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EVALUATION OF ANTI DIABETIC DRUG ALOGLIPTIN FOR THE TREATMENT OF OBESITY IN RATS

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ABSTRACT

Obesity is a complex disease caused by the interaction of a myriad of genetic, dietary, lifestyle, and environmental factors, which favors a chronic positive energy balance, and leads to increased body fat mass. The incidence of obesity is rising at an alarming rate and is becoming a major public health concern with incalculable social costs. Indeed, obesity facilitates the development of metabolic disorders such as diabetes, hypertension, and cardiovascular diseases in addition to chronic diseases such as stroke, osteoarthritis, sleep apnea, some cancers, and inflammation based pathologies. Standard reference Sibutramine produced a significant anti obesity activity in HFD induced obesity in rats. *Alogliptin* with medium and high doses exhibited a significant anti obesity activity by reducing the body weight, food intake, organ and fat pads weight and serum GLU, CHO, TRG, LDL and VLDL cholesterol levels with an increased HDL levels in HFD induced obesity models in rats.

Keywords: *Alogliptin*, Anti-obesity, Anti-diabetics, DPP-4

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INTRODUCTION

In the present Evaluation, we have selected a *Alogliptin* (Anti diabetic drug) in which variety of pharmacological features are abundant. However to date anti-obesity activities of this Drug have not been reported. Its medicinal properties of dipeptidyl peptidase 4 inhibitors (DPP-4) reported by the researchers to opt for the assessment of anti-obesity activities in various experimental animal models.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are novel oral antihyperglycemic agents for treating type 2 diabetes mellitus patients. Recent studies suggest that several DPP-4 inhibitors exert suppressing inflammatory reactions. However, whether or not DPP-4 inhibitors used as Anti-cancer drug. Alogliptin (2-({6-[(3R)-3-aminopiperidinyl-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)yl} methyl)benzonitrile monobenzoate) (AGP) is a selective DPP-4 inhibitor that has improves glycemic control. However, it remains unknown whether AGP has anti-cancer[1-7].

DPP4 was first discovered by Hopsu-Havu and Glenner in 1966. This protein is also called CD26 and is a ubiquitously expressed 110-kDa glycoprotein that belongs to the type 2 transmembrane protein family. As a member of the serine peptidase/prolyl oligopeptidase family, DPP4 is often sub classified based on its structure and function as follows: membrane-bound peptidase (fibroblast activation protein (FAP)/seprase), resident cytoplasmic enzyme (DPP8 and DPP9), and nonenzymatic member (DPP6 and DPP10). These proteins share a typical α/β-hydrolase fold. DPP4 comprises four domains: a short cytoplasmic domain, a transmembrane domain, a flexible stalk segment, and the extracellular domain, which is further separated by a glycosylated region, the cysteine-rich region, and the catalytic region. DPP4 can cleave dozens of peptides,

including chemokines, neuropeptides, and regulatory peptides, containing a proline or alanine residue at position 2 of the amino-terminal region. Despite the preference for prolines at position 2, alternate residues at the penultimate position are also cleaved by DPP4, indicating a required stereochemistry for cleavage. This DPP4 cleavage at post-proline peptide bonds inactivates peptides and/ or generates new bioactive peptides, thereby regulating diverse biological processes[8-11].

Obesity is a chronic disease consisting of the increase in body fat stores. However, measuring body fat requires quite sophisticated methods that make population-based measure of body fat almost impossible to perform. Consequently, there are not precisely defined normal values of body fat. Thus, for practical reasons, obesity is measured by means of the Body Mass Index (BMI), a calculation taking into account theweight for a given height: BMI is given as a ratio of Weight (kg)/height $(m)^2$ and highly correlates with total body fat and is very useful for epidemiological purposes. Based on the BMI and on its relationship with death from all causes, the WHO has established different cut-off points enabling the classification of obesity[12].

The psychogenic theory of obesity long held that obesity resulted from an emotional disorder in which food intake, relieved the anxiety and depression to which obese persons are usually susceptible. Stress associated with traumatic emotional events has been held responsible for certain cases of obesity and has been implicated in the pathogenesis of eating disorders such as night-eating syndrome and bulimia[13-17].

The exact etiology of obesity is unclear. The multiple causative factors like genetic, environmental, nutritional, physiological, psychological, social and cultural factors have been linked to its development and progression[18-21].

