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Research Article

EVALUATION OF ANTI DIABETIC EFFICACY OF POLYHERBAL FORMULATIONS IN EXPERIMENTALLY INDUCED HYPERGLYCEMIC RATS

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ABSTRACT

To evaluate the *in vivo* antidiabetic activity of polyherbal formulations extract and decoction of fresh leaves of *Annona squamosa, Psidium guajava, Tenospora cardifolia, Bougain villia, Aegle marmelos* and fruits of *Terminalia chebula, Ficus carica, Emblica officinale* and flowers of *Hybiscus rosasinensis*, *Cassia* auriculata and rhizome of *Zingiber officinale*. Methods: The albino rats divided into nine groups, Group I - Normal animals treated with vehicle (0.5%w/v CMC), Group II - Diabetic animals treated with Alloxan150mg/kg, Group III - Diabetic animals treated with Glibenclamide 5mg/kg/day P.O), Group IV - Diabetic animals treated with EPHF, 200mg/kg P.O, Group V - Diabetic animals treated with EPHF, 400 mg/kg. P.O, Group VI - Diabetic animals treated with DPHF, 200mg/kg. P.O, Group VII - Diabetic animals treated DPHF, 400mg/kg. P.O, Group VIII - Diabetic animals treated with PPHF, 200mg/kg. P.O, Group IX - Diabetic animals treated with PPHF, 400mg/kg. P.O. The fasting blood glucose levels were monitored for all animals in 1, 3, 5, 7 days of drug treatment by using glucometer (Model- Accucheck, Roche Germany). The fasting blood glucose levels in polyherbal formulations treated animals was compared with that of diabetic control group animals. The collected data was subjected to appropriate statistical tests including one way ANOVA (Analysis of Variance), followed by an appropriate Dunnett's post hoc test. Conclusion: Decoction of Polyherbal formulation had less significance of anti-diabetic activity compared with that of extract formulation. Powder formulation had less potency compared to decoction and extract Polyherbal formulation. Polyherbal formulation was capable in control of Hyperglycemia. So it can conclude that the secondary complications of diabetes are treated passively with Polyherbal formulation.

Keywords: Anti-diabetic, Antioxidant, Alloxan, Glucose.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production or inefficient insulin utilization. In diabetes, hyperglycemia generates reactive oxygen species (ROS) which in turn cause lipid peroxidation and membrane damage and thus, plays an important role in the production of secondary complications in diabetes mellitus such as kidney, eye, blood

vessel, and nerve damage. Antioxidants have been shown to prevent the destruction of β-cells by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes. Plants containing natural antioxidants (tannins, flavonoids, vitamins C and E) can preserve β-cells function and prevent diabetes induced ROS formation[1-3].

In general, current type 2 diabetic drugs are not only expensive but also they have their limitations and are known to produce serious side effects, therefore, the search for safer, specific and effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering potentials for discovery of new antidiabetic drugs[4, 5].

Poly herbal formulations, the name itself are indicating as multiple ingredients of different herbal origin. The plant ingredients may have wide spectrum of biological activities. Polyherbal formulations are mainly used to enhance the activity or to counteract the toxic effects of compounds used from the other plants. These formulations may be give synergetic effect, due to presence of multiple ingredients and also may be show synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves. These formulations having different active constituents with different mechanism of actions which can produce combined action against various complications of diabetes (Ebong PE et al 2008, (Chandrashekar joshi *et al*., 2007).

MATERIALS AND METHODS

Plant collection and Extract preparation

The fresh leaves of *Annona squamosa, Psidium guajava, Tenospora cardifolia, Bougain villia, Aegle marmelos,* fruits of *Terminalia chebula, Ficus carica, Emblica officinale*, flowers of *Hybiscus rosasinensis*, *Cassia* auriculata and rhizome of *Zingiber officinale* was collected locally from Kakinada, Andhra Pradesh and the other crude drugs, including the leaves of *Stevia rhubidiana* was collected from Yercaud botanical garden, Salam, Tamilnadu and Tea leaves was collected from Ooty tea gardens, Coimbatore, Tamilnadu.

PREPARATION OF POLY HERBAL FORMULATIONS

The composition and ratio of herbal ingredients are selected according to the potency of anti-diabetic activity which was stated in previous references.

a. Powder form of Polyherbal formulation.
b. Decoction of Polyherbal formulation.
c. Crude extract of Polyherbal formulation.

