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EVALUATION OF ANTI-OBESITY AND ANTI-DIABETIC EFFECT OF HISTIDINE DIHYDROCHLORIDE IN ZEBRAFISH MODEL

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ABSTRACT

The present study was intended to investigate the anti-obesity and anti-diabetic effect of histidine dihydrochloride in Zebrafish. Obesity induction by the model: diet induced obesity in zebrafish and glucose induced hyperglycemia by the model: glucose induced hyperglycemia in zebrafish. The effect of Histidine dihydrochloride on diet induces obesity in zebrafish model were determined by body weight; lipid estimation- (TG, Cholesterol, HDL, LDL); histopathological of liver and the effect of Histidine dihydrochloride on glucose induced hyperglycemia in zebrafish model were determined by the fasting blood glucose level. All the result we found the significant. HDC drug showed significant effect by decreasing in the body weight, lipid profile estimation – TG, Cholesterol, HDL, LDL, Fasting blood glucose level. HDC drug shows the anti-obesity and antidiabetic activity. This data indicates that in-vivo treatment with the HDC drug anti-obesity and antidiabetic condition in the diet induced obesity and glucose induced hyperglycemia zebrafish model.

KEYWORDS: Anti-obesity, anti-diabetic, body weight, HDC, glucose, hyperglycemia.

1. INTRODUCTION

Obesity is characterized as excess body fat, and is an epidemiological issue worldwide. The underlying cause of obesity and overweight is an energy disparity between consumed calories and spent calories. Changes in patterns of dietary and physical activity are also the result of environmental and social trends in sectors such as wellness, agriculture, transport, urban planning, economics, food processing, consumption, marketing, and education.[1] Obesity has

long been a significant source of type II diabetes mellitus, elevated blood pressure and dyslipidemia (National Institutes of Health and National Heart Lung and Blood Institute, 1998; National Task Force on Prevention and Treatment of Obesity, 2000).[2]

Diabetes mellitus is considered one of the world's five principal causes of death. Diabetes-related deaths are estimated at approximately 9% of global death. India is leading the way in any given country with the largest number of diabetic subjects. The number of diabetes in India is expected to increase by 2025 by 57.2 million.[3,4]

Histidine is an essential amino acid and has many biological functions such as anti-inflammatory, defensive tissue, and marked antioxidant activities such as scavenging free radicals, binding divalent metal ions, and anti-glycating activity have been reported in several studies.[5] Zebrafish has been established as a successful animal model for researching vertebral physiological and pathological conditions. Zebrafish's powerful resources can increasingly be used to model human diseases, such as obesity and diabetes.[6-11] In Zebrafish, glucose absorption / absorption takes place in the gills and intestines by a glucose transporter called GLUT. We have described here that glucose overload in live fish water can induce symptoms associated with diabetes mellitus pathophysiology.[12-15]

2. MATERIALS AND METHODS

2.1.Experimental animals

A local commercial distributor obtained adult male and female "wild type" zebrafish (Maruthi Aquarium, Saptagiri, Bengaluru). All fish were given at least 3 weeks to acclimatize to the laboratory environment, and housed in a 40-L tank group. The tanks were filled with filtered water (facility) and maintained room temperature, and provided a 12-hour cycle (light on at 6:00 h, light off at 18:00 h) in accordance with zebrafish care standards. All the fish used in this study were experimentally inexperienced and were fed Tetramin Tropical Flakes (Taiyo) and Artemia twice daily. For more research the fish will be subjected to hypothermia through freezing cold contact, and then decapitation as the effect. In accordance with CPCSEA guidelines, the animals were kept for the care and use of laboratory animals. Institutional Animal Ethics Committee approved the study protocol (IAEC/ABMRCP/2018-2019/17), Acharya & BM Reddy College of Pharmacy, Bengaluru.[16]

2.2.Acute toxicity study

For dose calculation and to determine the toxicity level of the synthesized product on the experimental zebrafish, an acute toxicity study was conducted. The observation was done preferably for 96 h. 7 fishes was used for each test concentration including the control groups. Different concentrations in a geometric series with a factor preferably not more than (2.2). Fishes will be considered dead if there will be no visible movement. According to OECD 203 guidelines.[17]

i. 7 fishes were taken in each group and treated with 0.5 g, 1 g, 1.5 g, 1.75 g, 2 g/ 100 ml respectively to check the toxic dose from low range to high range of dose.

ii. Drugs in different concentration was measured and dissolved in water and then missed up with 100 ml water.

iii. 7 fishes in each group were kept in different container containing different drug solution and was observed for 96 h.

