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Study of Pharmacodynamic Interaction of Sitagliptin, *Gymnema sylvestre*, *Zingiber officinale* on Experimental Rat Model



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ABSTRACT

Since ages, natural herbs and nutraceuticals have been used along with prescription drugs in treatment of chronic Type II diabetes mellitus and its associated alteration in lipid levels. This inevitable concomitant use of herbs along with allopathic medicines might lead to herb-drug interactions resulting in hypoglycaemic shock or therapeutic failure. The proposed study was undertaken to assess any interaction existing at pharmacodynamic level when herbs like *Gymnema sylvestre* (400 mg/kg) and *Zingiber officinale* (4 ml/kg) are concomitantly administered with synthetic drug Sitagliptin Phosphate (20 mg/kg) in STZ and high fat diet induced diabetic rats. Pharmacodynamic interactions were evaluated by determining the effect of combination of respective herbs with Sitagliptin Phosphate on serum glucose and lipid levels. The combination groups did not show decrease in the serum glucose levels comparable to individual herbs, rather they showed results equivalent to that of Sitagliptin Phosphate group. There was significant interaction ($p < 0.05$) found between the combination groups with their respective herbs. In case of regulation of lipids, the combination groups showed significantly ($p < 0.05$) better results when compared to individual herbs. Histopathological studies of pancreas, kidney and liver were carried out and it revealed that both the combination groups showed profound results in regeneration of beta cells in pancreas, regeneration of tubular epithelium in kidneys and regeneration of liver tissues. These observations showed that combination groups significantly did not prove to be better than monotherapy in regulating glucose levels, but showed better results in curing altered lipid levels which are secondary complications to chronic diabetes.

INTRODUCTION

The use of complementary and alternative medicine (CAM) is growing attention worldwide, to treat chronic diseases⁽¹⁾. CAM treatments such as herbal supplements have become increasingly popular in most of the countries, especially among older patients and those with chronic pain⁽²⁾⁽³⁾. Metabolic syndrome characterized by insulin resistance is becoming a threat worldwide predominantly in United States, Europe, and Asia⁽⁴⁾. Despite the current existence and availability of synthetic drugs for the treatment of diabetes mellitus (DM), the use of complementary and alternative medicine system is inevitable⁽⁵⁾. Along with the increase in blood glucose levels, the lipid profile of an individual is also altered leading to hyperlipidemia, hence we can say that hyperlipidemia is one of the primary disorder associated with diabetes mellitus. With the tremendous expansion in the use of traditional medicines worldwide as a concomitant therapy to allopathic medicines, the safety and efficacy of total therapeutic effect has become an important concern^(6, 7). Herbal remedies are classified as dietary supplements *i.e.* they are exempted from the safety and efficacy regulations that the U.S. Food and Drug Administration (FDA) requires for prescription and over-the-counter medications. As a result, individual herbal remedies have not been thoroughly evaluated in large clinical trials, and little information is available on the interactions between drugs and herbs. Many herbal products are marketed as 'natural', which may lead consumers to assume the products are safe, even when taken with prescription medicines. Furthermore, the continuous consumption of synthetic drugs may cause fatal adverse drug reactions, while those medications provided from natural sources are more affordable and have shown lesser adverse effects⁽⁸⁾. The complications encountered with the use of natural products need to be identified for its safety and efficacy^(6, 7). The market for herbal medicines is booming and the evidence for their effectiveness is growing, but inadequate regulations and absence of proper standards have hampered their use⁽⁶⁾. The concomitant use of synthetic drug and herbs is not been extensively studied. Also, there is a very poor communication between the physicians and the patients regarding the concomitant use of herbal products and the prescribed conventional allopathic medicines. This poor communication regarding the usage of herbal medicine and natural nutraceuticals without informing the doctor can result in various herb drug interactions⁽⁷⁾. Adverse reactions are under-reported because herbs have a complex composition and there is lack of information on the toxicity of medicinal herbs or their constituents⁽⁹⁾. Concomitant use of herbals and nutraceuticals with synthetic drug