Table I: Composition of Polyherbal Formulation

S. No.	PLANT NAME	PERCENTAGE %
1	*Aegle marmelos*	2.66%
2	*Annona squamosa*	9.32%
3	*Bougain villia*	2.66%
4	*Cassia auriculata*	6.66%
5	*Emblica officinale*	8.00%
6	*Ficus carica*	13.30%
7	*Hibiscus rosa sinensis*	6.66%
8	*Psidium guajava*	6.66%
9	*Stevia rebaudiana*	9.32%
10	*Tea leaves*	2.66%
11	*Tenospora cardifolia*	10.66%
12	*Terminalia chebula*	8.00%
13	*Zingiber officinale*	13.30%

a. Preparation of Powder of Polyherbal Formulation (PPHF)

The coarsely powdered plant materials was sieved through sieve no. 60. It helps to get uniform sized powder and according to the weight of individual powder, which are present in table no. I has taken in to a beaker and mixed well by using suitable blender to get homogenous powder formulation. Packed in suitable container and kept in dry place.

b. Preparation of Decoction of Polyherbal Formulation (DPHF)

All individual powders was taken in a beaker and mixed well with suitable blender until to get uniform powder formulation. Then packed the formulation in water diffusible paper to make easily dip in the hot water for making of decoction.

c. Crude Extract of Polyherbal Formulation (EPHF)

The Polyherbal formulation is a mixture of all the individual different parts of plant extracts was

carried out by maceration process for 24 hrs. All are dried and pulverized plant ingredients was taken in an individual conical flasks. Then added some amount of water up to rinse the powder, gentle agitated for first 6 hrs and kept a side for remaining 18 hrs. After 18 hrs filtered them individually, finally evaporated the solvent at 100^{o}C, To get paste like preparation. Then the different weights of the extracts according to the table.I taken in to a beaker mixed them properly with a little amount of water and evaporated the water to get a paste like preparation and kept in refrigerator after packed in a suitable container. (Sengottuvelu *et al*., 2008)

Animals

Male *Albino Wistar* strain rats (50-60 days old) was obtained from "Sri Venkateswara Enterprises", Bangalore, India. They were housed in plastic cages under controlled conditions (28±2^{0}C, 50% humidity and 12 h light/12h dark cycle) fed with normal rat chow marketed by Hindustan Lever Limited, Mumbai, India and was provided with clean drinking water *ad libitum*. The animals care and procedure of the whole experiment followed as per the principles and guidelines of the ethical committee of Nanda college of Pharmacy (Tamil Nadu, India) and Indian National Law on animal care and use (CPCSEA).

Diabetes induction in rats

The alloxan is widely used to induce diabetes (type I) in the experimental laboratory animal model [9]. The rats was injected (intraperitonelly) with alloxan monohydrate dissolved in sterile normal saline (120mg/kg b.w.). The rat was observed for four days, then the moderate diabetes of rats observed by Benedict's test [10] for urine glucose and then the blood was collected from tail vein for glucose estimation (Strip method). After a week the blood glucose range 250-300mg/dl rats was used for the experiment.

Experimental design

The albino rats was divided into nine groups with 6 rats in each as follows: Group I - Normal animals treated with vehicle (0.5%w/v CMC), Group II - Diabetic animals treated with Aloxxan150mg/kg, Group III - Diabetic animals treated with Glibenclamide 5mg/kg/day P.O), Group IV - Diabetic animals treated with EPHF, 200mg/kg P.O, Group V - Diabetic animals treated with EPHF, 400 mg/kg. P.O, Group VI - Diabetic animals treated with DPHF, 200mg/kg. P.O, Group VII - Diabetic animals treated DPHF, 400mg/kg. P.O, Group VIII - Diabetic animals treated with PPHF, 200mg/kg. P.O, Group IX - Diabetic animals treated with PPHF, 400mg/kg. P.O. The fasting blood glucose levels was monitored for all animals in 1, 3, 5, 7 days of drug treatment by using glucometer (Model-Accucheck, Roche Germany). Blood was collected from tail tip of the animals after 3 hours of drug administration and the tail wound is applied with Povidone Iodine ointment, to prevent any infection. The fasting blood glucose levels in polyherbal formulations treated animals was compared with that of diabetic control group animals.

