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Contents

Protective effect of Fagonia arabica (L.) against Alloxan monohydrate-induced Diabetes in albino Wistar rat

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الخلاصة:
مرض داء السكري هو اكثر امراض او اضطرابات الغدد الصماء او الغدد الافرازية شيوعا، لهذا التطوير العلاجي على امتداد الطب الغربي (allopathic)) هو في اغلب الاحيان محدد في الكفاءة ويحمل خطر التاثيرات المضادة وهو غالبا غالي الثمن خصوصا في العالم النامي. استنادا الى الادبيات السابقة وجد التاثير المفيد للفاكونيا اربيكا Fagonia arabica المضاد لداء السكري في داخل الجسم ولهذا تم اختيار الفاكونيا اربيكا. من اجل ان تشخص مناهج بديلة ومكملة للادوية الحالية درسنا الامكانية المضادة للسكري للفاكونيا اربيكا (L.) ضد آلوكسان الهيدرات Alloxan Monohydrate المستحث لداء مرض السكري في فئران ويستار البيضاء. المستخلص الايثانولي للفاكونيا اربيكا (L.) النباتي حضر بواسطة جهاز السكسوليت ثم جفد المستخلص بواسطة جهاز التجفيد للجصول على مسحوق من المادة. استعمل المستخلص لعلاج داء السكري في الفئران من خلال الاعطاء الفموي.
قبل البدء بالدراسة وضعت الفئران في بيوت لمدة اسبوع للتاقلم على البيئة ثم نفذت الدراسة لمدة 45 يوم وفق بروتوكول مدروس وممنهج تضمن قياس وزن الجسم ومستوى تركيز الكلوكوز في الدم وبعض الانزيمات (AST, ALT, ALP)وانماط الدهون (HDL, Ch., TG and LDL) ودراسة الاختبار النسيجي للكبد. اخذ الكليبنكلامايد Glibenclamide كدواء ومحلول قياسي لمعالجة داء السكري وقورنت نتائجه مع الفاكونيا اربيكا وكانت النتائج مطابقة تقريبا له. تم تحضير خمس مجاميع كل مجموعة تحتوي 6 فئران، (وتم اختيار الجرع للفاكونيا و الكليبنكلامايد والالوكسان اعتمادا على دراسات وطرق مختبرية ولهذا اختيرت جرعة الفاكونيا 250 mg/ kg bw و (150 mg/kg bw) (Alloxan monohydrate)و Glibenclamide,10 mg/kg bw).الدراسة الحالية هي تقييم للفاكونيا اربيكا وما لها من تاثير وقائي ضد مرض السكري المستحث بواسطة الالوكسان في الفئران.

Abstract

Diabetes mellitus is a most common endocrine disorder, affecting more than 300 million people worldwide. For this, therapies developed along the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects, and are often too costly. In order to identify complementary or alternative approaches to existing medications, we studied the anti-diabetic potential of Fagonia arabica (L.). We have selected F.arabica (L.). Ethanolic extract of the plant was prepared by using Soxhlet apparatus with 70% ethanol. This extract was used to treat the diabetic rats through oral ingestion. Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β -cells of islets of langerhans. Diabetes was induced by a single I.P. dose Alloxan monohydrate (150 mg/kg body weight). It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia.

The study was carried out on a 45 day protocol and the body weights, blood glucose levels were measured on Day 1, Day 7, Day 14, Day 21, Day 28, Day 35, Day 42 and Day 45 of the treatment, along with assays of AST, ALT, ALP, Lipid profile studies and histopathological examination of liver on day 45. Maximum activity was shown by the active compound with a percent variation in blood glucose levels. Glibenclamide (10mg/kg body weight) was taken as the standard and the results were

quite comparable with it. Because of the anti-oxidant and hypo lipidemic activity of the F.arabica, we have selected to know the anti-diabetic property of the plant. There is no information available on the anti-diabetic activity of F.arabica. Hence the present study is designed to evaluate the anti-diabetic property of the F.arabica in Alloxan monohydrate induced diabetes in albino wistar rats.

