these methods of treatments are failed in managing and controlling of diabetes due to its limited efficacy and undesirable side effects.[3] Recently, the importance is gained for medicinal plants to use as complementary and alternative methods for the treatment of diabetic mellitus.[4] Since from ancient period the herbal medicines are using for the treatment of diabetes, but few of them are in practice remaining are not been explored scientifically so far.[5] This property indicates the medicinal importance of this species. Based on the above information this plant has been selected for investigation.

Ficus krishnae Linn. Family: (Moraceae) is a large tree distributed in India, tropical Africa and Sri lanka.[6] It is commonly known as 'Makkhann Katori' in Hindi or 'Krishna fig tree' and considered as holy tree of India. Information based on ethanomedicinal surveys reveales that the herbal preparations of different parts of Ficus krishnae had been considered to treat ulcers, vomiting, dysentery, fever, inflammation, leprosy, cancer and diabetes.[7,8] The plant also revealed for the presence of rich sources of bioactive compounds like flavonoids terpenoids, glycosides, steroids, phenols[9] etc. A biological activity of medicinal plant was closely related to their elemental composition. *F. krishnae* is also rich in Mg and Ca which generally have high potential of lowering blood glucose level.[10] To the best of our knowledge there was no previous comprehensive study to evaluate the antidiabetic activity of *Ficus krishnae* stem bark extract. Therefore the present study adds the important information about protective efficacy of *F. krishnae* as novel antidiabetic agent.

MATERIALS AND METHODS

Collection of plant materials

Ficus krishnae stem barks were collected from Dev Dev Vana botanical garden, Bidar, Karnataka. India. The stem bark was allowed to dry in shade for 2-4 weeks, after drying the bark was grinded into powder and stored in airtight container for further use.

Soxhlet extraction
The bark powder (100 g) was successively extracted by hot soxhlet extraction with methanol solvent (600 mL) for 6-8 hours. After extraction the methanolic extract were stored for future use.

Experimental animals
Experiments were performed using, sexually mature both male and female albino rats of Wister strain (160-220g), obtained from NIN, Hyderabad were housed in polypropylene cages and maintained at 25-37˚C: 45% humidity in 12 hr light/dark cycle. The rats were provided standard laboratory chow (VRK Nutrition Sangli, Maharashtra, India) and water was available *ad libitum*. All procedures for animal experiment were strictly fallowed by guidelines prescribed by the committee for the experimentation on animals (CPCSEA Reg. No-34800/2001) and approved by Institutional Animal Ethical Committee.

Acute toxicity studies
The acute toxicity of methanol extract was evaluated through established protocol in adult albino rats: All the rats were divided into four groups containing six animals in each group.[11] Before experiment the rats were fasted for 16-18 hrs provided with water ad libitum. The methanol extract was prepared and administrated orally at different concentration i.e 250, 500 and 1000mg/kg body weight respectively. Control rats receive the vehical DMSO-80% only. The animals were examined for any signs of behavioral changes and mortality for the 72hrs and maintained at 25-37˚C: 45% humidity in 12 hr light/dark cycle. On the basis of this experiment an LD_{50} 1000 mg/kg was established for methanol extract and two doses 200 and 400 mg/kg per day were used in all further experiment.

Induction of diabetes
The rats were kept fasting for 24 hrs provided with water ad libitum and administered alloxan monohydrate 120 mg/kg (normal saline) intraperitoneally. After 1 hr the rats were fed with pellets and provided with glucose water for 24 hrs. The blood glucose level was checked before Alloxan treatment and 24 hrs after Alloxanisation.

Experimental design
The rats were considered as diabetic with the blood glucose level was observed beyond 250 mg/DL of blood. This condition was noticed after incubation of time after alloxanisation. The rats were separated into five groups of six rats in each group.[12]
Group I- Consists normal rats and received only vehicle (2ml/kg p.o) 80% DMSO.
Group II- Consists diabetic control rats receive water and food only.
Group III- This group consists of diabetic rats and received methanol extract of *Ficus krishnae* (200mg/kg/day p.o) in 80% v/v DMSO.
Group IV- This group consists of diabetic rats and received methanol extract of *Ficus krishnae* (400mg/kg/day p.o) in 80% v/v DMSO.
Group V- This group rats received Glibenclamide (5mg/kg p.o) suspended in saline.
Multidose study: the selected rats were treated with similar test sample; blood glucose level and body weight was measured on 0, 3, 7 and 14 days of treatment. After 14 days of treatment the rats of different doses were sacrificed and collected blood sample for estimation of lipid profile such as Total Cholesterol, triglycerides, HDL, LDL, VLDL and serum glucose level.[13] And whole pancreas, liver and kidney were collected in 10% formaline solution and take section 5µ thickness was stained heamatoxyline and eosin (H & E) for histological evaluation.[14] The photomicrographs of histological studies are presented in Fig. 1.