Over the last few decades obesity research has been characterized by a dramatic change in the overall understanding about the adipose tissue and its role in pathophysiology. The secretory nature of adipocytes or fat cells which encompass 95 % of total body cells has shifted the concept of white adipose tissue (WAT) as a mere energy storing organ to that of an extremely active endocrine tissue. The large number of secreted proteins includes hormones, growth factors, enzymes, cytokines, complement factors, and matrix proteins, collectively termed as adipokines or adipocytokines. Since in obesity the adipose tissue is in a state of hypertrophy and the secretary activity of WAT is believed to be exaggerated and closely associated with obesity induced metabolic syndrome. Amid all these secreted products cytokines or better described as pro-inflammatory cytokines are thought to be fore-runners in obesity associated metabolic syndrome[22].

Sibutramine offers three types of benefit in weight management like enhancement of weight loss, improvement in weight maintenance and reduction in comorbidities. The mechanism of action of sibutramine involves two complimentary physiological effects, first it promotes and prolongs satiety after eating thereby reduces food intake, including snack consumption. Secondly it stimulates energy expenditure and limits the decline in metabolic rate that normally accompanies weight loss. Sibutramine has two fold pharmacological actions in that it is a monoamine reuptake inhibitor, and is particularly effective in blocking the reuptake of both Serotonin and Noradrenaline[23].

Obesity is caused by an imbalance between energy intake and consumption. It is frequently associated with dyslipidemia, cardiovascular risks, hypertension, and type-2 diabetes mellitus, and thus is recognized as one of the most serious public health problems. Numerous drugs targeted towards inhibition of amylase/α-glucosidase/lipase, loss of appetite, and improving fatty acid metabolism, have been approved for the treatment of obesity; however, most of them have been withdrawn from the market because of their serious adverse effects. Therefore, many studies have been conducted to find and develop a new anti-obesity drug or a dietary supplement with lesser side effects[24-26].

MATERIALS AND METHODS

Experimental animals:

Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 16-25 g were procured from National Centre for Laboratory Animal Sciences, C/0 Sri. Venkateswara Enterprises, Bangalore for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry condition.i.e[27].

Room temperature- 26 ± 2^0C

Relative humidity- 45 - 55%

Light/dark cycle- 12 : 12h

The animals were fed with a synthetic standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli, Maharashtra. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to Guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Innovative College of Pharmacy, Greater Noida, U.P. CPCSEA registration number was 1346/PO/Re/S/10/CPCSEA and all the procedures were followed as per rules and regulations.

Chemicals:

All chemicals used were of analytical grade. Sibutramine (Symed Laboratories, Hyderabad,India) was used as Standard control. Test compound (*Alogliptin*) was provided by Taj Pharma, Mumbai, India.

Grouping of animals: The animals are divided into 6 groups of six rats in each group and the treatment given once.

Method for collection of blood sample[28-31]

On the 41st day the blood (up to2.5 ml) collected from retro-orbital puncture under the influence of light ether anesthesia into centrifuge tubes, which were centrifuged at 3000 rpm at room temperature. Anti-obesity activity of the drug was determined by measuring serum levels

of enzymes (GLU, CHO, TRG, HDL, VLDL, LDL) and Liver weight, Uterus Fat Pads, Mesentric Fat Pads).

Statistical analysis:

All results will be expressed as mean ± SEM from 6 animals. Statistical difference in mean will be analyzed using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's't' test). $P< 0.05^{*}$, 0.01^{**} and 0.001^{***} will be considered as statistically significant

RESULTS

Biochemical parameters

In normal control animals glucose level is noted as 103.35 mg/dL. A significant increase ($P < 0.01$) in serum glucose level is noted in HFD induced obese rats as 311.13mg/dL. Sibutramine (5 mg/kg) significantly ($P < 0.01$) reduced the glucose level in HFD induced obese rats i.e.; 110.7 mg/dL. *Alogliptin* also significantly ($P < 0.01$) reduced the serum glucose level in a dose dependent manner i.e. 165.83, 111.12 and 104.75mg/dL respectively (Table 1 and Fig.1).

In normal control animals serum cholesterol is noted as 74.12 mg/dL. Asignificant increase ($P < 0.01$) in cholesterol level is noted in HFD induced obese rats as 184.55 mg/dL. Sibutramine (5 mg/kg) significantly ($P < 0.01$) reduced the serum cholesterol level in HFD induced obese rats i.e.; 102.29 mg/dL. *Alogliptin* significantly ($P < 0.05$) reduced the serum cholesterol with high doses only i.e. 120.44 mg/dL. But low and medium doses of the *Alogliptin* also reduced the serum cholesterol non significant extent i.e. 153.30 and 132.73 mg/dL respectively (Table 1 and Fig.2).

