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ANTIANXIETY EFFECT OF ETHYL PYRUVATE IN TYPE 2 DIABETIC RATS

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ABSTRACT

Diabetes is associated with several complications like neuropathy, retinopathy, nephropathy, hypertension etc. Apart from all these complications, psychological problems are also associated with diabetes. Patients with diabetes are at greater risk of developing anxiety and this association has impact on the quality of life of the patient. Anxiety itself is a major contributor in the worsening of glycemic control. Diabetic patients experience several stresses which may be due to diseased condition or treatment of disease and these stresses include fear of hypoglycaemia, regular insulin injections, changes in life style, poor glycemic control etc. Ethyl pyruvate (EP) is a lipophilic ester derivative of pyruvate. Pyruvate plays a central role in intermediary metabolism and is the final product of glycolysis and the starting substrate for the tricarboxylic acid (TCA) cycle. Ethyl pyruvate inhibits IDO enzyme (indoleamine 2–3-dioxygenase), an enzyme that induces the catabolism of tryptophan into TRYCATs (tryptophan catabolites along the IDO pathway), such as kynurenin which is a key mediator in the degradation of serotonin. Ethyl pyruvate is a known antioxidant and also known to scavenge the free radicles. **Objective:** To study the antianxiety effect of the Ethyl pyruvate in type 2 diabetic rats. **Materials and methods:** Rats were randomly divided into six groups of eight rats each. Type 2 diabetes was induced with the help of high fat diet followed by administration of low dose of the streptozotocin (35 mg/kg) injection. Type 2 diabetes model was validated by estimating the plasma glucose, Plasma triglycerides, plasma cholesterol levels and lipid profile which were estimated with the help of kits. Body weight of rats was measured at 0, 14, 21 and 42 day of the study. Various activity models like elevated plus maze model, light and dark box and open field model were used in the different groups. Drug treatments with ethyl pyruvate through (i.p.) (10 mg/kg, 50 mg/kg and 100 mg/kg) and combination of ethyl pyruvate (50 mg/kg) with the metformin (100 mg/kg) were given for 21 days. Thiobarbituric acid reactive substances (TBARS) and glutathione were estimated in the brain of the normal, type 2 diabetic and drug treated rats at the end of the study. Brain serotonin levels were estimated in the normal, type 2 diabetic and drug treated rats. **Results:** Ethyl pyruvate (10mg/kg) did not show much significant effect in diabetes induced anxiety. Treatment with Ethyl pyruvate has no significant effect on the body weight of rats. Significant decrease in the glucose, triglycerides, cholesterol and lipid profile of rats was found after the administration of Ethyl pyruvate. Anxiety behaviour and oxidative stress parameters were improved after the treatment with the Ethyl pyruvate. Levels of serotonin was also increased after the treatment with Ethyl pyruvate. **Conclusion:** Our current preclinical showed that Ethyl pyruvate and its combination with metformin is effective in type 2 diabetes induced anxiety in rat model by improving the glucose levels, cholesterol levels, lipid profile, increasing anti-oxidant enzyme levels and brain serotonin levels. Ethyl pyruvate (10 mg/kg) was not much effective. Further studies are encouraged to identify the mechanisms responsible for anti-anxiety activity.

KEYWORDS: Type 2 diabetes, Anxiety, Serotonin, TBARS.

INTRODUCTION

Diabetes is defined as a metabolic disorder, characterised by hyperglycemia (Souto and Miranda, 2011) and alterations in the metabolism of proteins, fat, carbohydrates (Bastaki, 2005). Changes in the life style, physical inactivity, unhealthy diets, smoking, alcohal intake etc are some of the major risk factors for the diabetes (Kaku, 2010).

Diabetes is associated with several complications like neuropathy, retinopathy, nephropathy, hypertension etc (Americian Diabetes Association, 2014). Apart from all these complications, psychological problems are also associated with diabetes (Johansen *et al.,* 2014). Psychological problems associated with diabetes are depression (Nouwen *et al.,* 2010, Dooren *et al.,* 2013, Bajaj *et al.,* 2012), anxiety (Edwards and Mezuk, 2012, Wu *et al.,* 2012, Kaur *et al.,* 2013), memory impairment (Vijayakumar *et al.,*2012, Bruehl *et al.,* 2009) etc.

Anxiety is one of the major psychological problems associated with the diabetes (Smith *et al*., 2012). Patients with diabetes are at greater risk of developing anxiety (Kendzor *et* al., 2014, Smith *et al*., 2012) and this association has impact on the quality of life of the patient (Kaur *et al.,* 2013). Anxiety itself is a major contributor in the worsening of glycemic control (Grigsby *et al*., 2002, Adl *et al.,* 2013).

