WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 16, 444-453. Research Article ISSN 2277– 7105

EVALUATION OF PROTECTIVE EFFECT OF POLYHERBAL FORMULATION ON STREPTOZOTOCIN AND HIGH FAT DIET INDUCED HYPERLIPIDAMIEA, ATHEROSCLEROSIS AND SEXUAL DYSFUNCTION IN DIABETIC RATS

MD. Faheemuddin[1]*, Dr. Pankaj Kumar Sharma[2], Dr. N. Siva Subramanian[1], Prof. Dr. Birendra Shrivastava[2]

1Department of Pharmacy, Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar.

2Department of Pharmacy, Jaipur National University, Jaipur.

Article Received on
13 October 2017,
Revised on 04 Nov. 2017,
Accepted on 24 Nov. 2017
DOI: 10.20959/wjpr201716-9900

***Corresponding Author**
MD. Faheemuddin
Department of Pharmacy,
Smt. Sarojini Ramulamma
College of Pharmacy,
Mahabubnagar.

ABSTRACT

Introduction: Diabetes is the metabolic disorder with multi aetiologies' characterized by chronic hyperglycaemia which is leads to many other disorders. Diabetic complications like cardiovascular disorders, renal failure, hepatic disorders, diabetic foot and many other disorders. (Josephine M. et al 2011). In the present study Ethanolic extract of *Verbascum Thapsus, Lotus corniculatus Carlina acaulis* were used to prepare poly herbal formulation and it was evaluated for the antiatherosclerosis, antihyperlipidamic and sexual potency in high fat diet and Streptozotocin induced diabetic activity. **Methods:** Animals weight of 120-140grams were selected and Diabetes was induced to the animals using Streptozotocin and provided with high fat diet to induce atherosclerosis, hyperlipidamiea and sexual dysfunction. After the inducing of diabetes blood glucose levels and lipid profile was determined which is considered as 0^{th} day and treatment was given till 45day. On the 45^{th} day all the blood and tissue pathological studies were carried out. The PHF toxicity studies were carried out and dose of 100 mg/kg and 200mg/kg body was given during the 45 days study period. For reference standard glibinclamide drug treated, toxic control and normal group was also used. **Results:** From the biochemical parameters and histopatological studies it was proved that the PHF is effective in controlling blood glucose levels, lipid profile along with sexual potency in male rats. It was having more significant value than that of standard drug to maintain the sexual potency in male rats.

KEYWORDS: Poly Herbal Formulation, Ethanolic extract, Glibinclamide, Streptozotocin, Vitamin D_3.

INTRODUCTION

Elucidation of the role of cholesterol in the pathogenesis of atherosclerosis, hyper lipidamia and sexual dysfunction is often referred to as one of the greatest discoveries of the 20th century. Some of the milestones on the road to acceptance of the 'lipid hypothesis' which proposed that hypercholesterolemia was a causative factor in human atherosclerosis. Atherosclerosis may be defined as degenerative changes in the Tunica intima of medium and large arteries. The degeneration includes accumulation of lipids, complex carbohydrates, blood and blood products and cellular waste products accompanied by the formation of fibrous tissues and calcium deposition in the intima of blood vessels. (Doron Aronson, 2002) Accelerated atherosclerosis occurs in animal models with engineered deficiencies in [apo (a)] or LDL receptors. Other genetic or acquired disorders (e.g., diabetes mellitus, Hypothyroidism) that cause hypercholesterolemia lead to premature atherosclerosis. Lowering serum cholesterol by diet or drugs slows the rate of progression of atherosclerosis, causes regression of some plaques, and reduces the risk of cardiovascular events. The diabetes also deposits adipose tissue at various parts that leads to the organ failure, similarly the sex organs in males due to various factors in diabetes leads to sexual dysfunction. (Jun Kusunoki, 2000).

MATERIALS AND METHOD

MATERIALS

All chemicals were of analytical grade and obtained locally. Cholesterol, Triglycerides and HDL-C kit were procured from Robonik diagnostics, Hyderabad, India.