2.3.Evaluation of Diet induced obesity in Zebrafish[1]

Table 1: Methods of diet induced obesity.

SI. No.	Groups	Treatment	No. of. fishes
1.	Negative Control	No treatment maintained on regular diet	15
2.	Positive Control	Given artemia thrice every day for development of obesity for 8 weeks	15
3.	Treatment 1 (Low Dose)	Given artemia thrice every day for development of obesity for 8 weeks, followed by treatment with histidine dihydrochloride (0.17 g/100 ml) for 3 weeks.	15
4.	Treatment 2 (High Dose)	Given artemia thrice every day for development of obesity for 8 weeks, followed by treatment with histidine dihydrochloride (0.35 g/100 ml) for 3 weeks.	15

2.4.Evaluation of Glucose induced Hyperglycemia in Zebrafish[6]

Table 2: Methods of diabetic induction by glucose.

SI. No.	Group	Treatment	No. of. fishes
1.	Negative Control	No treatment maintained on regular diet	20
2.	Positive Control	Placed in freshly prepared 2%Glucose solution for 14 days, followed by regular diet.	20
3.	Treatment 1 (Low Dose)	Placed in freshly prepared 2%Glucose solution for 14 days, followed by treatment with histidine dihydrochloride (0.17 g/100 ml) for 3 weeks.	20
4.	Treatment 2 (High Dose)	Placed in freshly prepared 2%Glucose solution for 14 days, followed by treatment with histidine dihydrochloride (0.35 g/100 ml) for 3 weeks.	20

Sample collection

- Blood samples of fishes in all groups were collected by anaesthetizing using ice cold water followed by excision of the zebrafish head using a scalpel and immediately placing the glucometer test strip directly on the docked portion.
- Blood samples for biochemical estimations were collected by anaesthetizing the fish followed by docking the tail at anterior portion, a drop of anticoagulant was added and placed in effendroff tube and centrifuged at 3000 rpm for 5min. Serum pooled from 3 fishes were used for estimation has the amount of blood obtained was less.[18]

2.5.Fasting blood Glucose estimation

The blood glucose levels were assessed during the therapy for 14 days and after therapy using Accu glucometer test.[6]

2.6.Lipid Profile Estimation[19]

a) Triglyceride

Triglyceride is estimated by as per the diagnostic kit by using the hemato analyser.

b) Cholesterol

Cholesterol is estimated by as per the diagnostic kit by using the hemato analyser.

c) High density lipoprotein (HDL)

HDL is estimated by as per the diagnostic kit by using the hemato analyser.

d) Low density lipoprotein (LDL)

LDL is estimated by as per the diagnostic kit by using the hemato analyser.

2.7.Body Weight

The body weight is increased significantly in male and female zebrafish was observed till 8 weeks. In every week weight is taken by placing the zebrafish in the ice-cold water and the movement of zebrafish stops the take out the fish immediately from the ice-cold water and dry on the tissue paper. Afterwards place that fish on the weighing balance (sensitive) and note the weight of the zebrafish. Then place that zebrafish back to the normal water and the zebrafish will show the normal behavior.[19]

2.8.Histopathological studies

Zebrafish liver was harvested from the euthanized obese zebrafish and control group and fixed at 4°C in paraformaldehyde solution at 4% for 24 hours and samples were embedded in Epson resin, one micrometer section of fixed liver was stained with toluidine blue and images

were collected using a photomicroscope (40X).The histopathological studies were carried out at Prakash Diagnostic Laboratory, Bengaluru, Karnataka.[20]

2.9.Statistical Analysis

All the results will be expressed as Mean ± SEM (n=20). Data will be analyzed using one-way ANOVA followed by Dunnett's as post hoc test and data will be considered significant at ***p<0.05.***

3. RESULTS

3.1.Acute Toxicity

Table 3: Acute toxicity observation for 96 h.

