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Protective Effect of Achyranthes Aspera Against High Fat Diet And Streptozotocin Induced Diabetes In Rats.

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Introduction

Diabetes mellitus is one of the most prevalent conditions in the world and it is one of the top most cause of death in the United States of America, India, Europe and Asia. There are several complications associated with it like cardiovascular disease, retinopathy, nephropathy, wound infection and so on. More drugs and research data are being done to manage and treat the condition. (Dowarah and Singh, 2020). The management of diabetes mellitus depends on the type. Human diabetes mellitus type 2 can be divided into various stages. One of which includes late or early diabetes mellitus type 2. (Gheibi et al, 2017). The common forms of management options include oral medications, administration of insulin via injections or medications, emphasis on lifestyle factors like exercise and diet. The secondary complications of diabetes mellitus are also managed by appropriate methods of treatment. (Kaul et al, 2012). This is crucial because the constant exposure to a hyperglycaemic state leads to an increase in secondary complications which in turn is responsible for the majority of the mortality and morbidity. (Baig and Panchal, 2020) Some of the secondary issues that arise out of diabetes mellitus include wound healing impairment, its association with vascular conditions, inflammations, lipid abnormalities and so on. Understanding the importance of the complications that arise out of diabetes is of vital importance in terms of research and developing potential therapeutic targets. (Schmidt, 2016). Prevention of diabetes mellitus type 2 is very much necessary, factors like diet and exercise play a crucial role. By incorporating regular physical activities and diet one can prevent the condition. It has been established that almost 60% of individuals with the diabetic condition are obese in nature. Apart from these, regular screening is necessary for individuals who are at higher risk. A good example of it would be a family history of the same (Chatterjee et al, 2017). In India, the prevalence of diabetes mellitus particularly type 2 is predominant when compared to the western population. It has also been found that the complication of microvascular and macrovascular are high in Indian populations which could be due to genetic factors associated with ethnicity.(Unnikrishnan et al, 2016). Several plants in Ayurveda can be used to effectively manage obesity (Chandrasekaran et al, 2012).This means that it prevents the body from absorbing some of the complex carbohydrates to prevent the plasma glucose levels to increase. This can be coupled with other long term remedies like diet and physical activities or medications to get the maximum benefit out of it. It also has antioxidant and anti-obese properties. (Deepak et al, 2015). Achyranthes aspera is a herb that belongs to 7 the Amaranthaceae family. In tropical and subtropical regions, it is predominantly found. It is used to treat colds, asthma, piles and so on. It contains alkaloids, flavonoids, saponins, steroids and terpenoids(Saba Hasan, 2014). In Sanskrit, it is known as Aghata. It is widely spread in India and is also found in the United States of America and Australia. The seeds, stems and roots are predominantly used for medicinal purposes. Earlier, the decoction of Achyranthes aspera was used by mixing it with sugar or honey for diarrhoea and dysentery (Srivastav et al, 2011). The extracts of root contain phytochemicals such as Betaine and Achyranthine. (Srinivas et al, 2010). The active components are either found directly in Achyranthes aspera or can be obtained as secondary metabolites. (Alam et al, 2009). It has been used in Ayurveda for vomiting, piles, dysentery and blood disease (Dwivedi et al, 2011). Diabetes mellitus is associated with a number of secondary conditions like cardiovascular disorders. Hence there is an ardent need to manage, treat and prevent this condition effectively. Though there are ongoing management and treatment

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options, some of them have side effects. According to the World Health Organization, the public interest in using a traditional form of medicine as their primary form of health care is about 80%. However, Achyranthes aspera which is used in Ayurveda is present in abundance in nature and also has relatively few side effects. This indicates that more number of people are open to using traditional medicines like Achyranthes aspera to manage and treat diabetes mellitus type 2 effectively. Despite this, Achyranthes aspera is not well established and hence more number of studies are required in terms of assessing its dosage, safety and efficacy. The aim of the present study was to evaluate the protective role alcoholic extract of Achyranthes aspera on experimentally induced (streptozotocin-STZ) diabetic and high fat diet administered rat model.

Methodology

Plant material: The Achyranthes aspera root parts were collected from the S.K. University Campus, Ananthapuramu, Andhra Pradesh, India. The botanical identification and authentication were performed by Botanist Prof. B. Ravi Prasad Rao, Department of Botany, S.K.University and a specimen was deposited in the herbarium (Voucher No: 57403).