PLANT MATERIAL

The fresh aerial shoots of *Verbascum Thapsus*[6], *Lotus corniculatus*[7] and *Carlina acaulis*[5] was collected from various parts of India. Identification of the plant was done by Dr. K. Madhava Chetty assistant professor, department of botany, Sri Venkateswara University, Tirupati, A.P, India.

ANIMALS

Wister albino adult male rats weighing 120-140g were selected and housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and dark

cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. The composition of atherogenic diet used during the study was as given. Each of these treatment groups consisted of six animals/group. The protocol of this study was approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (Reg No: 769/2010/CPCSEA).

METHOD

Preparation of the extract material

The aerial shoots plants were isolated, chopped into small pieces and dried under shade at room temperature for seven days. The dried plants were powdered and passed through the sieve (coarse 10/44). This powder was used for the preparation of Ethanolic extract.

Ethanolic extract

Ethanolic extract was prepared by Heat Soxhlet extractor. The dried coarse powdered of plants (250gm) were transferred to a round bottom flask, 99% of ethanol was added to the flask and soaked for 2 hours. This was then boiled for 4 hours. The extracts so obtained was decanted in a beaker and then concentrated to 1/6th of the total volume on a water bath. This was preserved by adding a few drops of chloroform and kept in the refrigerator. This extracts were mixed at a ratio of 1:1:1 using tween 80 as surfactant and a poly herbal formulation was prepared. PHF was administered to the animals by making the concentration.

Induction of diabetes

A freshly prepared solution of streptozotocin (45mg/kg in 0.1M buffer citrate solution) is injected to overnight fasted rats. After 48 hrs the rat's blood glucose levels of 250mg/dl or above were considered for the further study. (Suman Bala Sharma, 2010).

Induction of atherosclerosis and hyperlipidamiea

Although the cause and pathogenesis of atherosclerosis still remains largely unresolved, it is generally agreed that correlation exists between high blood cholesterol and cardiovascular diseases. Antischkow for the first time succeeded in inducing atherosclerosis in rabbits by feeding cholesterol containing diet. Development of atherosclerosis in rabbits usually takes at least 60 days of feeding atherogenic diet. Rat is said to be resistant to such dietary manipulations for the development of atherosclerosis, but with supplementation of very high doses of vitamin-D_3 along with atherogenic diet, success has been achieved in developing

atherosclerosis in rats in a short period. The atherogenic diet (AD) consisting of 2 g of cholesterol and 8 g of saturated fat and 100 mg calcium were added to 90 g of powdered standard commercial pellet diet and thoroughly mixed. The rats were fed with high fat diet along with weekly challenge of oral vitamin-D3 for one month through oral route. (Martha Srinivas, 2008).

Experimental protocol

In order to induce atherosclerosis, the method reported by Bopanna *et al.* was followed. The animals were divided into five groups of six rats each and they received the following diets with or without treatment for 45 days orally:

Group I: Normal diet

Group II: Atherogenic diet + STZ (45mg/kg)

Group III: Atherogenic diet+ STZ (45mg/kg) + Glibinclamide (5mg/kg/day)

Group IV: Atherogenic diet+ STZ (45mg/kg) + PHF (100 mg/kg/day).

Group V: Atherogenic diet+ STZ (45mg/kg) + PHF (200 mg/kg/day).

At the end of the treatment the rats were fasted overnight, blood was drawn from retro orbital plexus as per CPCSEA guidelines. Serum was separated and stored in refrigerator until assay

Measurement of various Parameters

Physical Parameters: The body weight was recorded on the first day and then last day of the study period in each group.

Blood glucose levels

The glucose levels were determined by using commercial glucometer kit on initial and final day of the experiment by collecting blood from rat's tail.

Biochemical Estimations

Lipid parameters were determined in blood serum, at the initial day and on final day of 45 days, animals were fasted overnight and blood was collected from retro orbital plexus under light ether anaesthesia, centrifuged at 2500 rpm for 20 minutes. The serum obtained will be kept at $4^{o}C$ until used. The quantitative estimation of lipid profile was carried out using Infinite triglycerides liquid for triglycerides, Infinite cholesterol liquid for total cholesterol and Autozyme for HDL-C, ACCUREX in laboratory. Estimation of VLDL-C and LDL-C will be done by using the Friedward's formula.