Group	Group Name	Treatment	24 h	48 h	72 h	96 h
I	Control	No Drug	NB	NB	NB	NB
II	Test 1	HDC 0.5 g/100 ml	Excessive gills movement No mortality	Normal behavior No mortality	Normal behavior No mortality	Normal behavior No mortality
III	Test 2	HDC 1 g/100 ml	Excessive gills movement No mortality	Settling at bottom No mortality	Normal behavior No mortality	Normal behavior No mortality
IV	Test 3	HDC 1.5 g/100 ml	Excessive gills movement No mortality	Settling at bottom No mortality	Settling at surface No mortality	Normal behavior No mortality
V	Test 4	HDC 1.75 g/100 ml	Excessive respiration 3 fishes died	Settling at surface Increased gills movement No mortality	Settling at bottom Decreased gills movement No mortality	Normal behavior No mortality
VI	Test 5	HDC 2 g/100 ml	All Fishes died	---	---	---

***NB** = Normal Behavior

Acute toxicity was conducted according to OECD 203 guidelines. Mortality was observed at 2 g/100 ml and it is toxic dose. 50% mortality was seen in 1.75 g/100 ml. Hence the LC50 value is 1.75 g/100 ml. Based on the LC50 value two doses were selected for study 1/5th & 1/10th of the LC50 value is 1.75 g/100 ml.

3.2.Diet induced obesity in Zebrafish

3.2.1. Body weight

The experiment results are shown in table 4 and fig 1. The both dose of HDC show the better activity than the positive control group but in case of 0.35 g/100 ml dose has better activity when it is compared with the 0.17 g/100 ml.

Table 4: Effect of HDC on Body weight.

SI. No.	Group	Before Obese 1st week	After Obese 8th week	Treatment 3rd week
1.	Negative Control	0.465 ± 0.0285	0.599 ± 0.0453	0.623 ± 0.00353
2.	Positive Control	0.609 ± 0.00551	0.744 ± 0.0238	0.752 ± 0.0355
3.	HDC (0.17 g/100 ml)	0.498 ± 0.00947	0.643 ± 0.0145	0.649 ± 0.0150
4.	HDC (0.35 g/100 ml)	0.452 ± 0.0259	0.528 ± 0.0186	0.429 ± 0.0307 ***

The values are expressed as Mean ± S.E.M (n=3). where "***" represents p<0.001 Compared to positive control using One-way ANOVA followed by Dunnett's post hoc test.

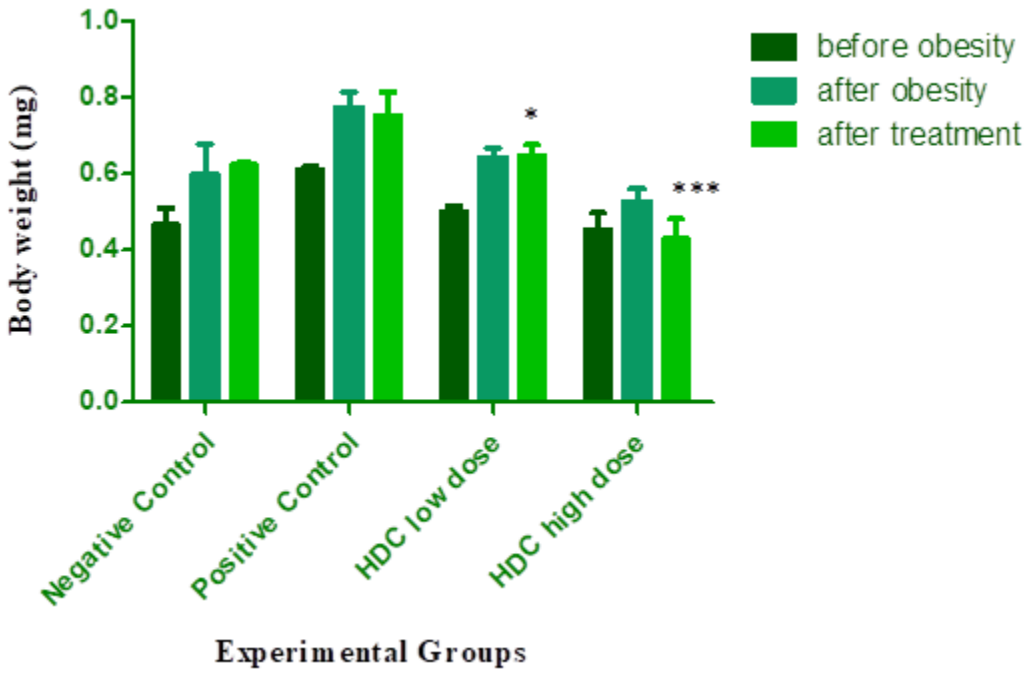


Fig 1: Effect of HDC on Body Weight.