may result in deleterious outcome if any. Herbal supplements may have a negative impact on patients and may interact with conventional medicines used to manage chronic conditions ⁽¹⁰⁾. Evaluation of the interaction between synthetic drug and natural product is important while practising combination therapy. Amongst the current synthetic drugs, Sitagliptin is one of the commonly used drugs in treating diabetes type 2 disorder. Sitagliptin (3R)-3-Amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl)-1-butanone is approved by regulatory authorities as an orally active, potent, and selective inhibitor of dipeptidyl peptidase IV (DPP-IV) used for the treatment of type 2 diabetes ^(11, 12). Along with this, people also consume various herbs to treat the increased blood glucose level and the altered lipid profile. *Gymnema sylvestre* belonging to the family *Asclepediaceae* is widely used herbs in India as well foreign countries for treating diabetes ^(13, 14, 15). In addition to diabetes, for increasing the quality of life people consume nutraceuticals like *Zingiber officinale* from the family *Zingiberaceae* ⁽¹⁶⁾. Both these herbs have a profound effect as antidiabetic. The objective of the present proposal is aimed to study the pharmacodynamic interaction between herbals like *Gymnema sylvestre* and *Zingiber officinale* and conventional allopathic medicine Sitagliptin phosphate (from the class of dpp4 inhibitors) in order to assess the concomitant use of these medicines.

MATERIALS AND METHODS

Animals

Rats were procured from the Bombay Veterinary College, Mumbai, India. Rats were housed in a 12 hour light-dark cycle, temperature ($24\pm 2^{\circ}\text{C}$) and relative humidity ($\text{RH}\leq 60\%$) controlled facility of the institute. Standard laboratory diet and water *ad-libitum* was provided to rats throughout the study. The experiment was carried out in accordance with the ethical guidelines of CPCSEA. The study was conducted after protocol been approved by Institutional Ethics Committee affiliated to CPCSEA.

Material

Sitagliptin was obtained as a gift sample from Glenmark generics, India. Herbal extracts of plants *Zingiber officinale* (Family: Zingiberaceae) and *Gymnema sylvestre* (Family: Asclepiadaceae) were procured from Konark Herbal and Health Care, Daman, India. The extract of ginger and gymnema contained 0.03% of gingerol (by HPLC analysis) and 75.62% of

gymnemic acids (by gravimetric analysis) respectively. Streptozotocin (STZ) was procured from, Huohua Industry Co. Ltd., China. High fat diet was procured from D.S. Trading, Mumbai, India.

Experimental Design:

Experimental Induction of Type II Diabetes Mellitus:

Rats were acclimatized for a week prior to initiation of study. Rats weighed 250-300gm were randomly divided in following groups containing equal number of animals (n=6). Group I- Normal Vehicle control, Group II-Disease control, Group III- Sitagliptin (STG; 20mg/kg) treatment, Group IV- Ginger extract (4ml/kg) treatment, Group V- Gymnema extract (400mg/kg) treatment, Group VI & VII were combination treatment STG + Ginger extract, STG + Gymnema extract respectively. Except for Group I all other groups were fed high fat diet throughout the study. STZ challenge was given to overnight fasted rats at the dose of 40mg/kg, on fifteenth day of initiation of study. After 24 hours of STZ challenge, rats were subjected to blood withdrawal for the purpose of evaluation of biochemical parameters in blood serum. Blood samples were collected from retro-orbital plexus. The fasting blood glucose, total cholesterol (TC), low density lipoprotein (LDL), High density lipoprotein (HDL), triglycerides (TG) levels were obtained. Fasting blood glucose levels more than 250mg/kg were considered diabetic.

Followed by induction of diabetes, animals were treated for 21 days once daily (*p.o.*). Biochemical parameters were evaluated at regular intervals during treatment. Rats were sacrificed on completion of study with overdose of carbon dioxide.

Liver, pancreas and kidneys were excised immediately for histopathological evaluations on the day of sacrifice. The organs were preserved in fresh solution of 10% formalin for the purpose of fixation and further evaluation

RESULTS

Effect on Blood Glucose Level

The sugar lowering effect of the treatment groups when compared to vehicle and disease control showed significant differences at $p < 0.05$. Herbs were found to reduce sugar level profoundly than sitagliptin and both the combination treatments. Obtained results showed that the activity by all treatment groups was highly significant on day 21 of the treatment. The mean differences showed the effect was lesser in first phase of treatment. Post treatment the blood sugar levels

were found to be reduced effectively with no harm of hypoglycaemia observed in any treatment group.

Effect on Total Cholesterol Level

The elevated total cholesterol was reduced significantly. The reduction in elevated cholesterol level was seen prominent till day 14 post treatment. Herbs and the combination treatments were found to reduce the cholesterol levels more efficiently than sitagliptin alone. The elevated values were found to reach to normal within 14 days of treatment, though reduction thereafter occurred with lower mean differences.