VLDL-C = Triglycerides/5

LDL-C = Total cholesterol – (HDL-C + VLDL-C).

Histopathology of Pancreas, Aorta and Testis

For histopathology, the rats were sacrificed by cervical decapitation and their aortas were dissected out. During the procedure, ice was used to keep the aorta samples fresh and avoid any degradation. The Pancreas, aortas and Testis were stored in 10% formaline solution and sent to a local pathological laboratory for hematoxyline and eosine staining. (Reenu Singh Tanwar, 2011).

Statistical Analysis

The results are expressed as mean ± standard error of mean (SEM). The data were analyzed using one-way analysis of variance (one-way ANOVA) followed by Dunnett multiple comparison test for comparison between groups. The criterion for statistical significance was $p<0.01$.

RESULTS

Atherogenic Diet (AD) Induced Atherosclerosis, hyperlipidamiea and sexual dysfunction in Diabetic *Rats*:

Table N0: 1 Effect of PHF extract on Body weight on in STZ induced diabetes, Hyperlipidamiea, Atherosclerosis and sexual dysfunction male rats.

Groups	Dose (mg/ kg)	Body Weight (Mean± S.E.M)	
		0^{th}day	45^{th}day
G I	Normal control	130.31±7.52	202.32±8.21
G II	Control(Vehicle)	130.67±5.72	286.57±7.54$^{\#\#}$
G III	10 mg/ kg	133.72±6.96	229.25±6.24**
G IV	100 mg/ kg	132.84±7.57	230.24±5.45*
G V	200 mg/ kg	138.21±6.42	220.32±7.45**

Table No: 2 Effect of PHF on Fasting Blood Glucose levels on in STZ induced diabetic, Hyperlipidamiea, Atherosclerosis and sexual dysfunction male rats.

Group	0^{th} day	15^{th} day	30^{th} day	45^{th} day
G I	98.45±6.25	101.43±5.52	102.01±7.35	99.75±8.41
G II	259.23±5.13$^{\#\#\#}$	290.12±8.45$^{\#\#\#}$	292.54±9.14$^{\#\#\#}$	295.31±7.15$^{\#\#\#}$
G III	267.24±8.35	209.5±7.21**	150.3±8.01***	111.3±9.54***
G IV	263.21±5.35	201.31±7.85*	167.21±8.12**	121.31±9.54***
G V	254.23±8.54	194.36±9.32**	134.28±7.36***	106.45±9.53***

Table No: 3: Effect of PHF on Serum Lipids levels in STZ induced diabetic Hyperlipidamiea, Atherosclerosis and sexual dysfunction male rats.

Group	G-I	G-II	G-III	G-IV	G-V
Initial HDL	41.67± 3.96	24.45±4.85	23.73±4.98	23.52±3.24	26.54±5.12
Final HDL	45.67±2.29	13.83±4.69$^{###}$	32.17±2.94**	34.57±5.32 **	42.84±5.21***
Initial LDL	18.8±1.57	62.67±4.86 $^{##}$	65.73±3.19	64.25±2.45	65.32±3.12
Final LDL	18.07±1.49	98.03±8.74$^{###}$	23.25±3.67***	31.25±3.45**	22.54±5.36***
Initial VLDL	14.87±1.21	22.78±2.38$^{##}$	23.42±3.28	22.17±2.12	21.75±1.85
Final VLDL	15.27±1.16	31.54±1.69$^{###}$	16.17±2.14***	17.54±3.42**	15.84±3.28***
Initial TC	73.5±5.35	108.23±7.18$^{##}$	112.32±6.28	110.83±8.36	109.84±9.31
Final TC	78.23±6.23	157.55±8.43$^{###}$	80.67±8.72***	84.32±9.86 **	78.69±6.23 ***
Initial triglycerides	74.33±7.16	123.93±8.94 $^{###}$	111.75±6.42	118.35±9.31	121.24±7.42
Final triglycerides	76.33±8.28	155.75±8.83$^{###}$	81.65±9.76***	90.54±8.41**	82.74±7.15***

Table No 4: Effect of PHF on Sperm Count levels in STZ induced diabetic Hyperlipidamiea, Atherosclerosis and sexual dysfunction male rats.