Key words: Fagonia arabica (L.), Alloxan monohydrate, Diabetes, Acute toxicity study, AST, ALT, ALP, Lipid profile, histopathological and Glibenclamide.

Introduction

Herbal products are often perceived as safe because they are "natural". In India, herbs exert different mechanism of actions including the mechanism of actions of synthetic oral hypoglycaemic drugs. Fagonia arabica L., belonging to the family Zygophyallaceae, commonly known as Dhamaso in Gujarati, Ustarkar in Hindi, Dhanvyas in Sanskrit is a wildly grown perennial plant. The plant is globally distributed in Africa, Arabia, Pakistan and India. It contains several triterpenoid saponins and mainly used in skin diseases(1).

Aerial parts of plant contains several triterpenoid saponins which gave sapogenin, nahagenin, oleanolic acid. It also gave diterpenes, fagonone and its derivatives, besides flavonoids (2, 3). Triterpenoid and sterol glycosides are reported in the plant (4). Leaves of plant contain flavonoids, quercetin and kaempferol and fruits contain ascorbic acid. The plant is useful in wound healing, small pox, vertigo, foul, abscesses, scabies, infected ulcers, scrofulous glands wounds and for dermatosis (5). It also used as astringent, antiviral, antimicrobial, antiseptic, anti-inflammatory, antioxidant (6) and in liver cancer, thrombolytic action (7). The plant shows synergistic effect with Heteropneustes fossilis extracts against myocardial, cerebral infarction, and embolism disorder in mice (8). Different parts of this herb have been used to cure various ailments, namely hematological, neurological, endocrinological and inflammatory disorders (10-12, 13-15). Diabetes mellitus can be defined as a metabolic disorder with impaired glucose utilization, characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Diabetes is a severe health problem and its incidence is increasing at alarming proportions. Type 2 diabetes results from the interaction between a genetic predisposition, behavioral and environmental risk factors. Although the genetic basis of type 2 diabetes has yet to be identified, there is strong evidence that modifiable risk factors such as obesity and physical inactivity are the main nongenetic determinants of the disease (9). T2DM is a non-autoimmune, complex, heterogeneous and polygenic metabolic disease condition in which the body fails to produce enough insulin, characterized by abnormal glucose homeostasis, its pathogenesis appears to involve complex interactions between genetic and environmental factors (16). T2DM occurs when impaired insulin effectiveness (insulin resistance) is accompanied by the failure to produce sufficient cell insulin (17). T2DM as a common and complex disease has been characterized by the following causes: Obesity (18), Abdominal adiposity (19), Imbalance of human metabolism, Genes, Ethnicity (20).

Materials and Methods

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and

recommendations. It includes in it all the steps from Extract preparation, observation, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner. Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Statistical Analysis:

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard deviation (S.D.) and analyzed for ANOVA and post hoc Dunnett's *t*-test. Differences between groups were considered significant at $P<0.001$ and $P < 0.05$ levels.

Acute toxicity testing

Acute toxicity studies revealed that Oral administration of graded doses (100, 250, 500, 1000, 1500 and 2000 mg/kg bw) of the alcoholic extract of *F. arabica* to rats. The ethanolic extract of *F. arabica* was safe up to 2000mg/ kgbw and did not produce any mortality and changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the 10 days observation period. No lethality or any toxic reactions or moribund state was observed up to the end of the study period. The obtained results signify that the use of the plant for treatment is safe. So the dose of 250 mg/ kg bw was selected to study the anti – diabetic property of the plant extract (FAEXT).