In normal control animals serum triglycerides level is noted as 94.25 mg/dL. A significant increase ($P < 0.01$) in serum triglycerides level is noted in HFD induced obese rats as 217.35 mg/dL. Sibutramine (5 mg/kg) significantly ($P < 0.01$) reduce the triglycerides level in HFD induced obese rats i.e.; 122.78 mg/dL. *Alogliptin* also significantly reduce the serum triglycerides level in dose dependent manner i.e. 159.33($P< 0.05$), 156.02($P < 0.05$) and 155.88 mg/dL ($P < 0.05$) respectively (Table 1 and Fig.3)

In normal control animals serum HDL cholesterol level is noted as 20.19 mg/dL. A significant decrease ($P < 0.01$) in HDL cholesterol level is noted in HFD induced obese rats as 14.0 mg/dL. Sibutramine (5 mg/kg) significantly ($P < 0.01$) increased theHDL cholesterol level in HFD induced obese rats i.e; 36.7 mg/dL. *Alogliptin*significantly ($P < 0.01$) increased the serum HDL cholesterol levels at medium and high doses i.e. 16.1 and 21.94 mg/dL respectively. At low doses *Alogliptin*exhibited a non significant increase in serum HDL cholesterol levels i.e. 31.01mg/dL respectively (Table 1 and Fig.4)

In normal control animals VLDL cholesterol level is noted as 18.85 mg/dL. A significant increase ($P < 0.01$) in serum VLDL cholesterol level is noted in HFD induced obese rats as 43.47 mg/dL. Sibutramine significantly ($P < 0.01$) reduced the serum VLDL cholesterol level in HFD induced obese rats i.e.; 24.55 mg/dL. *Alogliptin* also significantly reduced the serum VLDL cholesterol level in dose a dependent manner i.e.31.86 ($P < 0.05$), 31.20 ($P < 0.05$) and 31.17 mg/dL ($P < 0.05$) respectively (Table 1 and Fig.5).

In normal control animals LDL cholesterol level is noted as 38.57 mg/dL. A significant increase ($P < 0.01$) in serum LDL cholesterol level is noted in HFD induced obese rats as 207.27 mg/dL. Sibutramine (5 mg/kg) significantly ($P < 0.01$) reduced the serum LDL cholesterol level in HFD induced obese rats i.e.; 70.91 mg/dL. *Alogliptin* also significantly ($P < 0.05$) reduced the serum LDL cholesterol level at high doses only noted as 90.73mg/dL, Low and medium doses of *Alogliptin* reduced serum LDL cholesterol level to non significant extent as 147.46and 104.87 mg/dL respectively (Table 2 and Fig.6).

In normal control animals liver weight is noted as 3.39 g. A significant increase ($P< 0.01$) in liver weight is noted in HFD induced obese rats as 4.75 g. Sibutramine (5mg/kg) significantly ($P < 0.01$) reduced the liver weight in HFD induced obese rats i.e.3.71 g. *Alogliptin* significantly ($P < 0.01$) reduced the liver weight withmedium and high doses recorded as 4.22, and 3.96 g. Low doses of *Alogliptin* also reduced the liver weight to non significant extent noted as 4.55 g ($P>0.05$) respectively (Table 2 and Fig.7).

In normal control animals uterus fat pads weight is noted as 3.34 g. A significant increase ($P < 0.01$) in uterus fat pads is noted in HFD induced obese rats as 5.26 g. Sibutramine (5 mg/kg) significantly ($P < 0.01$) reduced the uterus fat pads in HFD induced obese rats i.e.; 3.97 g. *Alogliptin* also significantly ($P < 0.01$) reduced the uterus fat pads in dose a dependent manner i.e. 4.85, 4.60 and 4.24 g respectively (Table 2 and Fig.8).

In normal control animals mesentric fat pads weight is noted as 1.71 g. A significant increase ($P < 0.01$) in mesentric fat pads weight is noted in HFD induced obese rats as 3.25 g. Sibutramine (5 mg/kg) significantly ($P < 0.01$) reduced the mesentric fat pads in HFD induced obese rats i.e; 2.09 g. *Alogliptin* also significantly ($P <0.01$) reduced the mesenteric fat pads weight in dose a dependent manner i.e. 2.96, 2.63 and 2.34 g respectively (Table 2 and Fig.9).

Table 1: Effect of *Alogliptin* on serum biochemical parameters in HFD induced obesity in rats.

Group	Treatment	GLU	CHO	TRG	HDL	VLDL
I	Normal Pellet diet	103.35± 4.4	74.12± 8.06	94.25± 6.9	20.19± 1.3	18.85± 1.3
II	HFD	311.13± 23.5	184.55± 20.28	217.35± 23.8	14.0± 0.5	43.47± 4.7
III	SBT	110.7± 4.7	102.29± 7.79	122.78±5.1	36.7± 3.4	24.55± 1.0
IV	*Alogliptin* 1mg/kg	165.83± 30.8	153.30± 14.63	159.33± 8.2	31.01± 1.4	31.86± 1.6
V	*Alogliptin* 2mg/kg	111.12± 4.8	132.73± 12.9	156.02± 10.08	21.94± 1.1	31.20± 2.0
VI	*Alogliptin* 3mg/kg	104.75± 4.4	120.44± 18.09	155.88± 11.8	16.1± 0.45	31.17± 2.3

Table 2: Effect of *Alogliptin* on serum biochemical parameters in HFD induced obesity in rats.