Diabetic patients experience several stresses which may be due to diseased condition or treatment of disease and these stresses include fear of hypoglycaemia, regular insulin injections, changes in life style, poor glycemic control etc (Zambanini *et al.,* 1999, Green *et al.,* 2000, Shiu and Wong, 2002).

Pathophysiology of diabetic anxiety is complex and includes several biochemical changes such as hyperactivity of HPA axis (Edwards and Mezuk 2012, Zarate *et al.,* 2012), changes in the neurotransmitter level like serotonin (Sandrini *et al.,* 1997, Miyata *et al.,* 2007, Abraham *et al.,* 2010), catecholamines (Meyer *et al.,* 2006, Goddard *et al.,* 2010).

Diabetic complications and decreased synthesis and metabolism of serotonin (5-hydroxy tryptamine, 5-HT) are considered to affect the behavioural and cognitive dysfunctions in patients with Diabetes (Rajashree *et al*., 2011). The changes in brain 5-HT synthesis rate in diabetic rats are related to the various behavioural and psychological changes (Abraham *et al*., 2010).

During diabetes, persistent hyperglycaemia leads to increased production of reactive oxygen species (ROS) (Rousselot et al, 2000). There are some pathways of oxidative stress in DM including enzymatic, non-enzymatic and mitochondrial pathways. The most dominant factor among all these is glucose auto-oxidation that results in the development of free radicals (Zatalia and Sanusi, 2013).

IDO is the rate-limiting enzyme in the L-TRP–kynurenine pathway. Overstimulation of IDO leads to depletion of plasma concentrations of TRP which leads to reduced synthesis of 5-HT in the brain. Several metabolites are formed in the kynurenine pathway which is shown to have neurotoxic effects. Thus increased production of 3-OH-KYN and QUIN playing a role in neuronal damage and which can then cause cognitive decline infections of the central nervous system, ischemia and epilepsy. In addition, they may play a role in the development of psychiatric diseases such as anxiety, depression and schizophrenia (Wichers and Maes, 2003).

Ethyl pyruvate (EP) is a lipophilic ester derivative of pyruvate. Pyruvate plays a central role in intermediary metabolism and is the final product of glycolysis and the starting substrate for the tricarboxylic acid (TCA) cycle (Fink 2007). It inhibits IDO enzyme (indoleamine 2–3-dioxygenase), an enzyme that induces the catabolism of tryptophan into TRYCATs (tryptophan catabolites along the IDO pathway), such as kynurenin which is a key mediator in the degradation of serotonin (Maes *et al*., 2008).

Metformin is used as the first line treatment to treat the type 2 diabetes (Tendon 2007). Metformin comes under the category of bigunides which act as insulin sensitizers (Kittappa 2012). Metformin helps in improving the diabetes by reducing the hepatic gluconeogenesis (Tendon 2007).

MATERIALS AND METHODS

Animals

Spargue- Dawley rats, weighing 200-250 gm were employed in the present study (procured Panecea Biotech, Lalru). They were maintained on the standard laboratory diet and tap water. They were housed in the animal house of Rayat and Bahra Institute of pharmacy (RBIP), Sahauran and were exposed to natural cycle of light and dark under standard laboratory conditions The experiments were conducted in a semi sound proof laboratory. The experimental protocol of the study was duly approved by the Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (RBIP/IAEC/CPCSEA/2014/ PROTOCOL NO 21).

Chemicals and drugs

All the drug solutions were freshly prepared before use. Ethyl pyruvate and Metformin were purchased from Sigma- Aldrich, Chandigarh. Glucose estimation kit, Total cholesterol estimation kit, HDL estimation kit were purchased from accurex Biomedical Pvt. Ltd. Mumbai. Unless stated, all other chemicals and biochemical reagent of highest analytical grade were used for the study.

Treatment Schedule

All the animals were acclimatized to laboratory environment for atleast 3 days before testing.

To evaluate the effect of drug treatment on the development of anxiety in rats, treatment was started after validation of anxiety in the diabetic rats and continued for 3 weeks.

Induction and assessment of diabetes in rats

Induction of type 2 DM in rats was as per the method of Srinivasan *et al.*, 2005. The rats were allocated dietary regimen by feeding HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) for the initial period of 2 weeks. After the 2 weeks of dietary manipulations, rats were injected intraperitoneally (i.p.) with low dose of STZ (35 mg /kg), while the respective control rats were be given saline solution in a dose

volume of 1ml/ kg, i.p, respectively. High fat diet was continued till the end of study. Only the rats with blood glucose concentration more than 250 mg/dl after STZ injection were considered as diabetic and were taken for further study.