Results & Discussion

Effect of ethanolic extract of *F.arabica* on body weight of Alloxan monohydrate induced diabetic rats: The present study was an attempt to elucidate the anti-diabetic effect of F.arabica ethanolic extract (FAEXT) which showed to be anti-hyperglycemic. Before the supplementation of FAEXT and Glibenclamide, there were no significant differences of baseline body weight of the rats. The FAEXT (250 mg/kg) and Glibenclamide (10 mg/kg) treated rats, showed significant increase in body weight as compared with diabetic groups after 6 weeks of study. Before treatment, the fasting glucose level was significantly higher ($P\leq0.05$) in all groups when compared with normal group. After 4 weeks, groups treated with FAEXT showed dose dependent reduction of fasting glucose versus diabetic group ($P\leq0.05$). After 45 days of FAEXT supplementation to the diabetic rats, there was a significant elevation in insulin level in respect to diabetic control group in dose dependent manner ($P\leq0.05$, figure 1). From the dose fixation studies, 250 mg/kg bw was found to be the most effective in reducing fasting glucose levels. Data represented as mean ± S.D values of 6 animals each. *$p<0.001$, **$p<0.05$ (Dunnett t-test); diabetic control was compared with the normal, extract and standard treated groups were compared with the diabetic control.

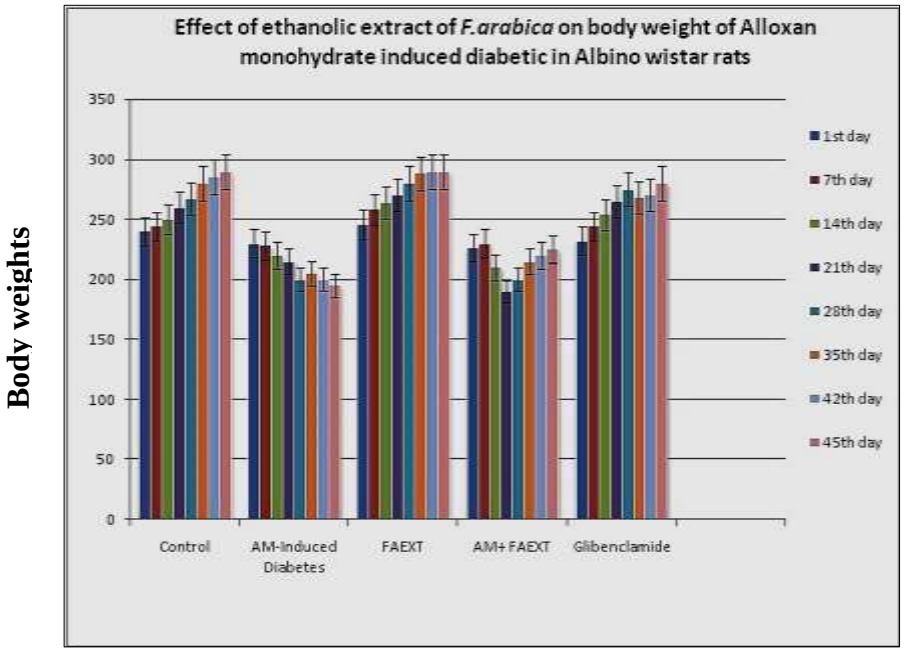

Fig.1. Effect of ethanolic extract of *F.arabica* on body weight of Alloxan monohydrate induced diabetic rats.

Effect of ethanolic extract of *F.arabica* (FAEXT) on fasting blood glucose levels: As shown in Table, the induction of diabetes has caused significant initial increase in the fasting blood glucose levels of all the groups. The diabetic control group shows significant increase throughout the study period when compared with the normal control group ($p<0.001$). However, the extract treated groups and the standard treated group shows significant decrease in the fasting blood glucose levels when compared with diabetic control ($p<0.001$) which was determined on the 45th day of experiment. The effect is more pronounced in standard (10mg/kg) group, followed by ethyl acetate (500mg/kg) group, aqueous (500mg/kg) group, ethyl acetate (250mg/kg) group, aqueous (250mg/kg) group, methanol (500mg/kg) group and methanol (250mg/kg) group as shown in figure 2. On the basis of these observations only ethyl acetate and aqueous extracts were selected for further analysis of antidiabetic activity.