Effect on Triglyceride Level

There was significant reduction in increased TG levels. Mean differences in activity of treatment groups and disease control confirms prominent effect on day 14 and 21 of treatment. The effect of both the herbs and combination treatments seems more efficacious in reducing the elevated levels when compared to sitagliptin treated animals.

Table 1. Effect of STG, Ginger extract, Gymnema extract, and combination treatments on serum glucose levels of STZ induced diabetic rats

Groups	Day 0	Day 1	Day 7	Day 14	Day 21
Group I	135.83 1.25	± 136.51 ± 1.63	139.75 ± 2.43	143.55 ± 2.91	136.95 ± 1.76
Group II	136.90 2.41	± 342.66 18.17	± 320.20 12.93	± 292.43 ± 8.74	295.95 ± 14.83
Group III	120.10 5.81	± 360.6 ± 15.54	331.11 14.91	± 271.53 ± 7.46*	202.25 ± 12.73*
Group IV	137.51 4.05	± 372.25 17.64	+ 311.43 19.74	± 232.05 17.24*	± 127.86 ± 8.89*
Group V	134.40 5.37	± 350.33 + 9.38	312.36 ± 8.93	257.98 16.56*	± 201.73 ± 20.93*
Group VI	151.50 3.58	± 360.1 + 8.05	308.80 ± 9.26	210.93 ± 8.33*	146.60 ± 14.95*
Group VII	132.11 4.71	± 356.66 15.43	+ 301.3 ± 13.98	251.51 11.88*	± 205.83 ± 18.67*

The values are represented as Mean \pm Standard Error Mean (SEM). The data is analysed by Two way anova at *p< 0.05.

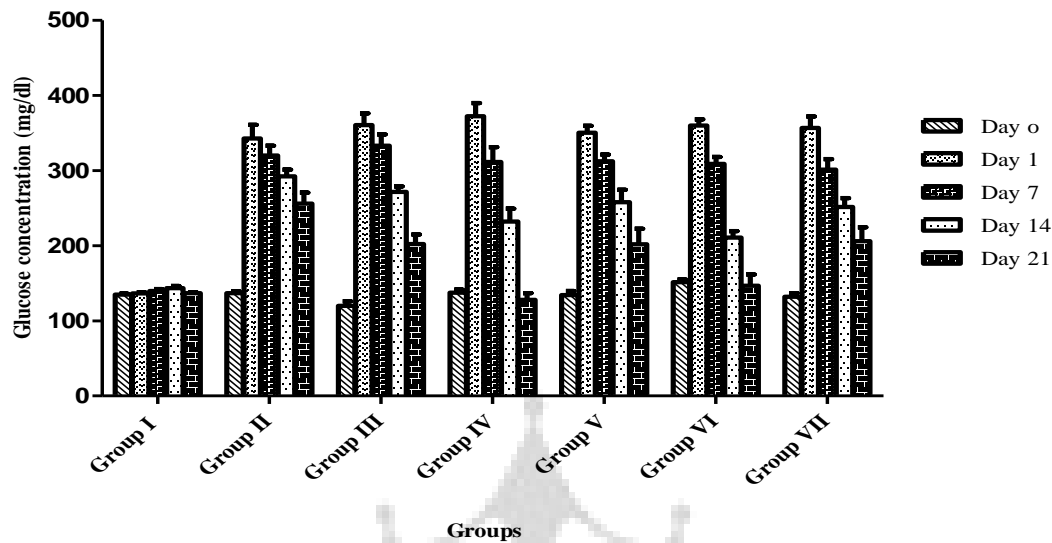


Figure 1: Effects obtained after treatment on Serum Glucose Levels

Table 2. Effect of STG, Ginger extract, Gymnema extract, and combination treatments on serum Total Cholesterol levels of STZ induced diabetic rats