Statistical Analysis
The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

RESULT AND DISCUSSION

Acute toxicity study
Animals which have showed good tolerance to testing for single doses of methanol extract of *Ficus krishnae* stem bark in doses of 1g/kg that were found to be non-lethal. Highest dose of extract did not show any noticeable changes/ signs of toxicity and mortality after 72 hrs administration of extract orally. Indicates the extract is safe without lithality.

Determination of the blood glucose levels of MFK
Blood glucose level (BGL) concentration was determined by using an OneTouch glucometer procured from market. Blood samples from the rats were colleted on tip of the strip at defined time at the glucometer and readings were recorded. The test conforms that the changes in BGL and lipid profile of treated, untreated and standard treated rats as shown in the Table-1. At the dose 400mg/kg b.w the methanol extract has shown 117±1.3 mg/DL, significant decreases in blood glucose level within 14 days of treatment, when compared with 200mg/kg and standard glibenclamide. In the present study at different concentration of methanol extract of *F.krishnae* has shown the significant decrease ($P < 0.05$) in blood glucose level. This indicates that the prasence of hypoglycemic agents in methanol extract. Flavonoids have been found to be an active principle in many herbal medicines[15] and are knowns to be good antioxidant that may be the protective function of organs against diabetic stress.

Effect of lipid profile
In diabetic rats, there was a significant increase of serum total cholesterol, triglycerides and decrease in HDL

cholesterol when compared to the normal/control rats. But after treatment with the plant extract at 200, 400mg/kg b.w and also standard drug have significantly decreased (P< 0.05) the level of cholesterol and triglycerides, whereas HDL cholesterol was improved as predicted in Table-3. The most common lipid abnormalities in diabetes are hyperchlolestrolemia and hypertriglyceridemia [16]. Repeatedly administration of methanol plant extract of F.krishnae for 14 days has significantly decreased the hyperchlolestrolemia and hypertriglyceridemia, observation of hypolipidemic may be due to decrease of cholestrogenesis and fatty acid synthesis.[17]

Table 1: Effect of *Ficus krishnae* stem bark extract on fasting blood glucose level in alloxan induced diabetic rats.
Animal: Albino Rats
Alloxan: 120 mg/kg, i.p.
Extract: p.o.

Groups	Treatment	Base Value	3rd days	7th day	14th day
I Group	Normal control	96.4±0.80[a]	92.2±0.32[a]	95.17±1.77[a]	93.33±0.15[a]
II Group	Diabetic control	256±0.4[a]	270±1.2[a]	278±0.40[a]	280±1.2[a]
III Group	Ficus krishnae methanol (200mg/kg bw)	246±0.2[a]	217.3±1.7[a]	178±1.4[a]	156±1.2[a]
IV Group	Ficus krishnae (400mg/kg bw)	256±0.8[a]	214.3±1.6[a]	144±1.2[a]	117±1.3[a]
V Group	Glibenclamide (5mg/kg)	252±1.2[a]	217.3±0.5[a]	186.22±1.8[a]	152±1.6[a]

Values are Mean ±S.E.M; n=6; a=****P < 0.0005 vs Diabetic Control

Table 2: Effect of *Ficus Krishnae* Stem Bark Methanol Extract On Body Weight In Alloxan Induced Diabetic Rats.

Groups	Treatment	Initial	3rd days	7th day	14th day
I Group	Normal control	190±1.2*	198±1.22*	200±1.8*	204±1.4*
II Group	Diabetic control	205±1.5*	215±1.7*	220±1.4*	277±1.7*
III Group	*Ficus krishnae* methanol (200mg/kg bw)	208±1.0*	185±1.3*	182±1.0*	178±1.2*
IV Group	*Ficus krishnae* methanol (400mg/kg bw)	206±1.2*	195±1.7*	182±1.2*	162±1.8*
V Group	Glibenclamide (5mg/kg)	202±1.4*	191±1.9*	184±1.0*	166±1.6*

Values are Mean±S.E.M; n=6 *P < 0.001 vs Diabetic Control.