Group	Treatment	LDL	Liver weight	Uterus Fat Pads	Mesentric Fat Pads
I	Normal Pellet diet	38.57± 2.11	3.39± 0.09	3.34± 0.10	1.71± 0.03
II	HFD	207.27± 3.16	4.75± 0.05	5.26± 0.02	3.25± 06
III	SBT	70.91± 4.50	3.71± 0.06	3.97± 0.12	2.09± 0.08
IV	*Alogliptin* 1mg/kg	147.46± 3.64	4.55± 0.09	4.85± 0.05	2.96± 0.08
V	*Alogliptin* 2mg/kg	104.87± 4.66	4.22± 0.08	4.60± 0.03	2.63± 0.06
VI	*Alogliptin* 3mg/kg	90.73± 8.49	3.96± 0.17	4.24± 0.05	2.34± 0.05

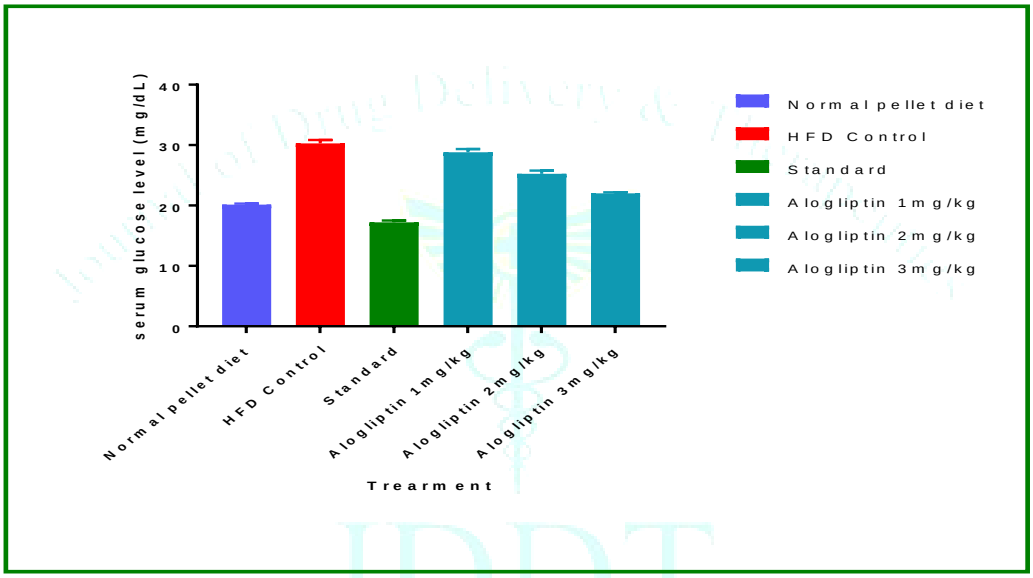

Figure 1: Effect of Alogliptin on serum glucose level (mg/dL) in High Fed Diet (HFD) induced obesity in rats

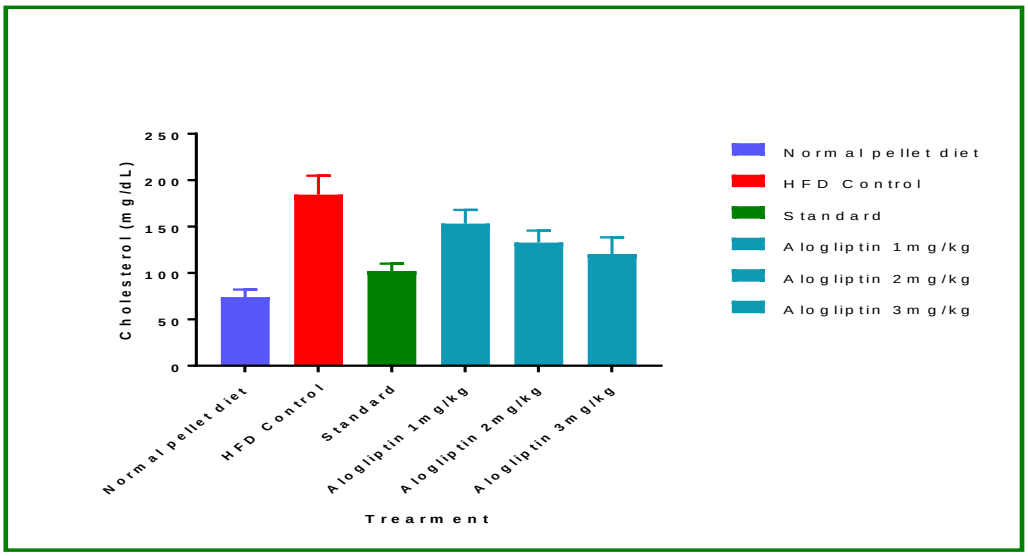

Figure 2: Effect of Alogliptin on serum cholesterol (mg/dL) in High Fed Diet (HFD) induced obesity in rats