3.3.Lipid Profile Estimation

3.3.1. Triglyceride

The experiment results are shown in table 5 and fig 2. The both dose of HDC show the better activity than the positive control group but in case of 0.35 g/100 ml dose has better activity when it is compared with the 0.17 g/100 ml.

Table 5: Effect of HDC on Triglyceride.

SI. No.	Group	Triglyceride
1.	Negative Control	9.87 ± 4.51
2.	Positive Control	54.7 ± 2.40
3.	HDC (0.17 g/100 ml)	67.5 ± 0.982
4.	HDC (0.35 g/100 ml)	21.8 ± 3.31 ***

The values are expressed as Mean ± S.E.M (n=3). where "***" represents p<0.001 Compared to positive control using One-way ANOVA followed by Dunnett's post hoc test.

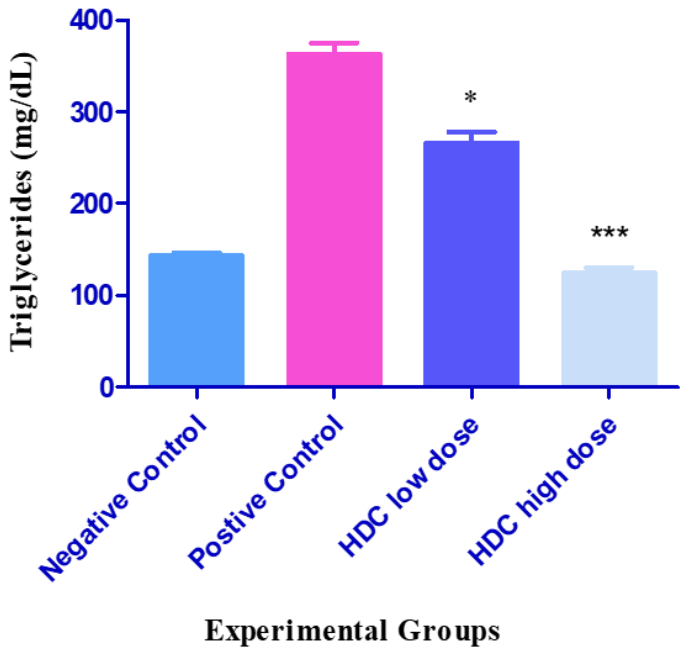


Fig 2: Effect of HDC on Triglycerides.

3.3.2. Cholesterol

The experiment results are shown in table 6 and fig 3. The both dose of HDC show the better activity than the positive control group but in case of 0.35 g/100 ml dose has better activity when it is compared with the 0.17 g/100 ml.

Table 6: Effect of HDC on Cholesterol.

SI. No.	Group	Cholesterol
1.	Negative Control	213 ± 5.81
2.	Positive Control	684 ± 6.01
3.	HDC (0.17 g/100 ml)	603 ± 5.61
4.	HDC /100 ml)	189 ± 1.67 ***

The values are expressed as Mean ± S.E.M (n=3). where "***" represents p<0.001 Compared to positive control using One-way ANOVA followed by Dunnett's post hoc test.

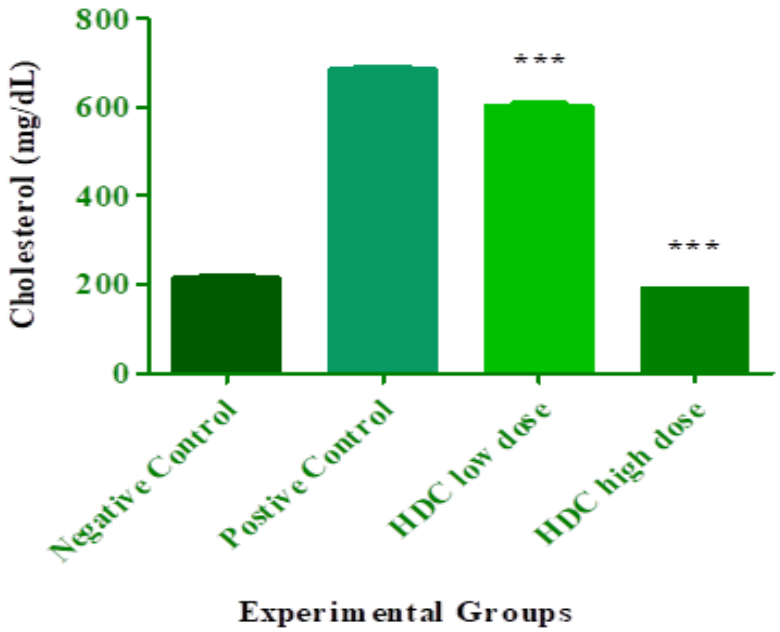


Fig 3: Effect of HDC on Cholesterol.