Table 3: Effect of various groups of *Ficus krishnae* on serum profile in alloxan induced diabetic albino rats after 14 days of treatment.

Groups	Treatment	Cholesterol	Triglycerids	HDL Cholesterol	VLDL Cholesterol	Cholesterol/HDL
I Group	Normal control	156.44±1.2*	95.9±0.43*	53.9±1.1*	20.18±1.9*	1.4±0.6*
II Group	Diabetic control	220.24±2.2*	174.54±4.4*	37.33±1.6*	69.36±3.8*	1.8±1.43*
III Group	*Ficus krishnae* methanol (200mg/kg bw)	69.1±1.4*	74.9±1.7*	47.8±3.2*	19.2±1.8*	1.3±1.6*
IV Group	*Ficus krishnae* (400mg/kg bw)	64.1±1.3*	64.9±2.4*	50.2±3.4*	12.1±1.5*	1.4±1.2*
V Group	Glibenclamide (5mg/kg)	168.4±1.6*	78.2±1.8*	48.2±2.6*	15.16±2.4*	1.5±1.3*

Values are Mean±S.E.M; n=6 *P < 0.005 vs Diabetic Control.

HISTOPATHOLOGICAL STUDIES

Histology of Pancreas

Histologic studies of our results as accordance with.[18,19] The cross sections of the pancreas of control albino rats exhibit the prominent cytoplasm with normal acini and cellular population in the islets of Langerhans (Group I) as shows in Fig-1A. In Group-II, extensive damage of islets of Langerhans and reduced dimensions of islets with reduced acini was seen in diabetic rats (Fig-1B). After treatment with methanol extract of *F.krishnae* at the dose of 200 and 400 mg/kg b.w the partial rejuvenation of normal cellular population of islets and enlarged size of *β*-cells with hyperplasia are seen in Group-III and IV as shown in figure-1C and D respectively.[20] Methanol extract at the higher concentration has shown a good rejuvenation of islets of langerhans in pancreas when compared with standard glibenclamide (Group V) treated group were healthy acini are shown in Fig-1E. The pancreatic tissues are highly susceptible to the action of alloxan induction because increase in formation of free radicals which causes the tissue injury. The *F.krishnae* stem bark of

methanol extract was rich in polyphenols and has *in vitro* antioxidant activity. Treatment of FK methanol extract to diabetic rats has reversed the effect due to antioxidant potential of plant which brings protective effect on the organs.

Histology of Kidney

Histology of kidney (Figur-2) in normal animals shows healthy structure of tissue (A). In diabetic rats, mild thickening of the basement membrane of the arterioles of glomeruli and mild change in density of mesangial mesangium was observed (B). After *F.krishnae* methanol extract treatment at 200 and 400 mg/kg b.w to diabetic rats but after *F.krishnae* extract treatment indicate that it inhibit the histopathological changes of the kidney in alloxan induced diabetic rats (C and D). The standard glibenclamide (E) has also shown good protection of the kidney tissue.

Histology of Liver

The histopathologic study of the liver from allaxon induced rat has showed degenerative changes in the hepatocytes as represented by disorganization of the hepatic cords, congestion of the central vein with mild hepatocellular necrosis due to stress by free radicals produced from allaxon as shown in Fig-3B (Group II). The liver of *F.krishnae* methanol extract treated rats at 200 and 400mg/kg b.w (Group-III and IV) has revealed a slight improvement in the structure of hepatic tissue compared to those of the untreated diabetic as shown in fig-3C and D. whereas (Group-V) standard glibenclamide treatment also shows in Fig-3E the improvement in hepatic tissue and the liver similar to the normal control rats (Group-I, Fig-3A).

The organs of Diabetic rats treated with F.krishnae have subjected with histological studies has shown the protective effect on pancreas, kidney and liver consists of healthy structures. Antidiabetic action of the plant extract in diabetic rats may be possible the insulinomemitic action or by stimulating the mechanism of glucose uptake from peripheral tissue, or by activation of gluconeogenesis in liver and muscle. Although, antidiabetic activity of plant may be the presence of bioactive compounds in extract is the responsible for hypoglycemic effects.[21,22]