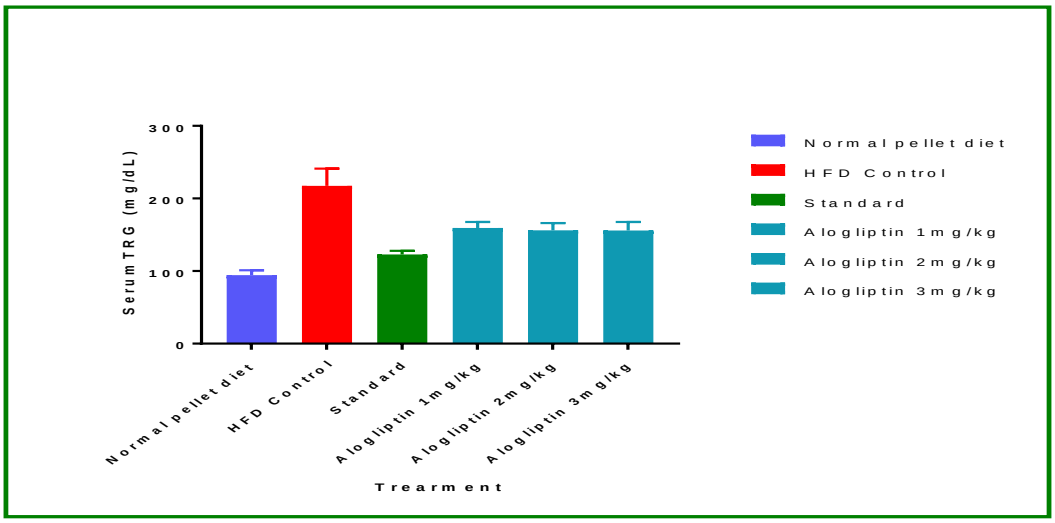

Figure 3: Effect of Alogliptin on serum triglycerides (mg/dL)in High Fed Diet (HFD) induced obesity in rats

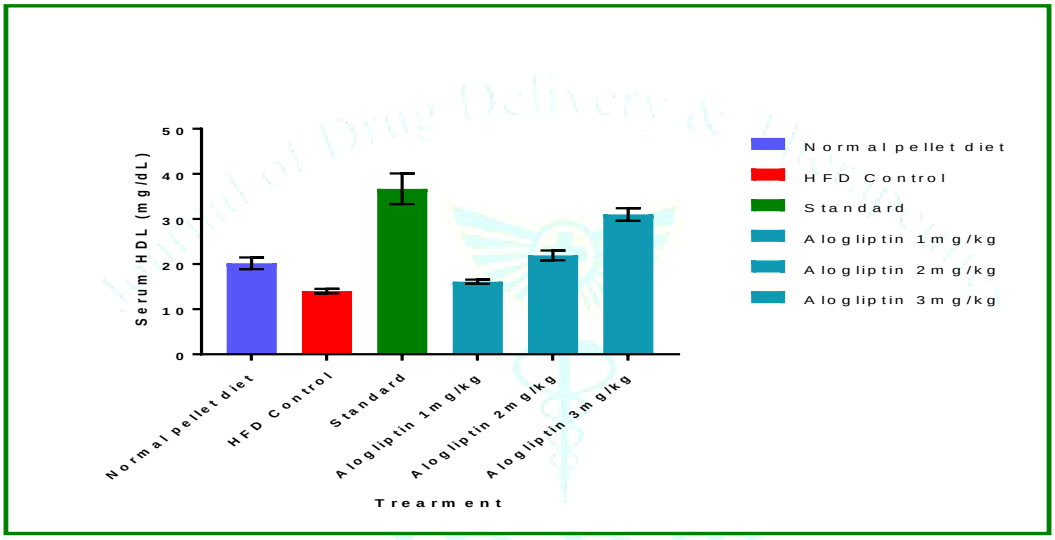

Figure 4: Effect of Alogliptin on serum HDL cholesterol (mg/dL) in High Fed Diet (HFD) induced obesity in rats