3.3.3. High Density Lipoprotein (HDL)

The experiment results are shown in table 7 and fig 4. The both dose of HDC show the better activity than the positive control group but in case of 0.35 g/100 ml dose has better activity when it is compared with the 0.17 g/100 ml.

Table 7: Effect of HDC on HDL.

SI. No.	Group	HDL
1.	Negative Control	174.7 ± 3.930
2.	Positive Control	655 ± 5.003
3.	HDC (0.17 g/100 ml)	459 ± 19.55
4.	HDC (0.35 g/100 ml)	292.7 ± 14.11 ***

The values are expressed as Mean ± S.E.M (n=3). where "***" represents p<0.001 Compared to positive control using One-way ANOVA followed by Dunnett's post hoc test.

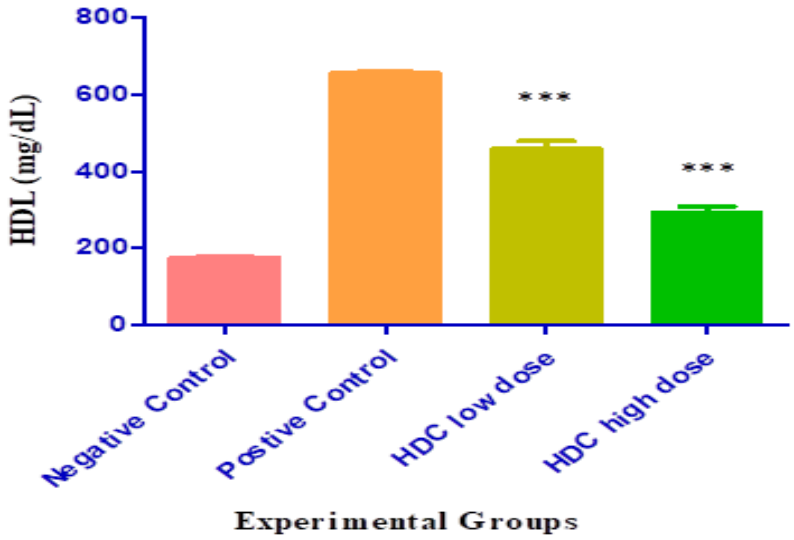


Fig 4: Effect of HDC on High Density Lipoprotein.

3.3.4. Low Density Lipoprotein (LDL)

The experiment results are shown in table 8 and fig 5. The both dose of HDC show the better activity than the positive control group but in case of 0.35 g/100 ml dose has better activity when it is compared with the 0.17 g/100 ml.

Table 8: Effect of HDC on LDL.

SI. No.	Group	LDL
1.	Negative Control	9.87 ± 4.51
2.	Positive Control	61.3 ± 1.64
3.	HDC (0.17 g/100 ml)	46.5 ± 1.53
4.	HDC (0.35 g/100 ml)	21.8 ± 3.31 ***

The values are expressed as Mean ± S.E.M (n=3). where "***" represents p<0.001 Compared to positive control using One-way ANOVA followed by Dunnett's post hoc test.

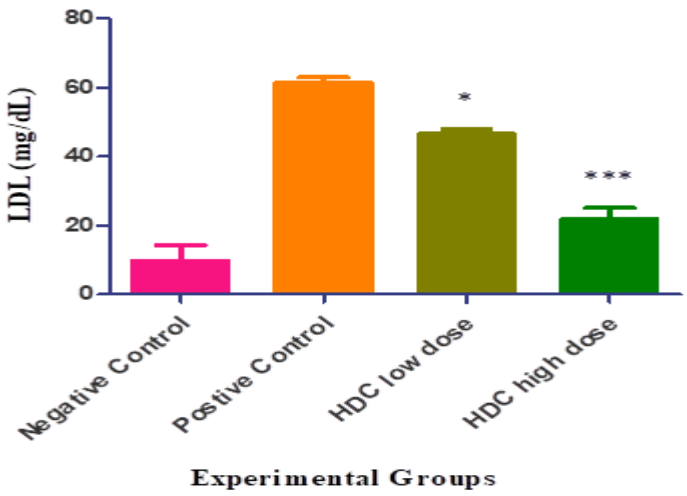


Fig 5: Effect of HDC on Low Density Lipoprotein.