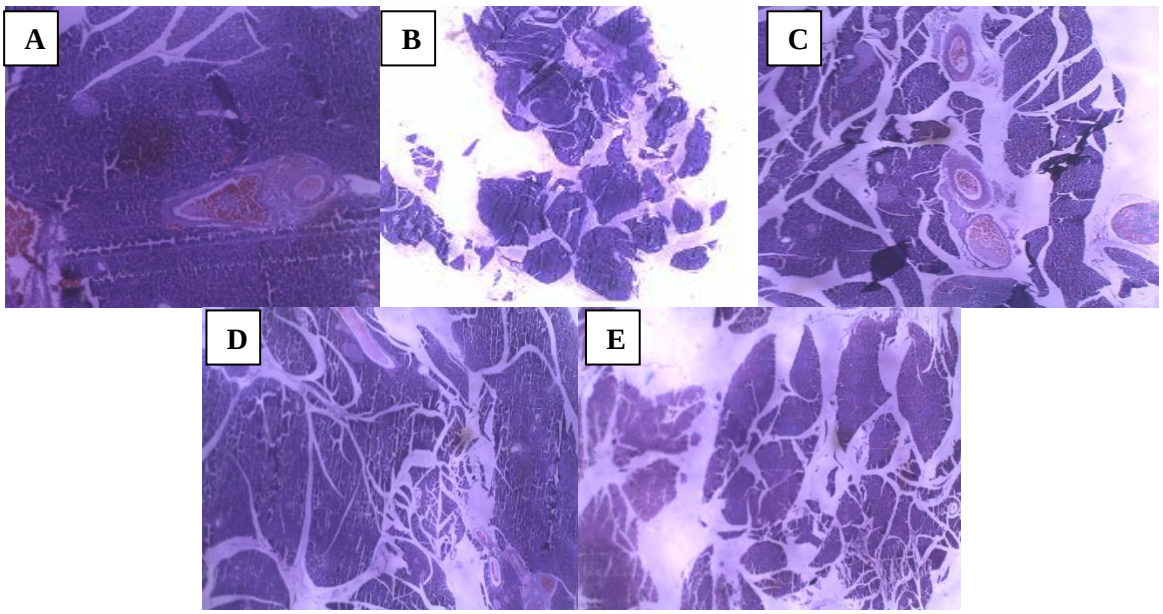
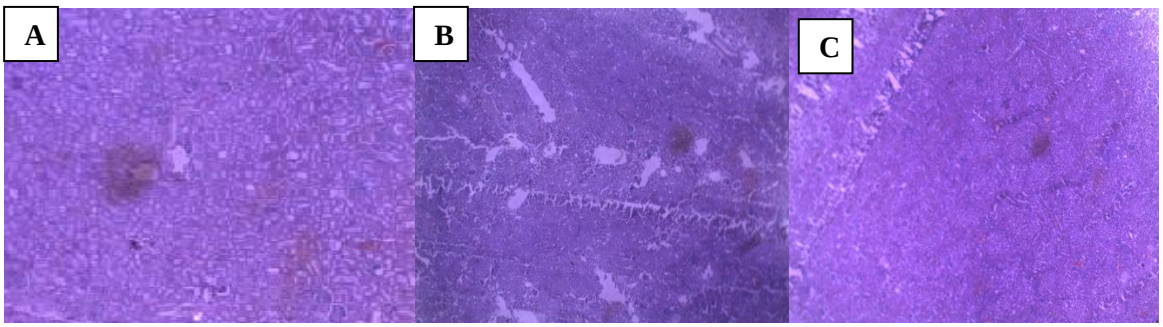


Figure 1: Shows Pancrea A- control, B-Alloxan rat, C- FK (200mg/kg), D-FK (400mg/kg) and E-Glibaclamide (Std).



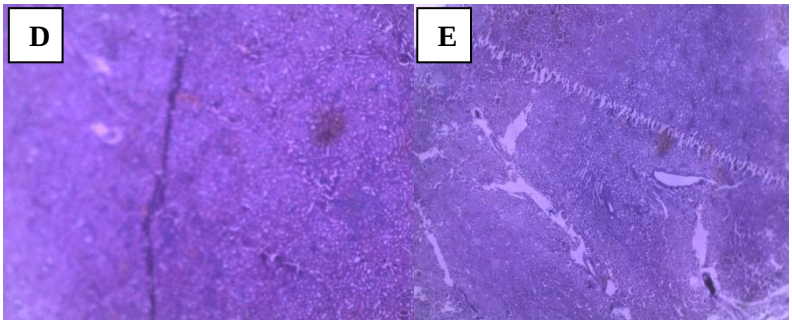


Figure 2: Shows Kidney A-Contro Rat, B-Alloxan Rat, C-Fk (200mg/Kg), D-Fk (400mg/Kg) And E- Standard (Glybanclamide).