Statistical Analysis: The collected data was subjected to appropriate statistical tests including one way ANOVA (Analysis of Variance), followed by an appropriate Dunnett's post hoc test. P values of less than 0.05, 0.01 and 0.001 was considered as less significant, significant and more significant respectively. The analysis was carried out using Graph pad prism software of version 4.

RESULTS

Acute oral toxicity of Polyherbal formulations in mice

The mice in acute toxicity study daily received Polyherbal formulations at dose levels of 300, 1000, 2000mg/kg. The duration of this study is 14 days. By comparing with control group it was observed that extract of Polyherbal formulation at doses 1000 and 2000mg/kg showed drowsiness. Except this it was not shown any effect to the function of Kidney, CNS, Liver, Gastro Intestinal Tract, Respiratory system and color of Skin, so no behavioral changes and no mortality was observed in mice with Polyherbal formulations.

Screening of anti-hyperglycemic activity of Polyherbal formulations in alloxan induced diabetic rats

The effects of Polyherbal formulations (EPHF, DPHF, and PPHF) on fasting blood glucose level in alloxan induced diabetic rats was given in table No.-II and Figure-1. The lower dose of EPHF, 200mg/kg shows decrease in fasting blood glucose level, whereas the higher dose400mg/kg produced a significant reduction in blood glucose level. The lower dose of DPHF (200mg/kg) produced less significant reduction and higher dose 400mg/kg produced significant reduction in blood glucose level and the PPHF

high dose only produces less significant reduction in blood glucose level on 7th day when compared with diabetic control groups. The Effect of Polyherbal formulations on liver function and kidney test parameters. In table No-III and IV the represented data belongings to changing of liver and kidney test parameters due administration of various doses of Polyherbal formulations. Alloxan administration causes significant rise in SGOT, SGPT, and ALP levels and Glibenclamide reduces to normal level. In Polyherbal formulations mainly the extract and decoction formulation significantly reduces near to normal level. Alloxan rises the serum urea and serum Creatinine levels and decreases the serum total protein levels. In Polyherbal formulations extract 400mg/kg significantly decreases the serum Urea and Creatinine levels as well as significantly increases the total protein levels.

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin will be secreted. However, Alloxan is an oxygenated pyrimidine derivative betacytotoxin and is known to induce diabetes mellitus in a wide variety of animal species through the damage of pancreatic β-cells when there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results [27]. The previous studies for the treatment of diabetes with Polyherbal formulation exert good acceptable results. Eg. Umamaheswari *et al*., 2010, Antidiabetic Activity of a Polyherbal Formulation (DIABET). The Polyherbal formulation is assessed in diabetic rats at 500mg/kg showed its effectiveness in oral glucose tolerance test and Antidiabetic activity, but it does not produce hypoglycemic effect. Patil Manoj *et al*., 2010, evaluated the effect of Polyherbal formulation in obesity associated diabetes. The antidiabetic and antiobesity effect of the formulation was found to be nearly similar to that observed for glibenclamide and sibutramine respectively. The toxicological evaluation of Polyherbal formulation is useful to evaluate its toxic effects which was produced on long term administration. In the acute toxicity study of Polyherbal formulations mortality was observed at any dose. At dose levels of 1000 and 2000mg/kg P.O of extract of Polyherbal formulation only produces drowsiness, except this no behavioral changes was observed at different doses of Polyherbal formulations. The symptoms which was observed in acute toxicity representing the safety and no toxicity of Polyherbal formulation. (Sabu .MC *et al*., 2009). Alloxan induces a wide variety of animal species by damaging the insulin secreting pancreatic b-cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues **(**Sanjiv Singh, *et al*., 2010). β cells destruction is happens by the free radicals which are generated in Fenton reaction of alloxan metabolism. Formation of Hydroxyl radicals ($^{\cdot}OH^{-}$) from the Hydrogen peroxide H_2O_2 is called Fenton. Due to this blood glucose levels of rats were increased significantly. Glibenclamide is a standard drug which will significantly decreases the raised blood glucose levels. In β cells of pancreas Na^+ ion channels are responsible for depolarization which will increases the production of insulin and K^+ ion channels are responsible for repolarisation which will decreases the production of insulin. Glibenclamide increases the production of insulin by inhibiting the K^+ ion channels. In the present experiment, administration of Polyherbal formulations for seven days prevented a significant elevation of glucose levels in diabetic rats. This could be due to the result of improved glycemic control produced by the formulation. The extract of Polyherbal formulation at a dose of 400mg/kg P.O produced a significant reduction in fasting blood glucose level when compared with other doses of Decoction and Powder formulation. The pronounced anti hyperglycemic effect was observed because of the synergistic effect of various active principles in the ingredients of Polyherbal formulation. The reduced fasting blood glucose level may be either due to the increase in glycogenesis, decrease in glycogenolysis or increase in entry of glucose molecules to various skeletal muscles. **(**Umamahaswari *et al.,* 2010).