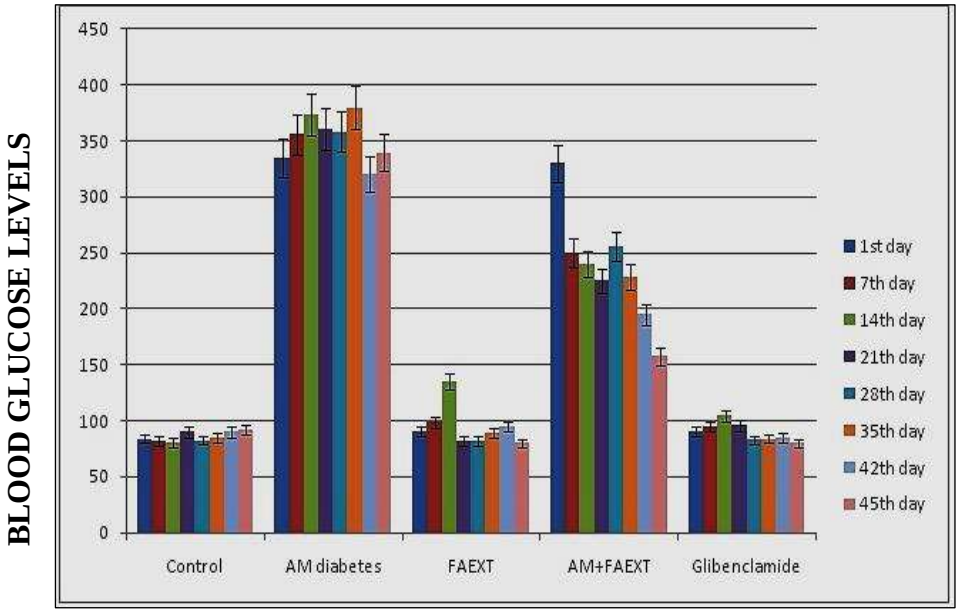

Fig.2. Fasting blood glucose levels of Alloxan monohydrate induced diabetic rats

Effect of ethanolic extract of *F.arabica* on AST, ALT and ALP levels: AST, ALT and ALP, as shown in Table 1 both the extracts show significantly lower levels of AST, ALT and ALP in comparison to the diabetic control group ($p<0.001$). Here the maximum reduction was observed for standard followed by FAEXT. An increase in the AST, ALT and ALP activities was recorded in diabetic rats in comparison with non-diabetic rats, indicating an altered liver function in diabetic condition. *FAEXT* significantly controlled AST, ALT and ALP values in the alloxan induced diabetic rats. In diabetic animals a change in the serum enzymes is directly related to changes in the metabolism in which these enzymes are involved. The increased levels of transaminases which are active in the absence of insulin because of increased availability of amino acids in diabetes (Bondy et al., 1949; Felig et al., 1970) are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes.

Parameters	Control	AM-induced diabetic rats (150mg/kgbw)	FAEXT (250mg/ kgbw)	AM-induced Diabetes + FAEXT	Glibenclamide (10mg/kgbw)
AST (IU/100ml)	35.1±6.30*	74.07±9.41**	15.96±7.41*	42±10.27*	25±5.90
ALT (IU/100ml)	28.78±5.40	52.2±10.11	24.01±3.80	38±2.07*	20.46±6.30**
ALP (IU/100ml)	12.9±1.4	23.6±2.1^{a**}	20.47±1.1	14.0±0.8^{b**}	12.5±1.5^{a**}

Table 1. Effect of FAEXT on AST, ALT and ALP levels.