Groups	Day 0	Day 1	Day 7	Day 14	Day 21
Group I	68.58 \pm 3.46	68.13 \pm 2.37	67.96 \pm 3.70	69.37 \pm 2.48	70.54 \pm 4.09
Group II	66.31 \pm 2.44	98.73 \pm 3.42	104.37 \pm 2.98	103.52 \pm 4.15	103.1 \pm 2.79
Group III	64.76 \pm 1.70	100.87 \pm 1.88	81.85 \pm 0.89	73.12 \pm 1.27	69.22 \pm 1.84*
Group IV	60.07 \pm 2.42	98.50 \pm 0.97	81.76 \pm 2.54	65.34 \pm 1.68	53.23 \pm 1.15*
Group V	70.97 \pm 3.53	100.65 \pm 2.15	87.12 \pm 1.19	65.29 \pm 2.71	54.36 \pm 2.71*
Group VI	67.66 \pm 1.51	96.96 \pm 1.08	84.14 \pm 1.71	68.33 \pm 1.52	55.52 \pm 1.68*
Group VII	68.79 \pm 1.51	102.65 \pm 2.45	86.16 \pm 1.83	65.84 \pm 2.23	54.45 \pm 0.98*

The values are represented as Mean \pm Standard Error Mean (SEM). The data is analysed by Two way anova at $*p < 0.05$.

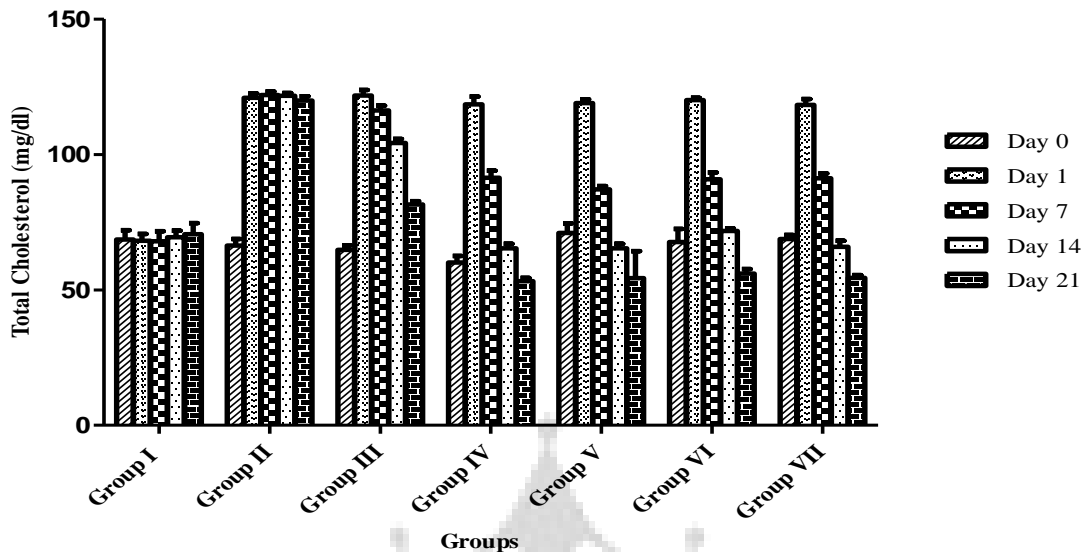


Figure 2: Effects obtained after treatment on Serum Total Cholesterol Levels.

Table 3. Effect of STG, Ginger extract, Gymnema extract, and combination treatments on serum Triglyceride levels of STZ induced diabetic rats

Groups	Day 0	Day 1	Day 7	Day 14	Day 21
Group I	72.82 \pm 4.65	69.67 \pm 4.58	72.67 \pm 5.90	70.81 \pm 3.81	70.07 \pm 3.95
Group II	68.33 \pm 3.86	250.61 \pm 12.77	252.68 \pm 13.42	238.99 \pm 13.66	252.45 \pm 14.93
Group III	64.22 \pm 3.99	251.96 \pm 5.12	214.50 \pm 3.97	159.16 \pm 6.53*	108.18 \pm 4.83*
Group IV	68.64 \pm 4.77	238.02 \pm 8.43	190.95 \pm 5.15	125.74 \pm 6.48*	74.69 \pm 3.45*
Group V	73.38 \pm 5.03	255.52 \pm 13.52	212.90 \pm 12.57	143.77 \pm 14.83*	70.07 \pm 2.66*
Group VI	72.68 \pm 4.76	252.52 \pm 14.20	195.43 \pm 10.50	136.57 \pm 5.19*	87.81 \pm 6.60*
Group VII	66.68 \pm 3.76	242.02 \pm 15.14	193.68 \pm 11.73	131.58 \pm 12.15*	79.56 \pm 4.42*

The values are represented as Mean \pm Standard Error Mean (SEM). The data is analysed by one way anova at $*p < 0.05$.