CONCLUSION

Polyherbal formulation consists of thirteen plant origin ingredients, which are individually used traditionally in the treatment of diabetes mellitus. Each plant act by different mechanisms to treat diabetes. On the basis of above results it could be concluded that extract of Polyherbal formulation exerts significant hypoglycemic activity. Decoction of Polyherbal formulation having less significance of anti-diabetic activity compared with that of extract formulation.

Powder formulation having less potency compared to decoction and extract Polyherbal formulation. Polyherbal formulation having the capable of control of Hyperglycemia. So it can conclude that the secondary complications of diabetes are treated passively with Polyherbal formulation. Further investigations in-depth has to be carried to find out the exact mechanism present behind the aqueous extracts of Polyherbal formulations and also the exact active ingredient responsible for the anti-diabetic activity.

Table II: The effect of Polyherbal 3-different Formulations on fasting blood glucose level in Diabetic induced Rats
Fasting Blood Glucose level (mg/dl)

No of Groups	TREATMENT	1st day	3rd day	5th day	7th day
I	Normal control (CMC -0.5%)	081.5 ± 1.15	81.6± 1.20	82.6 ± 1.70	83.4 ± 0.90
II	Diabetic control (Alloxan 120mg/kg)	211.7 ± 3.50**	237.7± 2.90**	249.4± 3.80**	268.7± 3.20**
III	Positive control (Glibenclamide 5 mg/kg)	206.4 ± 1.70	177.6 ± 2.06**	137.2 ± 0.88**	109.5 ± 0.50**
IV	E-PHF(200mg/kg)	197.5 ± 2.90	186 ± 0.50*	165.3 ± 2.00*	152.3 ± 1.70*
V	E -PHF (400mg/kg)	206.5 ± 3.05	182 ± 1.50*	152.5 ± 2.00**	126.8 ± 1.70**
VI	D-PHF(200mg/kg)	208.6 ± 5.30	192.3 ± 1.30	173.3 ± 2.08	165.7 ± 1.10*
VII	D-PHF(400mg/kg)	218.3 ± 0.80	184.6 ± 1.30	161.3 ± 1.40*	149.6 ± 1.70**
VIII	P-PHF(200mg/kg)	214 ± 2.60	204.1 ± 3.20	188.3 ± 2.30	175.2 ± 2.00
IX	P-PHF(400mg/kg)	221.6 ± 3.40	205.3 ± 2.10	174.6 ± 2.18	168.3 ± 5.30*

The represented data was in Mean±SEM. (n=5). *P<0.05 is less significant,
**P<0.01 is significant and compared to diabetic control