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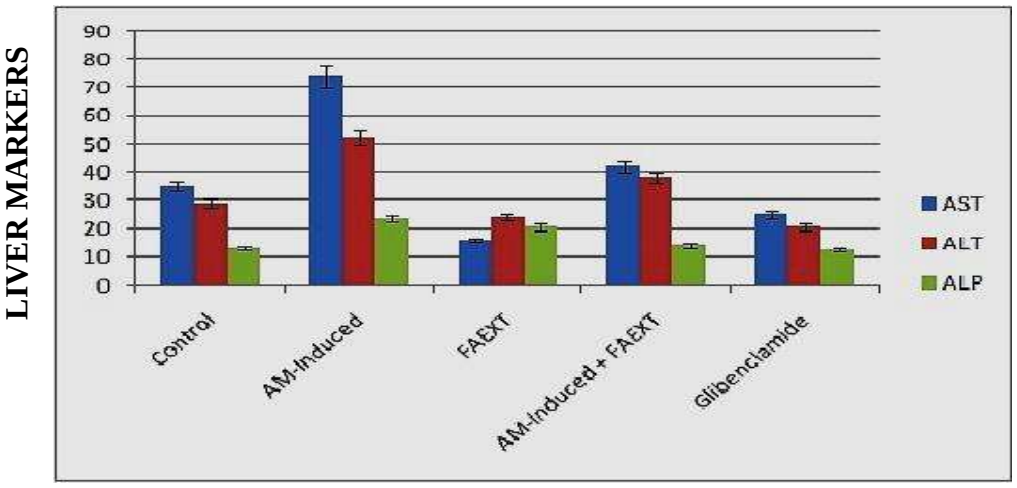

Fig.3. AST, ALT and ALP levels of Alloxan monohydrate induced diabetic rats.

In the present study, the *F.arabica* extracts significantly decreased AST and ALT enzyme activities. Hence, the improvements noticed in the levels of these

enzymes are as a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of AST and ALT levels after treatment also indicates a revival of insulin secretion. Elevation of ALP has been reported in diabetic rats (Mishima, 1967) and rabbits (Begum et al., 1978). This increase in ALP was significantly reversed by the extract of FAEXT.

Effect of ethanolic extract of F.arabica on Lipid profile: hyperglycemia in diabetic rats was associated with a high serum concentration of total cholesterol and triglycerides as present in the normal diabetic conditions (Georg and Ludvik, 2000). However, the active compound from FAEXT at a dose level of 10 mg/kg reversed the diabetes-induced hyperlipidemia compared to the diabetic control group. In FAEXT treated rats, there was a reduction in the levels of cholesterol and triglycerides, showing the hypolipidemic effect of this plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Sharma et al., 2003). In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after treatment with extracts of FAEXT may be directly attributed to improvements in insulin levels.

Table 2. Effect of ethanolic extract of F.arabica on Lipid profile

Groups	Lipid Profile Studies			
	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	138.4±3.51	78±5.01*	128±12**	68.56±5.6
AM-induced diabetic rats (150mg/kgbw)	204.77±7.51*	256.20±8.53*	51±7.48*	139.1±2.9
FAEXT (250mg/kgbw)	99.80±6.14	90.30±7.15*	162.04±5*	90±9.1
AM-induced Diabetes + FAEXT	166.10±5.75	220.80±7.67*	70±2.9	103±5*
Glibenclamide (Standard) (10mg/kgbw)	99.72±8.9	65±5.55*	135±5.78	79±5.67*