Groups	Sperms, Million/ ml
G I	239.67±17.95
G II	83.23±18.41$^{###}$
G III	177.84±16.15 **
G IV	148.85±18.65 **
G V	231.74±19.86 ***

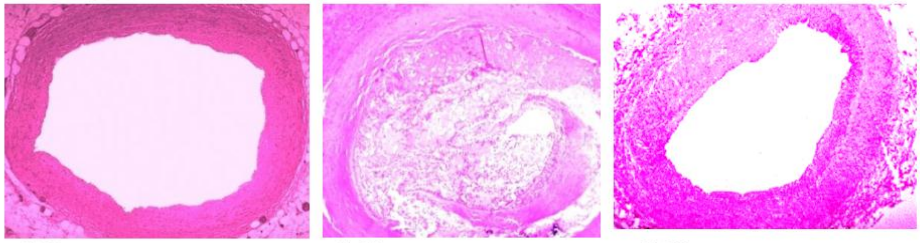
Values are expressed as (Mean ± SEM), n= 6, ns= not significant, one way analysis of variance (ANOVA) followed by dunnett multiple comparison. *** P<0.001 **P<0.01 and *P<0.005 as compared to control group. ^{a} P<0.001 ^{b}P<0.01 and ^{c}P<0.05 as compared to normal group.

Effect of PHF (100 and 200mg/kg P.O once daily for 45 days) administration / Glibinclamide (5mg/kg/P.O once daily for 30 day) on Histopathological changes in Pancreas, aorta and testis of rats fed with AD for 45 days.

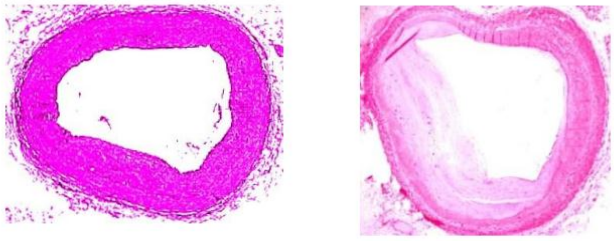
Effect of treatment on Aorta

Aorta of the normal group has shown normal aorta without any sign of atheroma or thrombi. In the above fig.2 GII shows the aorta of the toxic control rat which is almost totally covered by the atheroma, which will lead to total blocked of blood vessel that causes blocked of heart. In the image GIII is the standard drug treated rat aorta that has bit traces of atheroma development. That when compared to toxic control is very little, negligible. G-IV and G-V show the effect of PHF in preventing the plague formation in aorta. Its higher doe is more effective than that of its low dose and it most effective.