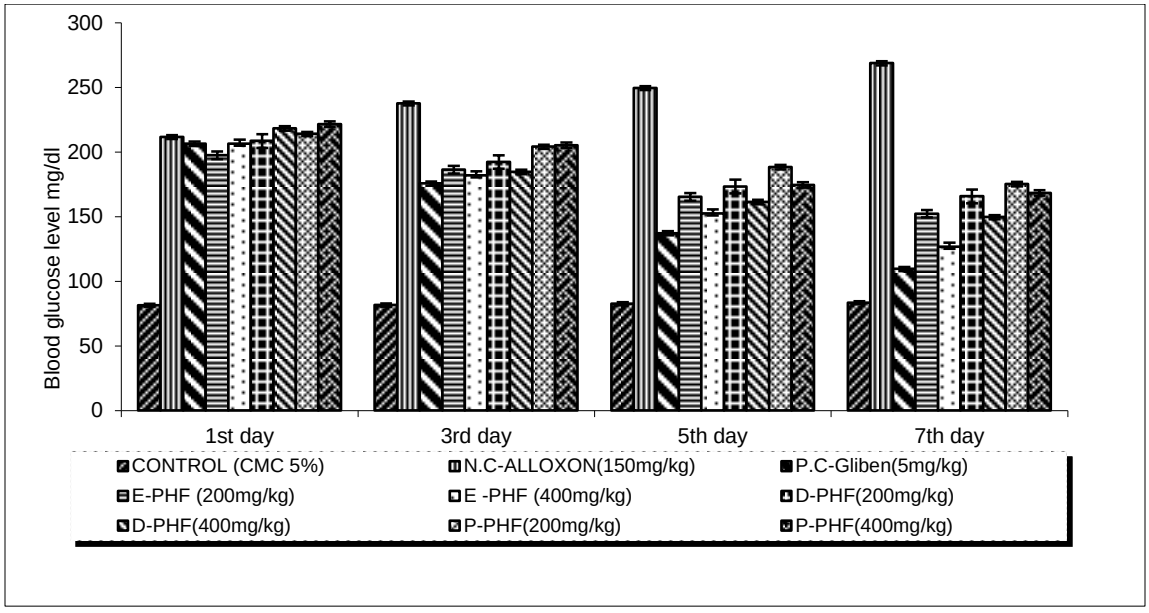

Fig. 1: The effect of Polyherbal formulation on fasting blood glucose level in alloxan induced diabetic rats

Table III: The Effect of Polyherbal formulation on liver function test parameters

No. of Groups	TREATMENT	Serum SGOT (IU/L)	Serum SGPT (IU/L)	Serum ALP (IU/L)
I	CMC (0.5%)	38.2 ± 0.3	26.4 ± 1.1	118.2 ± 1.14
II	Negative control (alloxan)	93.3 ± 0.5 **	81.3 ± 0.63 **	315.4 ± 2.7 **
III	Positive control (glibenclamide)	41.4 ± 0.6 **	30.1 ± 1.0 **	135.4 ± 2.9 **
IV	Extract of PHF (200mg/kg)	52.3 ± 0.8 **	53.4 ± 0.5 **	176.3 ± 6.3 **
V	Extract of PHF (400mg/kg)	43.5 ± 0.5 **	35.8 ± 1.4 **	160.1 ± 4.1 **
VI	Decoction of PHF(200mg/kg)	86.3 ± 0.88 *	66.9 ± 1.2 *	277.3 ± 2.08 *
VII	Decoction of PHF(400mg/kg)	53.6 ± 0.8 **	51.1 ± 0.8 **	262.5 ± 3.1 **
VIII	Powder of PHF (200mg/kg)	91.2 ± 0.5	77.4 ± 0.6	304.6 ± 0.8
IX	Powder of PHF (400mg/kg)	73.5 ± 1.1 *	68.5 ± 0.6 *	287.3 ± 1.4 *

The represented data was in Mean±SEM. (n=6).
*P<0.05 is less significant.**P<0.01 is significant and compared to diabetic control.