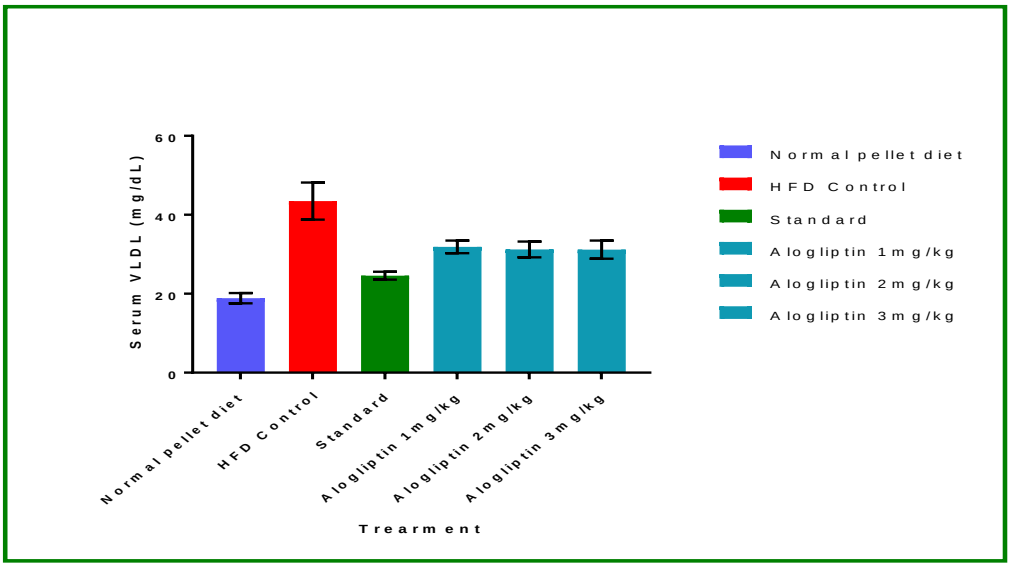

Figure 5: Effect of Alogliptin on serum VLDL cholesterol (mg/dL) in High Fed Diet (HFD) induced obesity in rats

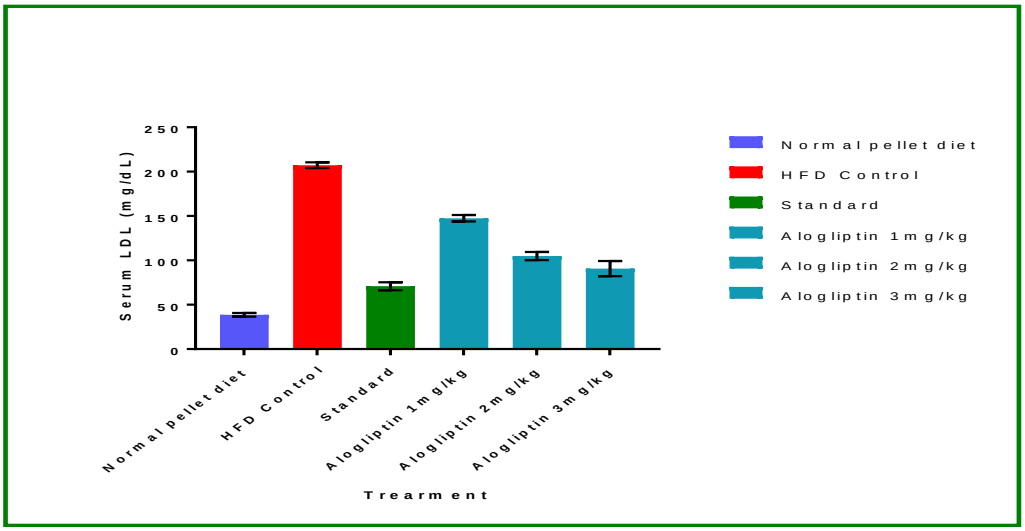

Figure 6: Effect of Alogliptin on serum LDL cholesterol (mg/dL) in High Fed Diet (HFD) induced obesity in rats