Extract preparation: The leaves of the plant were immersed in 93% ethanol (100 g/l) for one month. After this period, the filtration of the hydroalcoholic extract was done following which it was concentrated using a rotary evaporator and then lyophilized. Until further use, the storage of the dried material was done at 4 °C. The dilution of the dried extract was done in saline immediately prior to use. 4.2.3

Animals: Male albino wistar rats (n=36), weighing 150 to 200 g were used for the present antidiabetic study. The rats were placed in sterilized polypropylene cages and the temperature was maintained with a continuous 12 h light cycle and 12h dark cycle. The rats were provided with a standard rat diet and RO water ad libitum. All animal experiments were conducted after getting approval from the ethical committee and following the guidelines for the appropriate care, experimental induction and use of laboratory rats (Protocol No: IAEC/XIII/04/RIPER/2019).

Diabetes induction: The rats were fed with a high fat diet ad libitum for 28 days and then received a single dose of streptozotocin (40 mg/kg b. w., i. p.). After 7 days of streptozotocin injection, the fasting blood glucose levels of overnight-fasted rats were estimated and the animals which exhibit glucose levels of 170 ± 30 mg/dl are considered to be diabetic rats and to be included in further experiments. The rats are to be continued with HFD throughout the course of the study except for normal rats.

Experimental design: The animals were grouped into six groups (n=6) in each group. Group-I: Normal control rats were administered normal saline (0.5 ml/kg orally by oral gavages) daily. Group II: Diabetic rats induced with a single dose of streptozotocin (60 mg/kg b. w., i. p.). Group-III: Rats were administered with vanaspati + coconut oil (3:2) (0.5ml/kg.bwt) orally daily for 28 days to induce a high fat diet (HFD) and then diabetes induction was done. Group-IV: Diabetic rats were administered with HFD and co-treated with EAA (250 mg/kg b.w.) orally. Group-V: Diabetic rats were administered with HFD and co-treated with EAA(500 mg/kg b.w.) orally daily. Group-VI: Diabetic rats were administered with HFD and co-treated with metformin (250 mg/kg b.w.) orally daily. At the end of the experimental treatment days, the bodyweight of rats was measured using animal weighing balance.

Determination of serum biochemical parameters: After 24 hrs after the last dose, blood was collected from overnight fasted rats in each group by cardiac puncture for estimation of serum biochemical parameters. Blood was collected by puncturing the retro-orbital plexus after performing and serum was separated. Serum TG, TC, and HDL were estimated by semi-autoanalyser, (Erba Chem 7) using a diagnostic reagent kit. By using the formulae, the calculation of VLDL and LDL were done.

LDL –C(mg/dl) = A sample x conc. of calibrator (mg/dl) A calibrator

VLDL = Triglycerides (mg/dl)/5

Glycated haemoglobin was estimated by using a biochemical kit from Crest Biosystems, while serum insulin was measured by using an insulin ELISA kit for rats from Enzo diagnostics. The amount of serum insulin was quantified using an ELISA microplate reader (BIORAD) at 450 nm. Liver glycogen levels were estimated by an alkali method (Stetten and Katzen, 1961).The insulin resistance was measured by HOMA-IR by the following formula: HOMA-IR = (Fasting plasma glucose (mg/dl) x Fasting plasma insulin (µIU/ml) x (405)-1 4.2.7

Statistical analysis: The results were presented as Mean ± SEM for six animals in each group for each parameter. The statistical comparison was done by using SPSS software. A significant variation was observed, mean values were related using one-way ANOVA. A p-value less than 0.05 was meant statistically significant.

Results and Discussion

Initial body weight was normal in all the groups of rats (Figure 4.1). Thereafter, the difference increased throughout the study in high fat diet induced rats. The highest body weight value above 300 g was above in diabetes and diabetic+HFD rats whereas 175g was noted in normal rats. The body weight was ($p<0.001$)

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significantly lower in high doses of HEAA treatment in STZ+HFD rats. The weight reduction in HEAA (500mg) treated rats was similar to that of metformin-treated rats. The delayed bodyweight improvement was noted in HEAA (250mg) dose group.