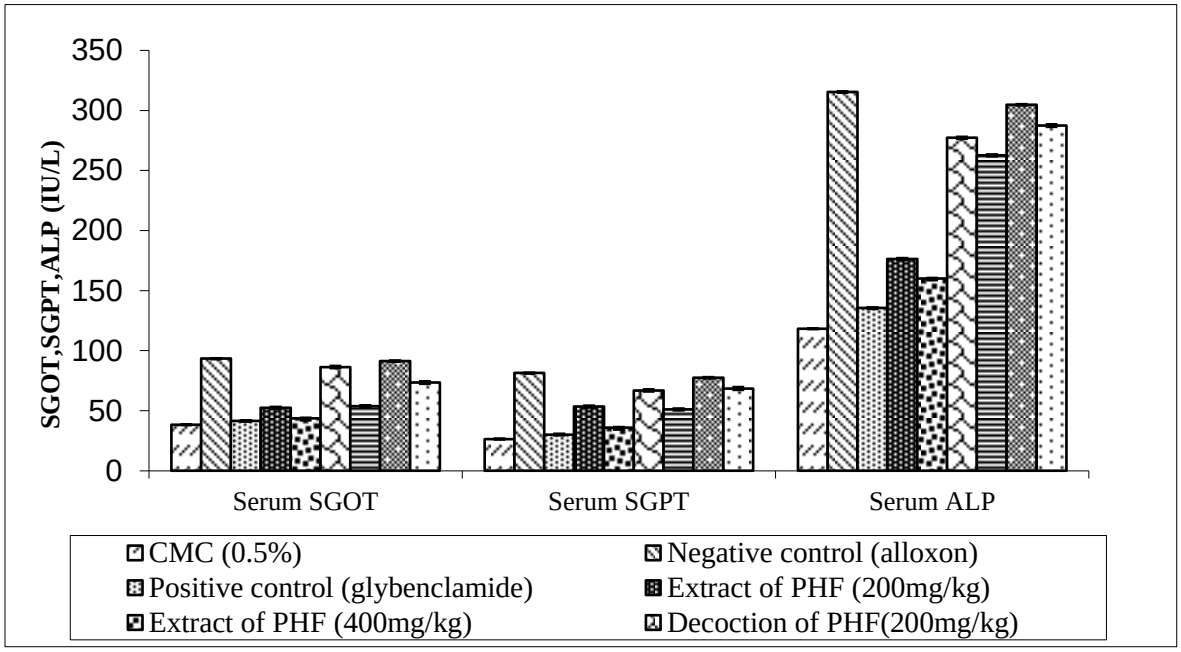

Fig. 2: The Effect of Polyherbal formulation on liver function test parameter

Table IV: The Effect of Polyherbal formulation on Kidney function test parameters in rats

No of Groups	TREATMENT	Serum Urea (n mol/l)	Serum Creatinine (mg/dl)	Serum Total protein (gm/l)
I	CMC (0.5%)	20.64 ± 0.88	0.46 ± 0.02	6.2 ± 0.10
II	Negative control (alloxan)	54.03 ± 3.50 **	1.78 ± 0.03 **	3.8 ± 0.10 **
III	Positive control (glibenclamide)	32.07 ± 0.51 **	0.39 ± 0.03**	6.06 ± 0.08 **
IV	Extract of PHF (200mg/kg)	41.46 ± 0.25 **	1.27 ± 0.03 *	4.5 ± 0.07 *
V	Extract of PHF (400mg/kg)	34.98 ± 0.54 **	0.54 ± 0.05 **	6.13 ± 0.12 **
VI	Decoction of PHF(200mg/kg)	49.74 ± 0.44	1.68 ± 0.03	3.97 ± 0.06 *
VII	Decoction of PHF(400mg/kg)	39.74 ± 0.45 **	1.04 ± 0.04 *	5.06 ± 0.08 **
VIII	Powder of PHF (200mg/kg)	57.16 ± 0.85	1.69 ± 0.06	4.05 ± 0.10
IX	Powder of PHF (400mg/kg)	49.66 ± 0.53	1.54 ± 0.05 *	4.5 ± 0.17 *

The represented data was in Mean±SEM. (n=6).
*P<0.05 is less significant **P<0.01 is significant and compared to diabetic control.