Experimental protocol
The experimental protocol used was as follows:

Group I (Normal group): Animals of this group were not subjected to any treatment. They were kept for 21 days. Body weight, blood glucose and lipid profile levels were assessed on different time intervals, i.e., 0, 14, 21 and 42 days. Behavioral tests were assessed on different time intervals, i.e., 0, 14, 21 and 42 days. All the animals were sacrificed on 42nd day of study and subjected to biochemical estimations of thio-barbituric acid reactive substances (TBARS), reduced glutathione (GSH) and estimation of serotonin.

Group II (Diabetic group): Animals in this group were served as negative control and were administered with High fat diet (2 weeks) followed by single dose of Streptozotocin (35mg/kg, i.p.). HFD was continued until the end of the study. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Group III (Diabetic + Ethyl pyruvate treated): Animals of this group were treated with administration of Ethyl pyruvate (10 mg/kg, i.p.) for consecutive 21days. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Group IV (Diabetic + Ethyl pyruvate treated): Animals of this group were treated with administration of Ethyl pyruvate (50 mg/kg, i.p.) for consecutive 21days. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Group V (Diabetic + Ethyl pyruvate treated): Animals of this group were treated with administration of Ethyl pyruvate (100 mg/kg, i.p.) for consecutive 21days. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Group VI (Diabetic + Ethyl pyruvate and metformin treated): Animals of this group were treated with administration of Ethyl pyruvate (50 mg/kg, i.p.) and metformin (100 mg/kg, p.o.) for consecutive 21days. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Measurement of the Animal Body Weight
In the present study, body weight of rats was measured at 0, 14, 21 and 42 day of the study.

Collection of Blood
Blood from the rats was withdrawn by retro orbital plexus technique. The collected blood was placed in eppendroff tubes. In the present study blood was collected on 0, 14, 21 and 42 day of the study.

Plama Separetion
A sample of 1.5 ml blood was centrifuged at 3000 rpm for 10 minutes and supernatant was carefully taken into a polypropylene tube and stored at 2-8^{0} C for measurement of glucose and lipid profile.

Estimation of the Glucose Level and Lipid Profile of Rats
Glucose level and lipid profile of rats was measured on the 0, 14, 21 and 42 day of the study with the help of commercial available kits.

Behavioural Studies
Behavioural tests were performed on the 0th, 14th, 21st and 42nd day of the study.

Elevated plus maze
Elevated plus maze model was as per the method of Pellow *et al.*, 1985. During this 5 min experiment, the behaviour of the rats was recorded as the following parameters:
(a) Number of entries into the open arms
(b) Number of entries into the close arms
(c)Time spent by animal in open arms

Open-field test
Open field test was as per the method of Kennett *et al.*, 1985**.** During 5 min period following parameters were recorded:
(a) The number of rows of crossed squares
(b) Number of grooming
A grooming episode will be defined by repetitive movements of front paws or mouth.

Light and dark box model
Light and dark box model was as per the method of Costall *et al.*, 1988. Following parameters will be recorded:
(a) Number of entries into the bright arena
(b) Time spend in the bright arena

Assessment of Lipid Peroxidation by Thiobarbituric Acid Reactive Substances (TBARS) Assay and Glutathione (GSH)
The tissue lipid peroxidation reaction was assessed by estimating thiobarbituric acid reactive substances (TBARS) by the method of Wills *et al.*1966. The reduced glutathione (GSH) level was assessed by the method of Ellman, 1959.

Estimation of Serotonin
Estimation of neurotransmitter serotonin was as per the method of Schlumpf *et al*., 1974.

Statistical Analysis
The observations were statistically analyzed with the help of InStat3. All of the results were expressed as mean

± standard error of the mean (S.E.M). All the behavioral parameters were statistically analyzed with the help of Two-way ANOVA followed by Tukey's multiple comparison tests whereas biochemical parameters were statistically analyzed by One-way ANOVA followed by Dunnet's multiple comparison test. The $p<0.05$ was considered to be statically significant.

RESULTS

Effect of ethyl pyruvate on body weight of rats

Type 2 diabetes caused significant increase in the body weight of rats. After the administration of EP (10 mg/kg, 50 mg/kg and 100 mg/kg) and combination of ethyl pyruvate (50 mg/kg) with the metformin (100mg/kg) no significant change was found in the body weight of rats.

Values are expressed as Mean ± SEM, n= 8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. a as compared with normal group, b as compared with diabetic group.