From the graph (Figure 4.2), it can be seen that the glucose levels are the highest in diabetic rats fed with a high-fat diet while it was less high in diabetic rats compared with normal the control group. There are ($p<0.001$) significant differences in the levels of glucose between each group. The STZ and STZ+HFD groups glucose values above 200 mg/dl. The high dose of HAAE (500mg) treatment for diabetic and HFD rats showed ($p<0.001$) reduced levels of glucose values proving its anti-diabetic properties, the efficacy is similar to that of metformin treatment. The lower dose of HAAE (250mg) also decreased the glucose level from the diabetic range but the significance was less compared to the high dose of the test drug. The insulin and glycogen levels are decreased in high-fat diet rats induced diabetes followed by diabetic rats. The results were comparable to that of metformin, a standard drug. The effect of HEAA(250 mg/kg bw& 500 mg/kg bw respectively)in diabetic rats was evident from the results of insulin and glycogen values(Figure 4.3). Animals treated with the standard drug also show a significant reduction in their blood glucose and HbA1C level compared to Group II ($p<0.05$) and Group III ($p<0.001$). Administration of a high dose of HEAA caused significant ($p<0.05$) elevation in liver glycogen levels (Figure 4.4).The metformin also significantly decreased the HbA1C and glycogen levels in diabetic rats. The HEAA 500 mg/kg significantly ($P<0.001$) reduced serum levels of total cholesterol, triglyceride, very low-density lipoprotein-cholesterol, and lowdensity lipoprotein-cholesterol while significantly increasing the high-density lipoprotein-cholesterol in STZ alone induced diabetic rats fed with high-fat diet less reduction compared to metformin treated rats. Additionally, 250 mg/kg of the extract significantly ($P<0.01$) reduced serum lipid profile and lipoproteins level (Figure 4.5 and 4.6). Furthermore, the AST and the ALT activity were also analysed across the different groups. The AST and ALT activities was in normal range in control group and the value was significantly ($p<0.001$) high in STZ induced and HFD+STZ group. The ALT and AST activities was controlled to normal in Metformin treated group. The HFD+STZ+HEAA (250 mg) group rats less significantly reduced these enzymes than HFD+STZ+HEAA (500mg) treatment rats. (Figure 4.7). From the results, it can be seen that Achyranthes aspera has both antidiabetic and anti-obesity properties. Significant reductions in glucose and lipid content were found after the administration of Achyranthes aspera thereby proving its therapeutic properties. The anti-diabetic properties of Achyranthes aspera have been established for a long time while recent studies have also shown its anti-obesity properties. A similar study proved that there is a reduction in the glucose levels by reducing the elevated blood glucose levels exhibited its hyperglycaemic activities. (Talukder et al, 2011) Apart from all these, an increased fat diet has been shown to increase the risk of hyperlipidaemia. It has also been established that Achyranthes aspera has been used in Ayurveda to treat hyperlipidaemia thereby proving its anti-lipidemic properties.(Latha et al, 2011) On the other hand, another study by Evelyn Njideka et al indicated that when Achyranthes aspera was administered in the form of tea, it showed reductions in glucose levels and lipid levels. However, HDL and LDL levels were not altered by it though there were significant reductions in the triglycerides levels(Evelyn Njideka et al, 2019). Another in vivo study which used streptozotocin to induce diabetes mellitus to understand the effects of Achyranthes aspera in diabetes obtained the same results as mentioned above. (Kumar et al, 2011). Apart from all the above-mentioned roles of Achyranthes aspera, also has antioxidant, anti-inflammatory and hepatoprotective roles which were further proved in different studies (Khan et al, 2014; Kumar et al, 2009). There is also evidence suggesting its role in hyperlipidemic and hypercholesteremic conditions (Krishnakumari and Priya, 2006). Due to the multiple roles of the herbal plant-Achyranthes aspera, it has significant scope in the pharmacology field in terms of treatment for diabetes mellitus associated with clinical alteration of high fat diet and the complications which arise out of it. The strong hypoglycaemic effect and the anti-obesity effects were proved in this chapter. The present study results concluded that Achyranthes aspera is practically a safe herbal medicine that can be used in the management of diabetes mellitus associated with hyperlipidemia and further research is necessary regarding its use in humans.