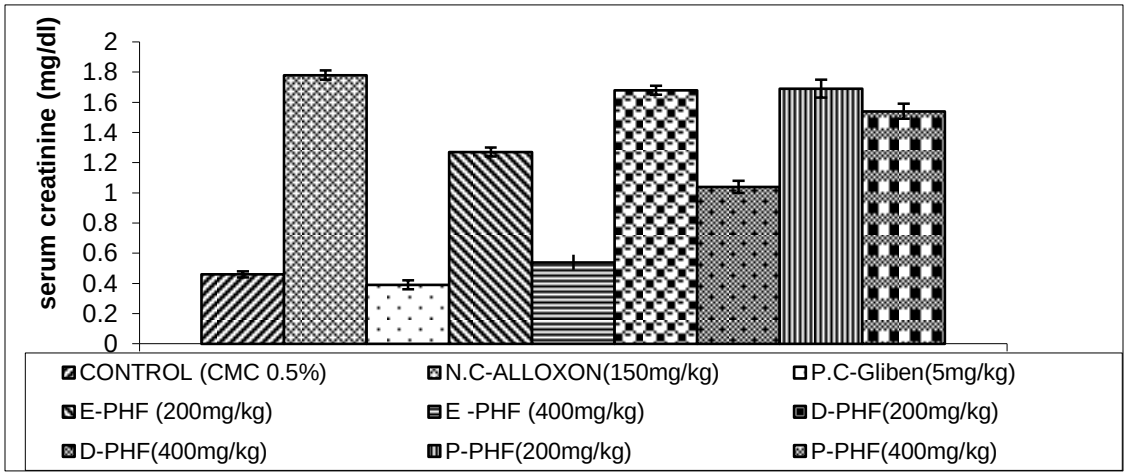

Fig. 3

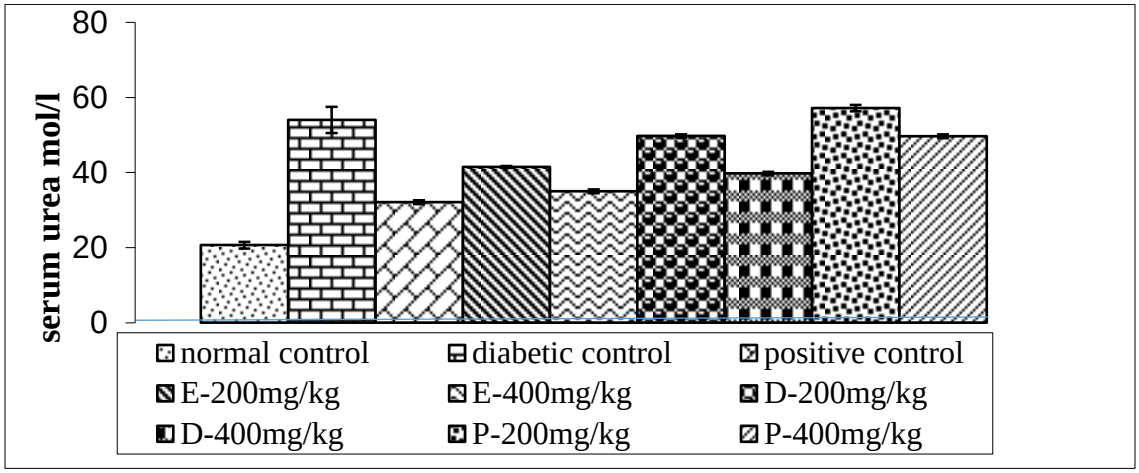

Fig. 4

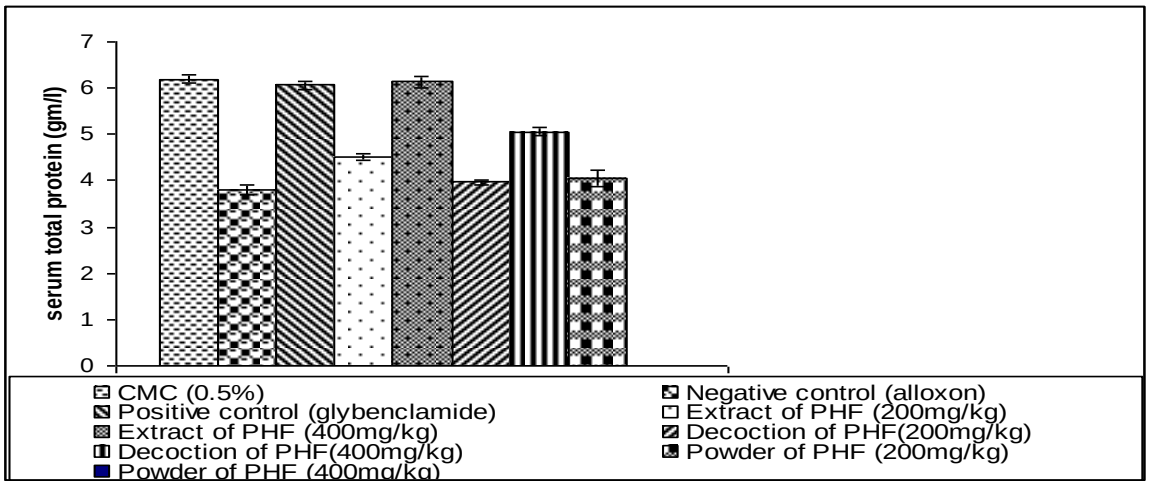

Fig. 5

Fig. 3 – 5: The Effect of Polyherbal formulation on Kidney function test parameters in rats

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