3.4.Evaluation of Glucose induced Hyperglycemia in Zebrafish

3.4.1. Effect of HDC on Fasting Blood Glucose Level

The experiment results are shown in table 9 and fig 6. The both dose of HDC show the better activity than the positive control group but in case of 0.35 g/100 ml dose has better activity when it is compared with the 0.17 g/100 ml.

Table 9: Effect of HDC on Fasting blood glucose level at 1st, 7th, 14th, 21st, 28th, 34th day.

Group	Treatment	Fasting Blood Glucose Level (mg/dl)					
		1st Day	7th Day	14th Day	21st Day	28th Day	34th Day
Negative Control	Normal water on regular diet	70.3 ± 0.33	78 ± 1.15	82 ± 1.2	82.7 ± 1.20	79.7 ± 1.20	81.3 ± 1.45
Positive Control	2% glucose for 14 days followed by regular diet	74 ± 0.57	181 ± 0.88	276 ± 0.88	259 ± 0.88	252 ± 0.88	249 ± 0.88
HDC (0.17 g/100 ml)	2% glucose for 14 days + 0.17g/100 ml Histidine Dihydrochloride	70.3 ± 0.88	174 ± 1.45	275 ± 0.58	189 ± 1.73 ***	180 ± 0.88 ***	172 ± 2.03 ***
HDC (0.35 g/100 ml)	2% glucose for 14 days + 0.35g/100 ml Histidine Dihydrochloride	70.3 ± 1.20	185 ± 0.88	275 ± 1.8	189 ± 1.73 ***	144 ± 3.06 ***	89.7 ± 1.45 ***

The values are expressed as Mean ± S.E.M (n=3). where "***" represents p<0.001 Compared to positive control using One-way ANOVA followed by Dunnett's post hoc test.