Effect of treatment on the Aorta of STZ induces atherosclerosis diabetic rat:-



G-I G-II G-III

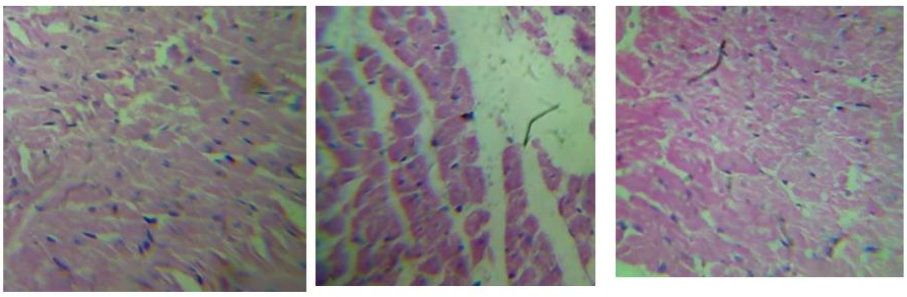


G-IV G-V

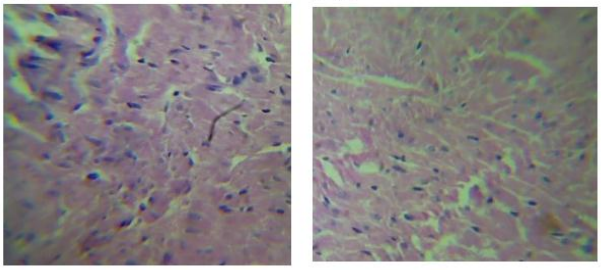
Effect of Treatment on Pancreas

In the below images G-I is the normal pancreas with normal arrangement of cells and no cell damage is seen. In the G-II image the cellular damage and spaces are observed that indicates that STZ had damaged the pancreatic cell which lead to diabetes in the rats. G-III image Glibenclamide restored its near to normal alignment of pancreatic cells. The black dots show the Pancreatic langerhans. G-IV and G-V show the effect of PHF which is more effective to retain the normal pancreatic cells even at lower dose and at higher dose it is showing almost normal pancreatic cell alignment.

Effect of treatment on the pancreas of STZ induces diabetic rats:-



G-I G-II G-III



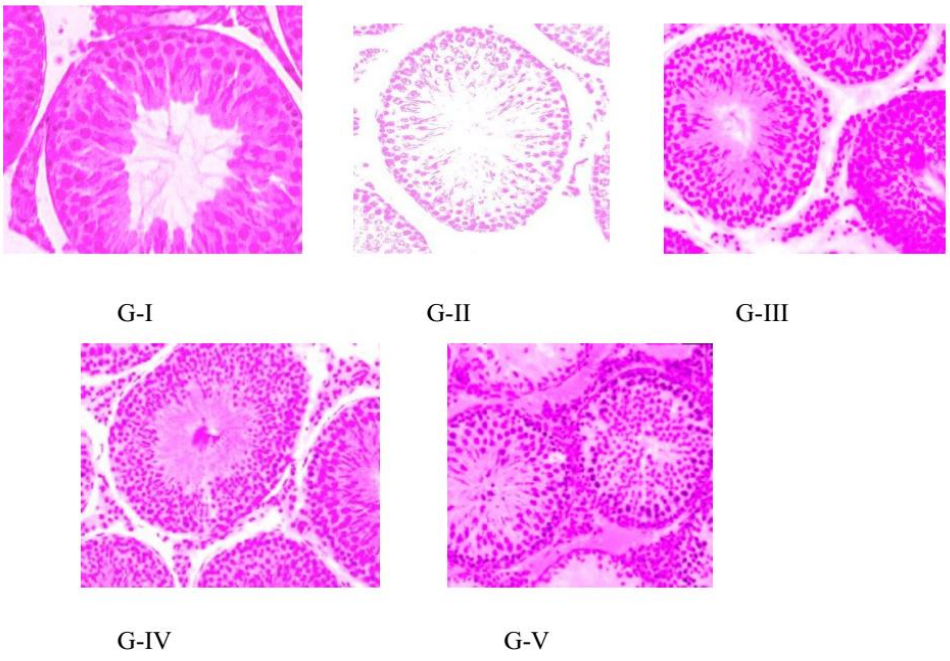
G-IV G-V

Effect of treatment on the testis of rats

In the below figure the images G-I to G-V are the images of testis of each group respectively. In the above images the No. of black dots indicates the no of Semermatids and black dotted with lining are the sperms. The medial hallow white space indicates the tubule lumen. The more no of spermatids and sperms rats is sexually potent and if the no is less it indicates the rat is less potent, similarly the large tubule lumen indicates the damage and less no of germinal, spermatid and sperms that indicate the animal is less potent sexually.

In the G-I the animal is normal and the image indicates the normal testis structure. The GII indicates less no of germ cell and spermatic and sperms that indicates that the animal is sexually less potent. In the standard treated group the no of germinal cell are more near to normal. The GIV and GV the testis has not showed any significant damage, near to normal structure of testis is retained in these groups.