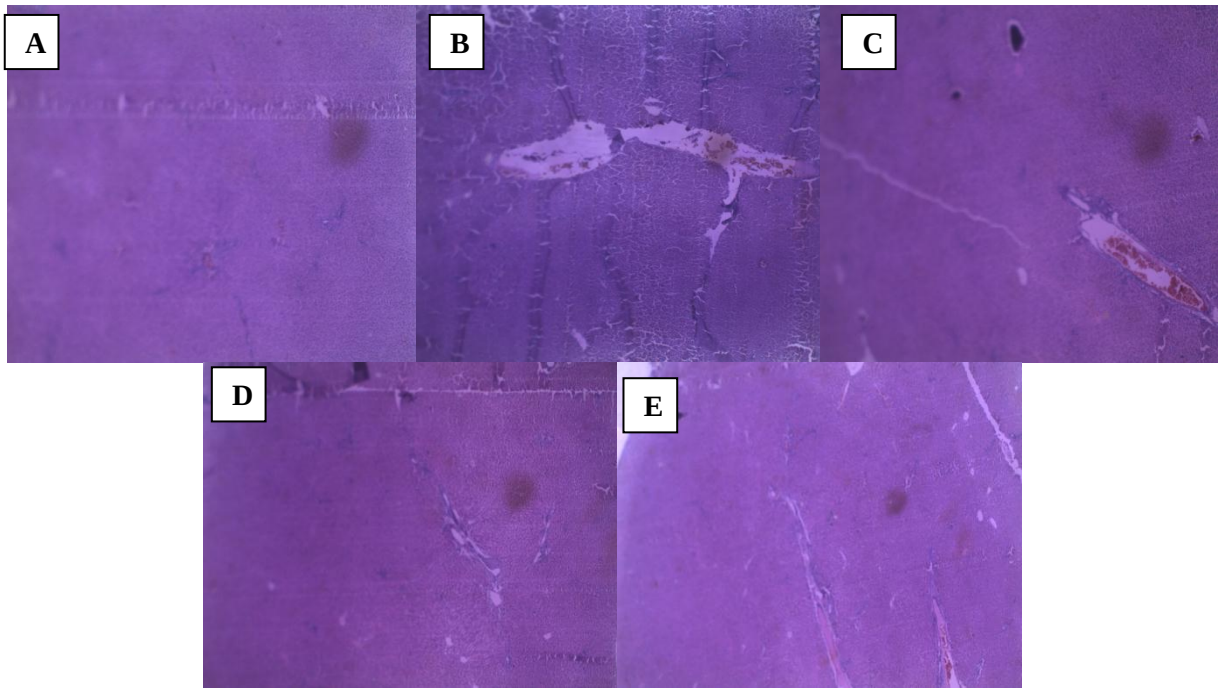


Figure 3: Shows Liver A-Control Rat, B-Alloxan Rat, C-Fk (200mg/Kg), D-Fk (400mg/Kg) and E- Standard (Glybanclamide).

CONCLUSION

In conclusion, the methanol extract of *Ficus krishnae* stem bark was found to exhibit a significant hypoglycemic and antihyperglycemic activity in normal and alloxan-diabetic rats. The plant extract exerted a dose-dependent protective effect on pancreas, kidney and liver similar to the reference drug glibenclamide. Together the results of present investigation shall provide the folkloric use of *F.krishnae* in the management of diabetes mellitus. Studies are in progress in our laboratory for elucidation of structure of the active components responsible for lowering the blood sugar level effects in experimental diabetis rats.