Conclusion: Achyranthes aspera treated rats showed good results in terms of their anti-diabetic and antilipidemic properties by decreasing the oxidative damage, increasing the insulin protein expression in the pancreas. Achyranthes aspera was also compared with metformin standard to find its therapeutic efficiency and the results were promising. More research is required in Achyranthes aspera to formulate an efficient treatment for diabetes mellitus type 2 associated HFD complications.

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Conflict of Interest: The authors declare no conflict of interest.

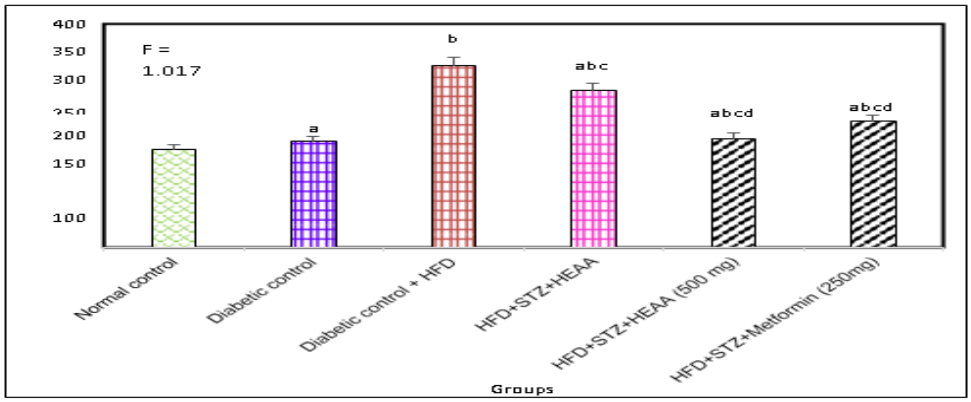

Figure 4.1: Effect of hydroalcoholic extract of Acyranthes aspera (HEAA), compared to metformin in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats on body weight.

Values are mean + SE (n=5)

The 'F' and 'P' values are one way ANOVA with Bonferroni 't' test.

[a]Significantly different from control group [b]Significantly different from STZ only group [c]Significantly different from HFD+STZ+ group

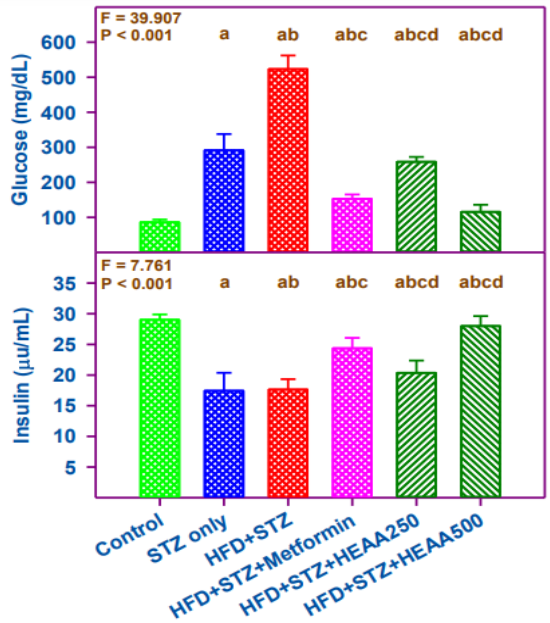

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Figure 4.2: Effect of hydroalcoholic extract of *Acyranthes aspera* (HEAA), compared to metformin in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats on glucose and insulin levels
Values are mean + SE (n=5)
The 'F' and 'P' values are one way ANOVA with Bonferroni 't' test.
[a]Significantly different from control group
[b]Significantly different from STZ only group
[c]Significantly different from HFD+STZ group
[d]Significantly different from HFD+STZ+Metformin group.