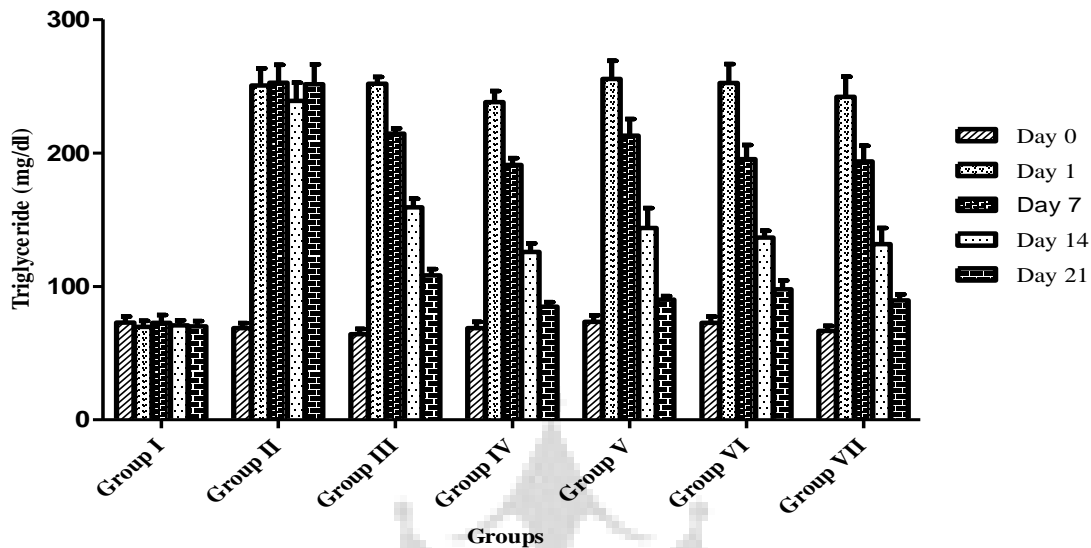


Figure 3: Effects obtained after treatment on Serum Triglycerides levels

Histopathology Investigations

There were no abnormalities found in the anatomy of pancreas, liver and kidney of vehicle control group.

Pancreas:

The disease control showed mild degenerative changes in pancreatic β -cells. Also, the comparative presence of islets of Langerhans was reduced showing depopulation of pancreatic cells. The degenerative changes in the treatment groups were found minimal in comparison to the disease control. The combination and herb treated animals showed increase in comparative presence of islets of Langerhans and increased average size of pancreatic cells. These regenerative changes were found to be more prominent in herb and combination treated group than others.

Liver:

There were mild granular degeneration changes observed in disease control. There were no abnormalities found in the herb treated animals. The degeneration changes observed in sitagliptin

and combination treated groups were found less compared to the disease control. The degenerative changes were found very minimal in both the combination treated groups.

Kidney:

In kidney, vacuolar and granular degenerative changes were observed on tubular epithelium. Also, cystic dilations of renal tubules had occurred. These changes were minimal and found to be improved with the treatments given in all experimental groups.