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REFERENCES

1. Nikhil K Sachin, Yatindra Kumar, Seema Pushkar. Antidiabetic potential of alcoholic and aqueous extracts of Ficus recemosa Linn. Bark in Normal and alloxon induced diabetic rats. International journal of pharmaceutical sciences and drug research, 2009; 1(1): 24-27.
2. Singh, U., Kochhar, A., Singh, S.: Blood glucose lowering potential of some herbal plants. J. Med. Plants Res., 2011; 5(19): 4691–4695.
3. Sunil Kumar, Vipin Kumar, Om Prakash. Antidiabetic, hypolipidemic and histopathological analysis of Dillenia indica (L.) leaves extract on alloxan induced diabetic rats. Asian Pacific Journal of Tropical Medicine, 2011; 347-352.
4. Rai, P.K., Rai, N.K., Rai, A.K., Watal, G. Role of LIBS in elemental analysis of *Psidium guajava* responsible for glycemic potential. Instrumentation Science and Technology, 2007; 35: 507–522.
5. Rakesh Kumar Singh, Shikha Mehta, Dolly Jaiswal, Prashant Kumar Rai, GeetaWatal. Antidiabetic effect of *Ficus bengalensis* aerial roots in

experimental animals Journal of Ethnopharmacology, 2009; 123: 110–114.
6. Biswas K, Observations on the systematic position of *Ficus krishnae*. Current Science, 1934; 3: 424-7.
7. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. In: Kirtikar KR, Basu BD (eds). Dehra Dun, India: International book distributors, 1987; 3: 2061-2062.
8. Chetty MK, Sivaji K, Rao TK., Flowering plants of chittoor district. 2nd ed, Students Offset Printers., Tirupati, 2008-09.
9. Amarvani P Kanjikar, Aruna LH, Ramesh L Londonkar. Novel investigation on in vitro anti-diabetic and volatile profile of bioactive compounds present in methanolic extract of *Ficus krishnae* stem bark. International journal of ChemTech research, 2017; 10(9): 220-228.
10. Amarvani P Kanjikar, Ramesh L Londonkar. Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Activity of Stem Bark of *Ficus Krishnae* International Journal of Pharmacognosy and Phytochemical Research, 2017; 9(5): 733-738.
11. Nayaka HB, Londonkar RL, Umesh MK. Evaluation of *Portulaca oleracea* L for anti-fertility effect in female albino rats. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(5): 86-89.
12. Rajnish Gupta, Manas Mathur, Vijay K. Bajaj, Pawan Katariya, Sunita yadav, Raka Kamal and Radhey S. Gupta. Evaluation of antidiabetic and antioxidant activity of Moringa oleifera in experimental diabetes Journal of Diabetes, 2012; 4: 164–171.
13. Sood R. Diabetes Mellitus. Medical laboratory Technology—Methods and Interpretations. Jaypee, 1999.
14. A.N. Nagappa, P.A. Thakurdesai, N. Venkat Rao, Jiwan Singh. Antidiabetic activity of *Terminalia catappa Linn* fruits Journal of Ethnopharmacology, 2003; 88: 45–50.
15. Mohammed Fazil Ahmed, Syed Mohammed Kazim, Syed Safiullah Ghori. Antidiabetic Activity of Vinca rosea Extracts in Alloxan-Induced Diabetic Rats. International Journal of Endocrinology, 2010; 841090.
16. Shanmugasundram ERB, Gopinath KL, Shanmugasundram KR, Rajendran VM. Possible regeneration of islets of langerhans in streptozotocin diabetic rats given Gymnema sylvestre leaf extract. Journal Ethnopharamacol, 1990; 30: 265-279.
17. Isaac A, Gopinath D, Murthy NS. Role of informal care providers in home based long term care in diabetes mellitus at Kaiwara Primary Health Center area, Karnataka, India. Asian Pac J Trop Dis., 2011; 1(2): 127-130.
18. Bonilla JV, Gilbertsville KY. Methods and Composition for regulation of blood cholesterol. Afri J Traditional Complement Altern Med., 2009; 6(4): 573–578.
19. Maisa MA Al-Qudah, Moawiya A Haddad, Jafar MF EL-Qudah. The effects of aqueous ginger extract on pancreas histology and on blood glucose in normal and alloxan monohydrate-induced diabetic rats. Biomedical Research, 2016; 27(2): 350-356.
20. Yadev JP, Saini S, Kalia AN, Dangi AS. Hypoglycemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan-induced diabetes rats. Indian J. Pharmacol, 2008; 40: 23-27.
21. Shanmugasundaram ER, Gopinath KL, Radha Shanmugasundaram K, Rajendran VM. Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given Gymnema sylvestre leaf extracts. J. Ethnopharmacol, 1990; 30: 265-79.
22. Mahalingam Gayathri And Krishnan Kannabiran. Antidiabetic And Ameliorative Potential Of Ficus Bengalensis Bark Extract In Streptozotocin Induced Diabetic Rats. Indian Journal of Clinical Biochemistry, 2008; 23(4): 394-400.