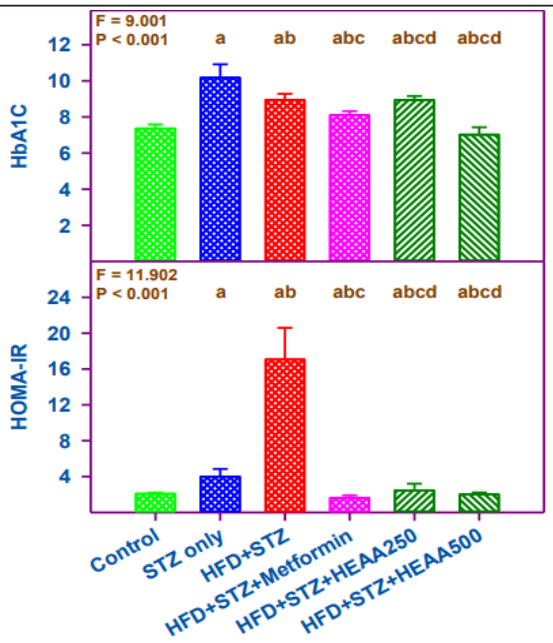

Figure 4.3: Effect of hydroalcoholic extract of *Achyranthes aspera* (HEAA), compared to metformin in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats on HbA1c and HOMA-IR
Values are mean + SE (n=5)
The 'F' and 'P' values are one way ANOVA with Bonferroni 't' test.
[a]Significantly different from control group
[b]Significantly different from STZ only group
[c]Significantly different from HFD+STZ group
[d]Significantly different from HFD+STZ+Metformin group.

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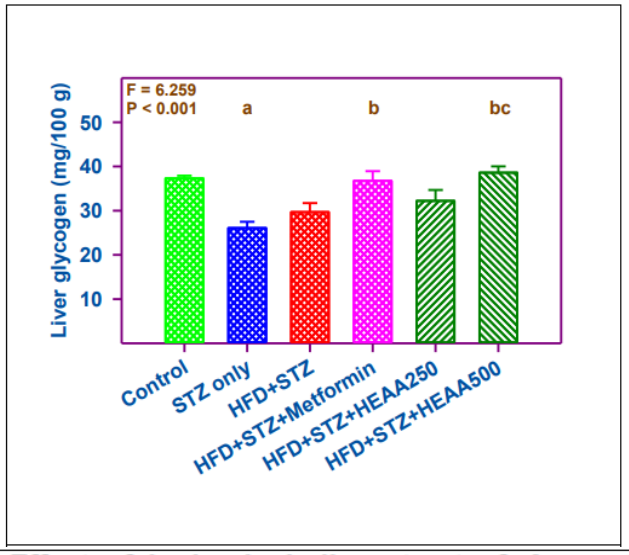

Figure 4.4: Effect of hydroalcoholic extract of *Acyranthes aspera* (HEAA), compared to metformin in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats on liver glycogen level.
Values are mean + SE (n=5)
The 'F' and 'P' values are one way ANOVA with Bonferroni 't' test.
[a]Significantly different from control group
[b]Significantly different from STZ only group
[b,c]Significantly different from HFD+STZ group

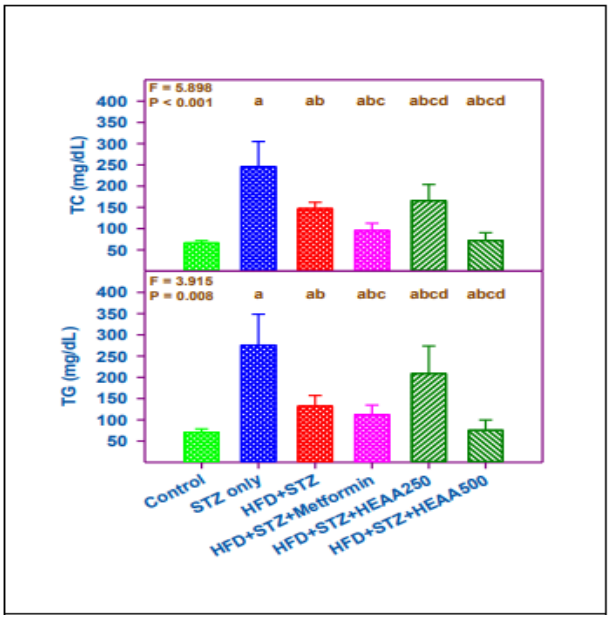

Figure 4.5: Effect of hydroalcoholic extract of *Achyranthes aspera* (HEAA), compared to metformin in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats on TC and TG
Values are mean + SE(n=5)
The 'F' and 'P' values are one way ANOVA with Bonferroni 't' test.
[a]Significantly different from control group
[b]Significantly different from STZ only group
[c]Significantly different from HFD+STZ group
[d]Significantly different from HFD+STZ+Metformin group.