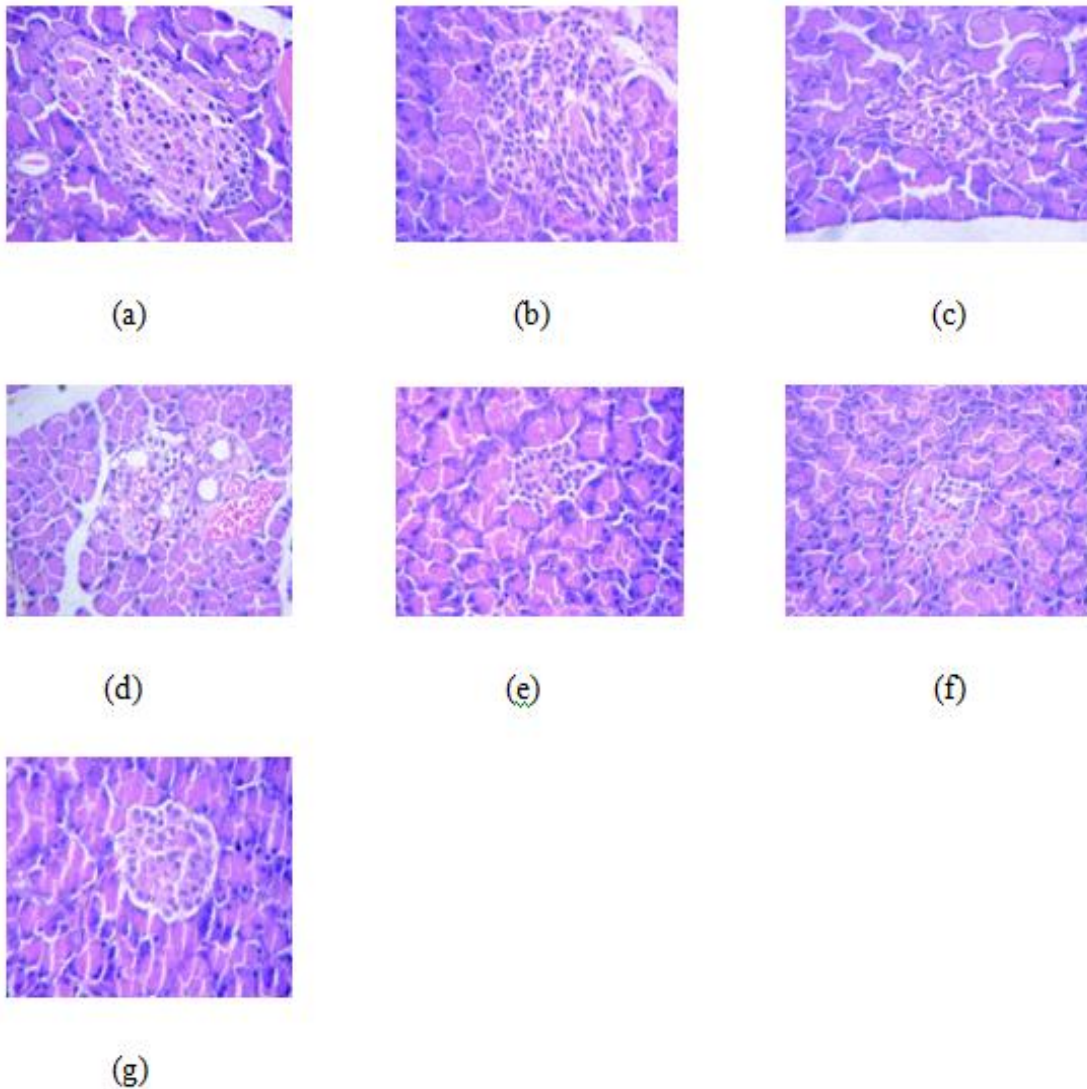


Figure 4: Histopathological images of pancreas (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V, (f) Group VI, (g) Group VII