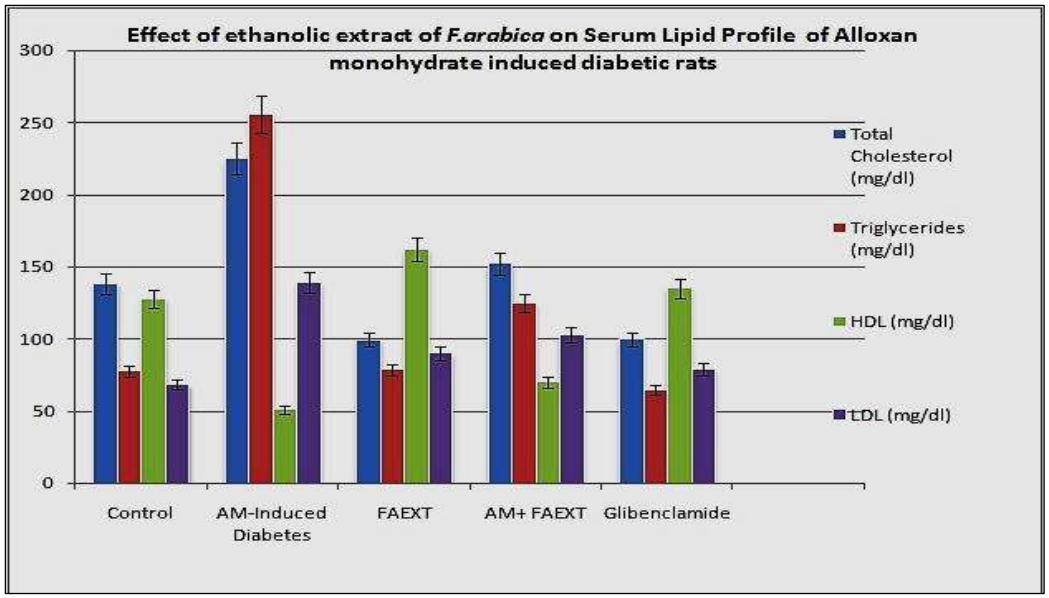

Fig.4 Effect of ethanolic extract of *F.arabica* on Lipid profile

Histopathological studies: Fat accumulation in liver and inflammation were reduced with the addition of active compound of FAEXT revealed. The presence of secondary metabolites that have been shown to possess antidiabetic effect in other plants (Marles and Fransworth, 1995; Saxena et al., 2004). Saponins (Abdel- Zaher et al, 2005), alkaloids (Li et al, 2004) and flavonoids (Coskul et al, 2005; Tanko et al, 2007) which were responsible for the anti-diabetic effect in other plants were also detected in the extracts of this plant. The standard treated group also shows recovery and tends to approach the histopathology of the normal rat liver. 'A' = Normal rats, 'B'= Alloxan monohydrate (AM) (150 mg/kgbw) rats, 'C' = FAEXT 250 mg/kgbw, 'D' = AM group (150 mg/ kgbw) + FAEXT 250 mg/kgbw, 'E' = Reference control i.e. Standard drug (Glibenclamide, 10 mg/kgbw).

Development of phyto-medicines is relatively inexpensive and less time consuming; it is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years. Tissue sections from liver of normal controls showed normal architecture. The diabetic control showed dilated blood vessels, nuclear vacuolation and focal fatty infiltration. Treatment with the active compound FAEXT at lower dose improved the lesions. Mild necrosis was observed in diabetic rats which received Alloxan monohydrate. Necrosis was seen at higher doses of both the extracts on non-diabetic rats as well. Toxic changes in the histology of liver were observed cytoplasmic vacuolation, hydropic changes, and inflammation of portal veins. Eosin (a red fluorescent dye that is a bromine derivative of fluorescein). haematoxylin (Tissues are stained in aqueous hematoxylin after mordanting in iron ammonium sulfate (iron alum)).