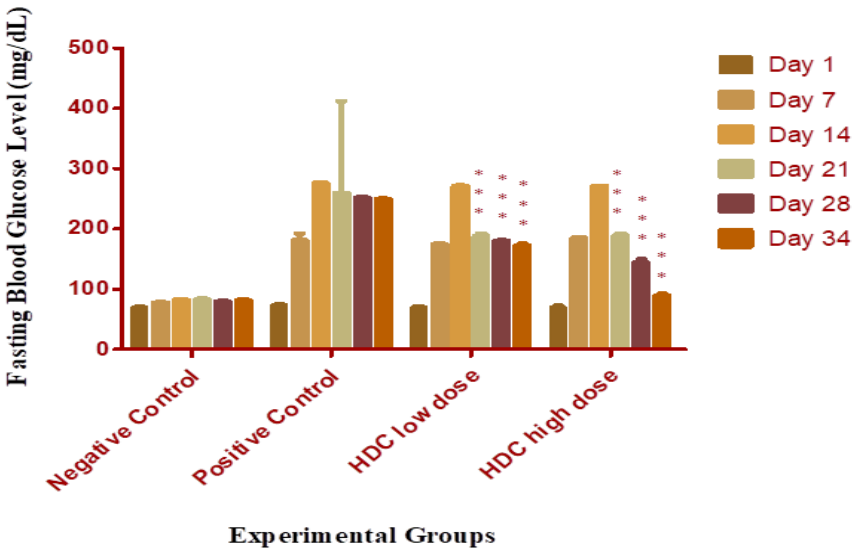
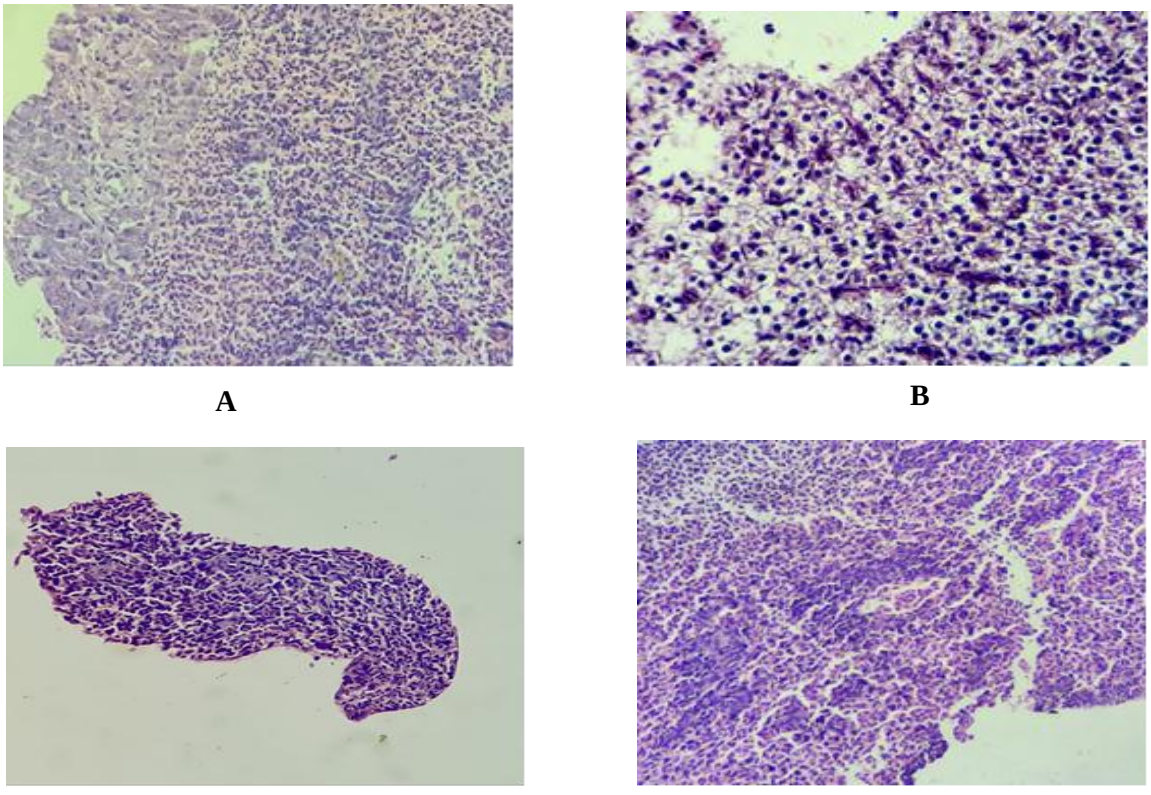


Fig 6: Effects of HDC on Fasting Blood Glucose Level at 1st, 7th, 14th, 21st, 28th, 34th day.

3.5. Histopathology of Zebrafish liver on diet induced obesity



A **B**

C **D**

Fig 7: Histopathology of Zebrafish liver on diet induced obesity.

A. Group I (Negative Control): Normal view; No hepatic tissue.
B. Group II (Positive Control): Presence of hepatocytes with droplets of fat like vacuole; lipid accumulation.
C. Group III (HDC 0.17 g/100 ml): No hepatic tissue; Normal histopathology.
D. Group IV (HDC 0.35 g/100 ml): Normal histopathology; No hepatic tissue.

4. DISCUSSION

In this study, the effect of Histidine dihydrochloride on diet induced obesity and glucose-induced hyperglycemia in the zebrafish model was investigated by evaluating the parameter effects such as body weight; lipid estimation- (TG, Cholesterol, HDL, LDL); histopathological of liver and fasting blood glucose level.

Diabetes mellitus is a metabolic dysregulation condition, initially diagnosed as hyperglycemia, which eventually results in damage to the blood vessels leading to several complications that all occur long after euglycemia is resolved by therapeutic action. Here we have detailed a protocol that allows the generation of diabetes models of both type 1 (alloxan) and type 2 (glucose) in zebrafish.[21]

Zebrafish are freshwater teleost, which control their amounts of internal water and overall solvents. They function hyper-osmotically, a technique of osmoregulation that requires increased intake of water as opposed to their freshwater ecosystem due to higher concentrations of internal salts.[22] The constant inflow of water results in the absorption of other molecules, including glucose, from their environment. So, zebrafish are an excellent candidate for use in early chemical screening for toxicity and drug effectiveness.[23-25] Previous experiments have shown that teleost's in general, and zebrafish in particular, have endocrine islet tissue that includes hormone-producing cells such as β cells, and that these cells merge into a central region similar to the Langerhans islet. Insulin from teleost is homologous to human insulin, developed at a frequency equivalent to that of humans.[26]