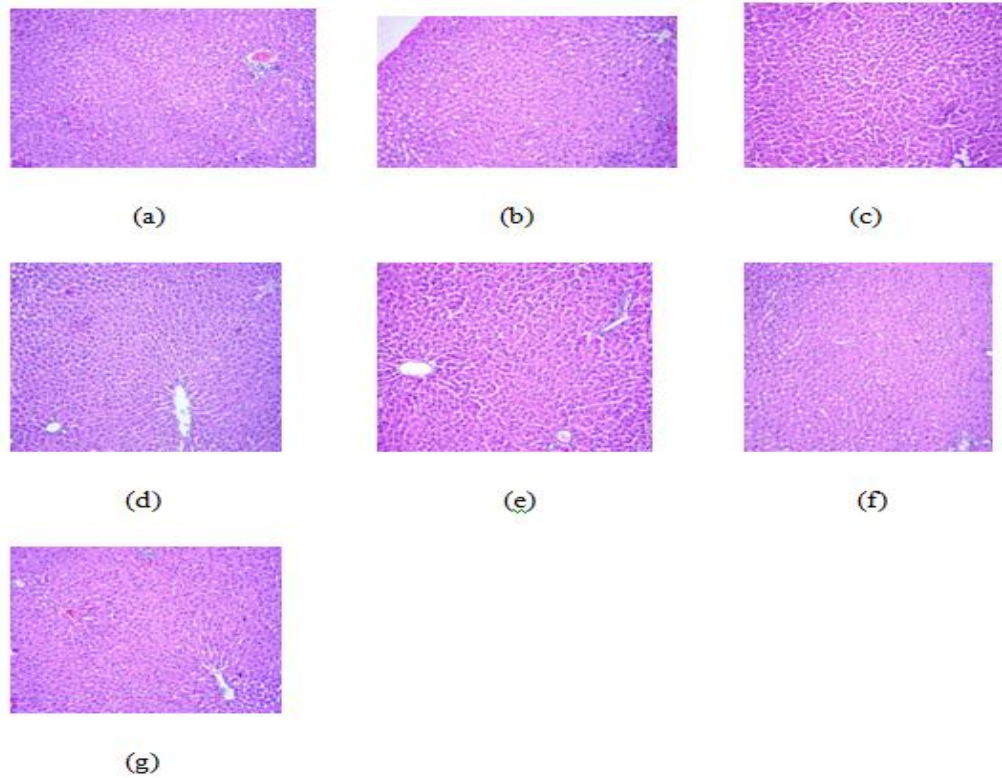


Figure 5: Histopathological images of Liver (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V, (f) Group VI, (g) Group VII

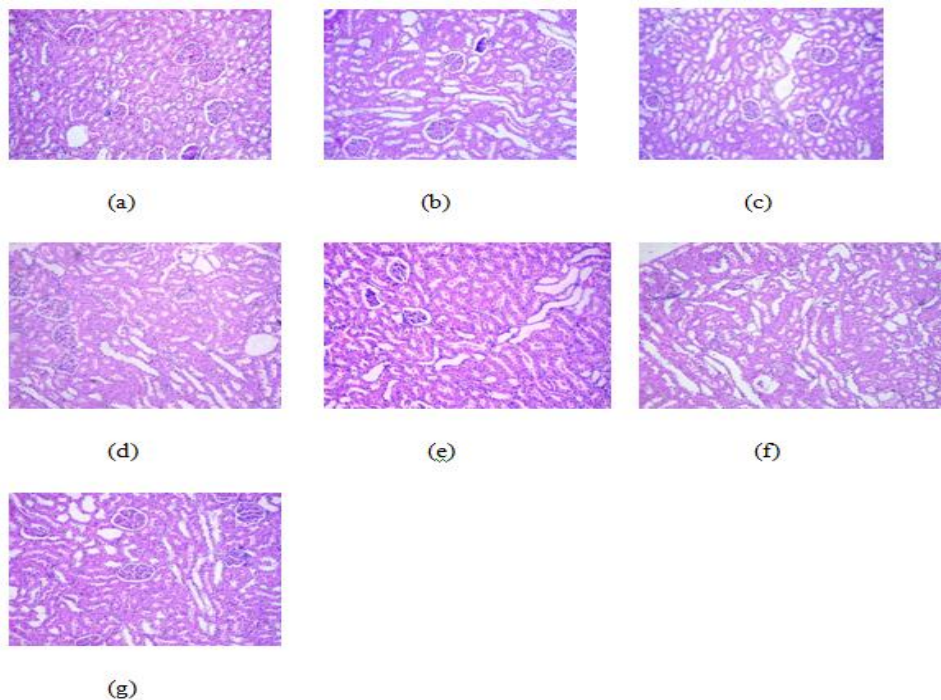


Figure 6: Histopathological images of Kidney (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V, (f) Group VI, (g) Group VII

DISCUSSION

Possible risk of hypoglycaemia is encountered when patients consume multiple therapies to treat type II diabetes mellitus. Concomitant therapy of herbal *churnas* and nutraceuticals along with allopathic drugs may either result in deleterious outcome if any or may lead to therapeutic failure. The study was carried out to assess any possible *in vivo* interaction of the herbs like Gymnema and Ginger on Sitagliptin Phosphate or *vice-a-versa* when taken concomitantly. The efficacy of the combination groups were compared with the individual groups for evaluation of any interaction.

There was increase in the levels of serum glucose and alteration in serum lipid parameters in the diseased groups indicating the induction of chronic diabetes and its related complications following single intraperitoneal administration of 40mg/kg streptozotocin (STZ) and continuous feeding of high fat diet in rats^(18, 19). Streptozotocin enters via glucose transport (GLUT 2) and alkylates the DNA of the cell, which leads to the activation of poly-ADP ribosylation. This activated pathway leads to depletion of cellular NAD⁺ and ATP. Increased in the ATP dephosphorylation, lead to formation of substrates for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also released. In addition to this, Streptozotocin also liberates toxic amounts of nitric oxide that inhibits *aconitase* activity and participates in DNA damage. Thus it leads to increase in the oxidative stress and destruction of beta cells in the pancreas⁽²⁰⁾. Diet rich in cholesterol and saturated fatty acids increases the availability of acetyl- CoA, a precursor for cholesterol biosynthesis. This in turn increases the activity of HMG-CoA reductase, the rate determining enzyme in cholesterol biosynthesis thus increasing the synthesis of cholesterol in the body. Thus inducing increase in lipid levels (except HDL) leading to secondary complications observed in chronic diabetes⁽²¹⁾.