Effect of treatment on testis of STZ induces sexual dysfunction diabetic rat:-



G-I G-II G-III

G-IV G-V

DISCUSSION

High fatty diet is a very common cause of heart disease and many other disorders. Particularly, with an increase in tendency towards fast foods, which are rich in saturated fats, an increase in coronary heart disorder (CHD) in diabetic patients and normal patients is being observed in the developing countries since past few decades. A one percent decrease in HDL-cholesterol is associated with a 3-4% increase in the risk of heart disease. In the present study

an increase in plasma HDL-cholesterol with a concomitant percentage decrease in glucose and other lipid parameters were observed (Tables 2 and 3).

It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and total protein which are actually raised in atherogenic diet, can be lowered significantly with PHF.

CONCLUSION

Treatment with PHF produced a significant decrease in the serum level of lipids and glucose in atherogenic diet induced Hyperlipidamiea, Atherosclerosis and sexual dysfunction in STZ male Diabetic rats. Hence by considering the effects observed in this model, the possible mechanism of PHF may involve increase of HDL-cholesterol, which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase (LCAT) enzyme. LCAT enzyme is involved in the transesterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL. Thus from the above results we can conclude that PHF has anti-hyperlipidamic, anti-atherosclerotic and ant-sexual dysfuntion activities in diet induced atherosclerosis in a STZ induced diabetic rats.

REFERENCES

1. Doron Aronson*1and Elliot J Rayfield., How hyperglycemia promotes atherosclerosis: molecular mechanisms, Cardiovascular Diabetology, 2002; 1-10.
2. G. C. Jain* and S. Agarwal, Favourable Effect of Cleome viscosa L. on Serum and Hepatic Lipids in Hyperlipidemic Rats, Asian J. Exp. Sci., 2006; 20(2): 331-336.
3. Jun Kusunoki, Katsumi Aragane, Tetsuya Kitamine *et al;* Postprandial Hyperlipidemia in Streptozotocin-Induced Diabetic Rats Is Due to Abnormal Increase in Intestinal Acyl Coenzyme A:Cholesterol Acyltransferase Activity, journal of American heart association, 7272, Greenville Avenue, Dallas, TX 75231 (http://atvb.ahajournals.org/content/20/1/171).
4. Martha Srinivas, Akula Annapurna & Yellu Narsimha Reddy, Anti-atherosclerotic effect of atorvastatin and clopidogrel alone and in combination in rats,Indian Journal of experimental Biology, Oct, 2008; 46: 698-703.
5. Sofija Đorđevića,*, Vanja Tadića, Silvana Petrovićb, Jelena Kukić-Markovićb, Silva Dobrićc, Marina Milenkovićd, Nihad Hadžifejzović: Bioactivity Assays On *Carlina*

Acaulis And *C. Acanthifolia* Root And Herb Extracts: Digest Journal of Nanomaterials and Biostructures, July – September, 2012; 7(30): 1213–1222.

6. *Muhammad Riaza, Muhammad Zia-Ul-Haqb, Hawa Z.E. Jaafarc,* Review Article on Common mullein, pharmacological and chemical aspects. Rev Bras Farmacogn, 2013; 23: 948-959.
7. Zagrobelny M., Bak S., Ekstrøm CT, Olsen CE, Møller BL. The cyanogenic glucoside composition of *Zygaena filipendulae* (Lepidoptera: Zygaenidae) as effected by feeding on wild-type and transgenic lotus populations with variable cyanogenic glucoside profiles. *Insect Biochem Mol Biol.,* 2007; 37(1): 10-18.
8. Reenu Singh Tanwar, SumanBala Sharma, Usha Rani Singh *et al;* Antiatherosclerotic Potential of Active Principle Isolated from *Eugenia jambolanain* Streptozotocin-Induced Diabetic Rats, Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine, 2011; 127641: 9.
9. Suman Bala Sharma, Reenu Singh Tanwar, Rini A C, *et al;* protective effect of *Morus rubra* L. leaf extract on diet-induced atherosclerosis in diabetic rats, Indian Journal of biochemistry & Biophysics, Feb, 2010; 47: 26-31.