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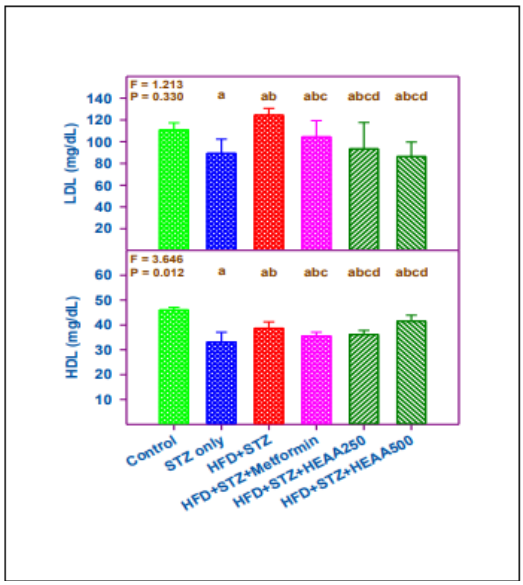

Figure 4.6: Effect of hydroalcoholic extract of *Achyranthesaspera* (HEAA), compared to metformin in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats on LDL and HDL

Values are mean + SE(n=5)

The 'F' and 'P' values are one way ANOVA with Bonferroni 't' test.

[a]Significantly different from control group

[b]Significantly different from STZ only group

[c]Significantly different from HFD+STZ group

[d]Significantly different from HFD+STZ+Metformin group.

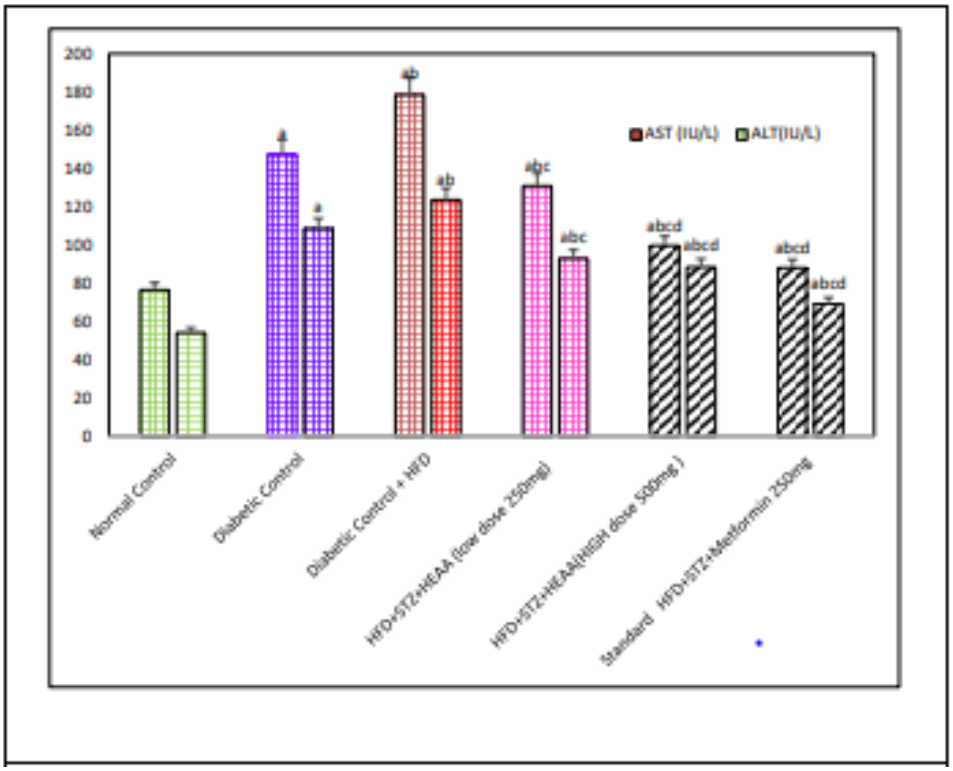

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Figure 4.7: Effect of hydroalcoholic extract of *Achyranthes aspera* (HEAA), compared to metformin in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats on ALT and AST enzyme activity.

Values are mean + SE(n=5)
The 'F' and 'P' values are one way ANOVA with Bonferroni 't' test.
[a]Significantly different from control group
[b]Significantly different from STZ only group
[c]Significantly different from HFD+STZ group
[d]Significantly different from HFD+STZ+Metformin group

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