The study found that, there was decrease in the serum glucose level in all the groups when compared to the diseased control group. Gymnema and Ginger showed significant decrease in the serum glucose levels as compared to Sitagliptin Phosphate^(12, 14, 16). However in both the combination groups, the serum glucose levels were found to be equivalent to that of Sitagliptin Phosphate, suggesting that an interaction might have taken place. According to statistical analysis, when groups of Gymnema and Ginger and their respective combination with Sitagliptin

were compared, there was significant interaction between them ($p < 0.05$), thus stating that *Gymnema* and *Ginger* showed no added advantage over individually monotherapy.

Sitagliptin being a DPP-IV inhibitor, competitively binds to the receptor and shows its inhibitory action, thus stimulating the release of Glucagon like peptide-1 (GLP-1) and Glucose insulinotropic peptide (GIP). These GLP-1 and GIP further inhibit glucagon release which leads to rise in insulin levels^(11, 12).

Gymnema sylvestre acts by neural inhibition brings about taste alteration and reduce the perception of sweetness. Gymnemic acids present in the *Gymnema* extract exhibit DPP-IV inhibitory activity too. Thus, unlike sitagliptin, they too enhance insulin secretion through increase pancreatic beta cells and improved cell function. It is also said to promote regeneration of islet cells. This has been well proved in the histopathological studies of pancreas treated *Gymnema*. It also inhibits glucose absorption from intestine.^(13, 14, 15)

It can be postulated that, since Sitagliptin and *Gymnema* both act on the same receptor, they might compete for the binding site which lead to the decreased efficacy in the combination group. Whereas, *Ginger* showed significant decrease in the serum glucose levels through its antioxidant potential thus reducing the oxidative stress in the beta cells⁽¹⁷⁾. Significant results have been demonstrated in the histopathology of pancreas. *Gymnema* has affinity to p-glycoproteins (P-gp) as well as organic anion transport proteins (OATP). Along with *Gymnema*, even *Ginger* is a substrate for P-gp because of which elimination might have taken place of both the herbs thus decreasing their effect in the combination group. Both the combinations have a profound effect on regeneration of tubular epithelium in kidneys because of the inherent properties of herbs stated in literature^(14, 17). The mechanism is still unclear that how the drugs might have interacted in rats and further studies are to be undertaken, taking in consideration various doses of the drug and the herbs.

The lipid lowering activity of both the combination groups was found to be the best when compared to the individual treatment groups, suggesting that the combination group worked more effectively in regulating the lipid levels. DPP-IV inhibitors act via neural mechanism and increase the inhibitory activity of GLP-1. This brings about increase in satiety, which decreases food uptake. Also, it lowers the pace of gastric emptying. DPP-IV inhibition brings about

incretin induced inhibition of hormone *gastric lipase*^(22, 23). All these mechanisms decrease the absorption of intestinal triglycerides, thus decreasing the level of lipids in blood. Both Sitagliptin and Gymnema are potent DPP-IV inhibitors which might act as stated above. The histopathological study of liver also supported these findings. Ginger and its combination with Sitagliptin, however, maintained the lipid levels due to the strong antioxidant activity and due to the inhibition of LDL oxidation and the suppression on the activity of HMG-CoA (3-hydroxy-3-methylglutaryl co-enzyme A) reductase⁽²⁴⁾ of the former and it showed significant results in the histopathological study of rat liver.

CONCLUSION

Although the combination of the herbs along with Sitagliptin Phosphate did not work in favour of practising multidrug therapy, it significantly controlled the secondary complications related to Type II diabetes mellitus. The combination profoundly regulated the lipid levels in rats.

There was no evidence of hypoglycaemia observed in any of the treatment groups at selected doses for the proposed work. Hence, further studies are required considering various other doses of Sitagliptin Phosphate, *Gymnema sylvestre* and *Zingiber officinale* extracts into consideration. Interactions need to be monitored pharmacodynamically as well as pharmacokinetically at various other doses in order to come to a proper conclusion.

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