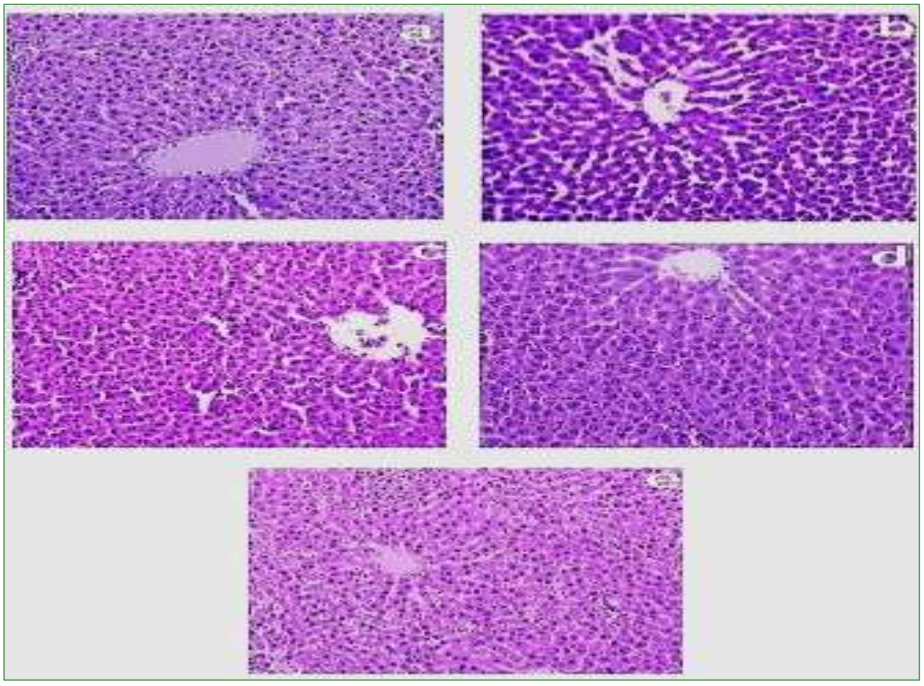

Fig. 5: Micrographs of rat liver stained by haematoxylin and eosin of A, B, C, D & E , Effect of FAEXT administration on liver Histopathological changes.

Conclusion

The alcoholic extract of *F.arabica* showed anti-diabetic activity against Alloxan monohydrate induced Diabetes in Albino wistar rats. The plant is safe for use as no mortality was recorded in the acute toxicity test. The study was performed to find out beneficial Anti-diabetic effects of the *F.arabica* through animal studies, revealed that the plant extract is having protective effect against Diabetes. Alloxan monohydrate was used induce the diabetes and Glibenclamide was used as standard drug. There are increase in the levels of Ch, TG, LDL and decrease of HDL level in group B compared to control group A by using AM induced diabetes. Decrease of Ch, TG, LDL levels and increase of HDL level in group C compared to the diabetic control group B by using FAEXT showing the hypolipidemic effect of this plant . In Glibenclamide treated rats, there are reduction in the levels of Ch, TG, LDL and increase level in HDL in group E compared to the diabetic control group B . The hypolipidemic effect may be due to inhibition of fatty acid synthesis. FAEXT (250mg/kgbw) was showed beneficial effects on blood glucose levels of normal in rat models and we found best results and the results also reveal that the significantly protect from other metabolic aberrations found in diabetes, physiological as well as biochemical aberrations.

Effective blood glucose and hypertension control is the key for preventing or reversing diabetic hypertensive complications. The results indicated that the FAEXT is most potent in lowering the fasting blood glucose level of the diabetic rats; the effect is dose dependent. Moreover, the extract showed improvement in parameters like body weights and fasting blood Glucose levels. The FAEXT also lower serum ALT, AST, and ALP levels which show the effect of the active compound in reversing the organ damage due to diabetes which is clearly observed by high levels of AST and ALT in diabetic control.

Histopathological examination of liver showed the recovery of damaged tissue when sections of treated groups are compared with diabetic control. The histopathological studies also indicated that F.arabica is effective in regeneration of insulin secreting β-cells and thus possesses anti-hyperglycaemic activity.

Recommendation

In order to maintain health where phytochemicals are involved, a daily recommended allowance similar to other nutrients is required. From the present study we observed that the ethanolic extract of the *F.arabica* (250mg/kgbw) is safe and showed protection against Alloxan monohydrate induced Diabetes. Therefore, there is a need for dosage allowance for each bioactive compound from *Fagonia arabica* (L.).

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وقائع بحوث المؤتمر العلمي الطبي الرابع

للمدة (20-21/أبريل/2016)

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