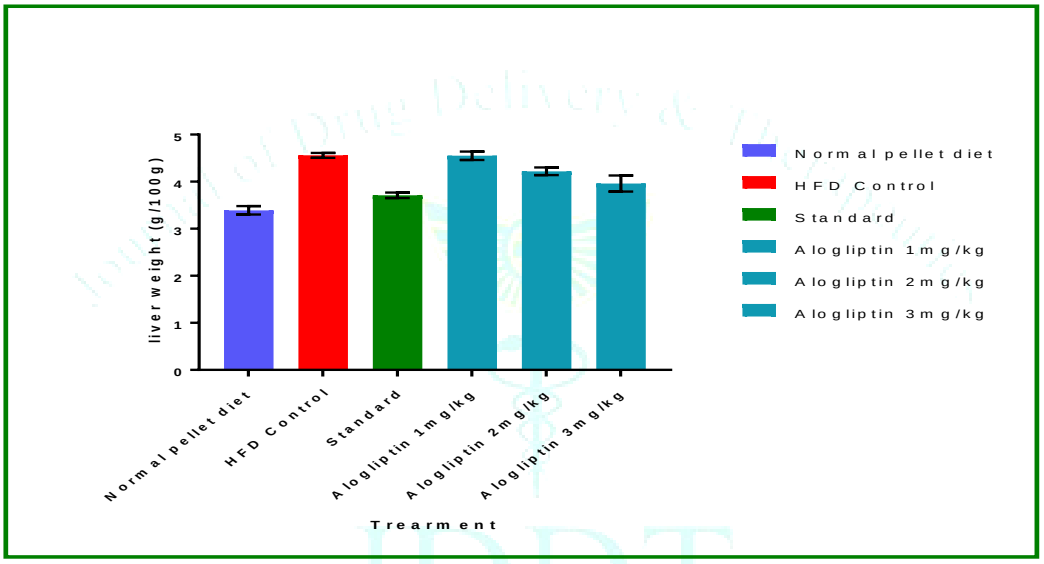

Figure 7: Effect of Alogliptin on liver weight (g/100g) in High Fed Diet (HFD) induced obesity in rats

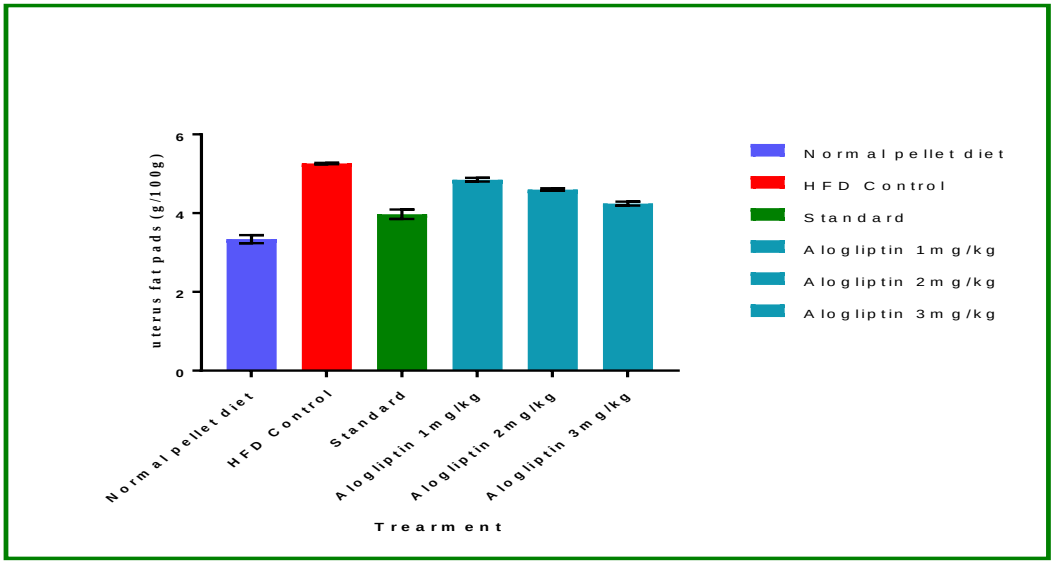

Figure 8: Effect of Alogliptin on uterus fat pads (g/100g) in High Fed Diet (HFD) induced obesity in rats