Zebrafish also expresses a homologous GLUT-1 receptor for the glucose transporter, which contains multiple genomic sequences with a strong glucose homology. Many molecules used in the insulin signaling pathway are also expressed in zebrafish, including the insulin receptor a and b, and a tyrosine-kinase substrate.[27-29]

Body weight is the parameter of the diet induced obesity in zebrafish model. The normal body weight in zebrafish ranges from 0.45 mg – 0.64 mg, in obese state it is reported that it is increased in the body weight. In our study body weight significantly increased in the diet induced model. The body weight was found to decrease in the treated group upon treatment with the HDC. For 0.17 g/ml HDC treatment it was observed that body weight was decreased significantly, whereas similar significant reduction was observed in 0.35 g/ml HDC treatment and it was seen that 0.35 g/ml HDC showed the body weight values nearly similar to the normal / negative control group.

Triglyceride is the parameter of lipid profile estimation in which our study shows that there is decreased in the treated group. For 0.17 g/ml HDC treatment it was observed decreased significantly, whereas similar reduction was observed in the 0.35 g/ml HDC treatment and it was seen that 0.35 g/ml HDC showed the triglyceride values nearly similar to the negative / normal group.

Cholesterol is the parameter of lipid profile estimation in which our study shows that there is decreased in the treated group. For 0.17 g/ml HDC treatment it was observed decreased significantly, whereas similar reduction was observed in the 0.35 g/ml HDC treatment and it was seen that 0.35 g/ml HDC showed the cholesterol values nearly similar to the negative / normal group.

High density lipoprotein (HDL) is the parameter of lipid profile estimation in which our study shows that there is decreased in the treated group. For 0.17 g/ml HDC treatment it was observed decreased significantly, whereas similar reduction was observed in the 0.35 g/ml HDC treatment and it was seen that 0.35 g/ml HDC showed the HDL values nearly similar to the negative / normal group.

Low density lipoprotein (LDL) is the parameter of lipid profile estimation in which our study shows that there is decreased in the treated group. For 0.17 g/ml HDC treatment it was observed decreased significantly, whereas similar reduction was observed in the 0.35 g/ml HDC treatment and it was seen that 0.35 g/ml HDC showed the LDL values nearly similar to the negative / normal group.

Fasting blood glucose level (FBGL) is the amount of blood glucose present. The normal fasting blood glucose in zebrafish ranges from 65 mg/dL – 90 mg/dL, in diabetic state FBGL

is reported to be increased. In our study the FBGL was found to be significantly increased in the diabetes induced group of glucose induced hyperglycemia model. On treatment with the HDC the FBGL was found to be decreased in the treated group. For 0.17 g/ml HDC treatment it was observed that FBGL was decreased significantly, whereas similar significant reduction was observed in 0.35 g/ml HDC treatment and it was seen that 0.35 g/ml HDC showed the FBGL values nearly similar to the normal / negative control group.

From the histopathological result of diet induced obesity of zebrafish liver shows normal histopathology and no hepatic tissue present in the liver.

5. CONCLUSION

In the present study it shows that there is increase in the body weight, lipid profile estimation – TG, Cholesterol, HDL, LDL, Fasting blood glucose level and when the treatment is done with the HDC drug it is decreased.

The HDC drug shows the anti-obesity and antidiabetic activity. This data indicates that in-vivo treatment with the HDC drug anti-obesity and antidiabetic condition in the diet induced obesity and glucose induced hyperglycemia zebrafish model.

Zebrafish liver when treated with the diet induced obesity group there was no hepatic tissue and normal histopathology results is seen when its given for histopathological study.

By the above data it shows that increase in the body weight, lipid profile estimation – TG, Cholesterol, HDL, LDL, Fasting blood glucose level and when the treatment is done with the HDC drug it is decreased and show the significant ($p<0.001$) value. And the results are seen in the 0.17 g/ml and 0.35 g/ml both but proper decreasing in the 0.35 g/ml HDC treatment is seen.

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7. Conflict of Interest

There aren't any conflicts of interest related to this publication.

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