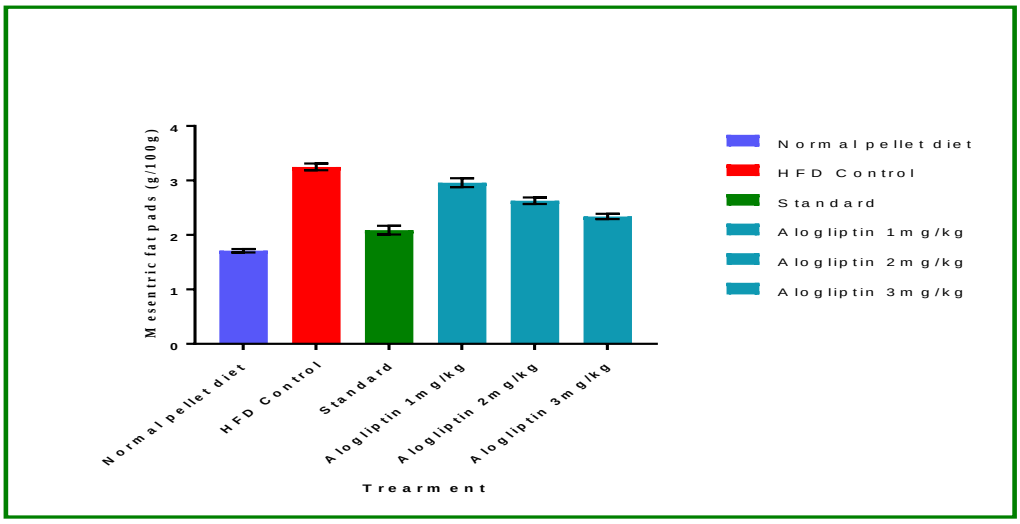

Figure 9: Effect of Alogliptin on mesentric fat pads (g/100g) in High Fed Diet (HFD) induced obesity in rats

DISCUSSION

In recent years there is a raise in development of anti obesity, anti cancer and anti inflammatory drugs as the incidence it is very high, multifactorial in nature with complexity and also lack of knowledge about the weight controlling system, even with cancer and inflammation strenuous research no effective medicament has come out as a one drug sofar. The main approach to control body weight rely on expenditure, thermogenesis, control of available substrate to cell and tissue through hormonal and other metabolic factors controlling the fate of available energy substrates, lethal obesity finally control of fat reserves through modification of eipogenesis and hipolysis in white adipose tissue.[13-21]

Sibutramine is a serotonin–noradrenaline reuptake inhibitor (SNRI). A reuptake inhibitor inhibits the neuronal uptake of neurotransmitters and prolongs the duration of responses to both exogenous and neuronal release, in this case, of serotonin (5-HT) and noradrenaline (NA). Sibutramine has also been shown to block the reuptake of dopamine (DA) but at about a threefold lower potency when compared to 5-HT and NA. More recent studies suggest that sibutramine increases extracellular DA concentrations at similar levels to 5-HT in an animal model. 5-HT is recognized to have an influence on food intake and macronutrient selection. [23]

The standard treatment of obesity is the use of High fed diets, often supplemented with exercise, food education and changes in eating habits. At present, the only option available to the severely obese is bariatric surgery, since relapses are extremely common with all treatments and in the morbidly obese most individual treatments do not achieve even a significant weight loss paralleled by improvement in the overall condition of the patient. In spite of its considerable danger and drawbacks, bariatric surgery is currently used as a last-resort therapy for life-threatening obesity.

High Fed Diet (HFD) is a widely used model of obesity. Presenting a varied and energy dense diet often leads to hyperphagia and weight gain. The ability of rats to select their own food items (and macronutrient content) introduces another source of variability between HFD.

HFD contains with a variety of highly palatable, energy rich, high carbohydrate foods elicited significant increase in body weights and fat pad mass. HFD have been previously reported to increase energy intake and cause obesity in humans as well as animals.

It is well known that hyperlipidemia is the leading risk factor for atherosclerosis. Epidemiological investigation revealed a positive correlation between the severe degree of atherosclerosis and the concentrations of plasma cholesterol as well as LDL. Numerous population studies have linked raised concentration of total cholesterol or LDL–cholesterol in plasma with increased incidence of atherosclerotic events.

HFD which contained high cholesterol, a significant increase of different plasma lipids such as TC, TG and LDL with a parallel decrease in HDL was observed compared with that of control group.

Consumption of HFD promotes obesity and fat accumulation in humans and several animal species, including rats, mice, and pigs.

In this present work, it was planned to Evaluation have been selected to study their anti obesity activity in experimental animals, rats. Literature survey revealed that the *Alogliptin* from other medicinal uses was used as an ethnic folklore medicine for obesity.

The *Alogliptin* had significantly reduced the physical parameters like body weight, food intake, organ and fat pads weight in HFD induced obesity models in rats.

Alogliptin had significantly reduced the serum biochemical parameters like GLU, CHO, TRG, LDL-CHO, VLDL-CHO levels and increased the HDL levels in HFD induced obesity models in rats.

Thus our present study suggests that *Alogliptin* possess potent obesity activity and increase life span.

CONCLUSIONS

The anti obesity activity of *Alogliptin* were evaluated in HFD induced obesity activities with different models respectively.

Standard reference Sibitramine produced a significant anti obesity activity in the selected models from this studies it can be concluded that *Alogliptin* with medium and high doses exhibited a significant anti obesity activity by reducing body weight, organ and fat pads weight and serum GLU, CHO, TRG, LDL and VLDL cholesterol levels with an increase in HDL levels in HFD induced obesity models in rats.

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Conflicts of Interest

I Mohd Fasih Ahmad (Author) declare that there are no conflicts of interest regarding the publication of this paper.

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