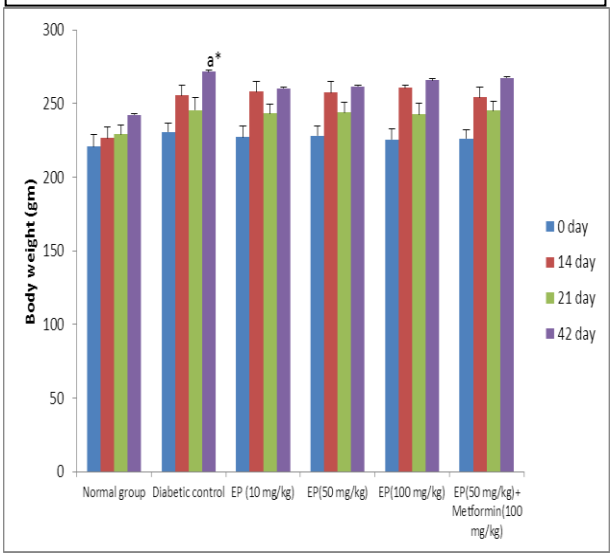

Fig. 1: Effect of Ethyl pyruvate on Body weight of rats.

Effect of Ethyl pyruvate on plasma glucose levels

Glucose levels were also significantly increased ($p< 0.001$) in the rats after HFD/STZ administration as compared to the NPD fed rats. Treatment with the Ethyl pyruvate (10 mg/kg, 50 mg/kg and 100 mg/kg) and combination of ethyl pyruvate (50 mg/kg) with the metformin (100mg/kg) decreased ($p< 0.05$, $p< 0.05$, $p< 0.01$ and $p< 0.01$ respectively) the glucose levels of the type 2 diabetic rats.

Values are expressed as Mean ± SEM, n= 8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. a as compared with normal group, b as compared with diabetic group.

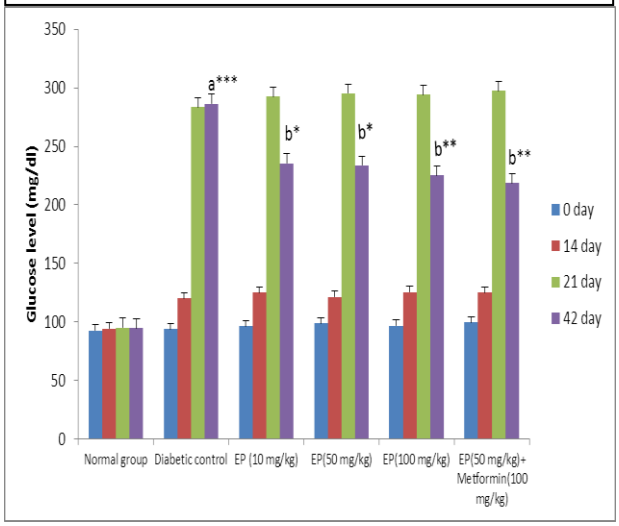

Fig. 2: Effect of Ethyl pyruvate on Glucose levels of rats.

Effect of Ethyl pyruvate on total cholesterol levels of rats

Combination of HFD/STZ showed significant increase in the total cholesterol level as compared to the NPD fed rats. Administration of ethyl pyruvate (10 mg/kg) did not show any significant change in the total cholesterol levels. On the other hand administration of Ethyl pyruvate (50 mg/kg and 100mg/kg) and its combination with metformin decreased cholesterol levels in type 2 diabetic rats ($p<0.05$, $p<0.01$ and $p<0.01$ respectively).

Values are expressed as Mean ± SEM, n= 8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. a as compared with normal group, b as compared with diabetic group.

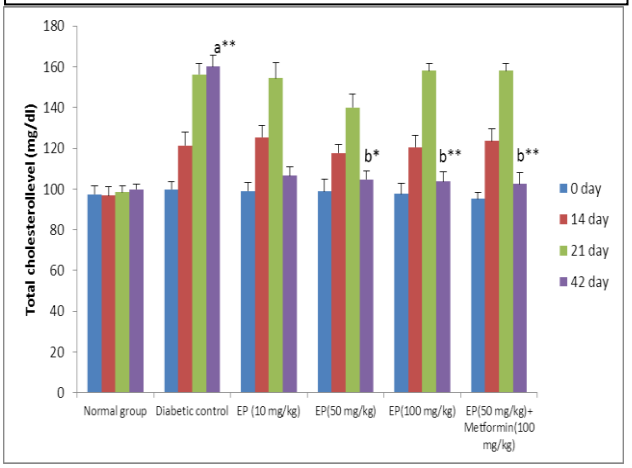

Fig. 3: Effect of Ethyl pyruvate on Total cholesterol levels of rats.

Effect of Ethyl pyruvate on triglycerides levels and lipid profile of rats

Combination of HFD/STZ showed abnormalities in the lipid metabolism as shown by significant increase in the levels of triglycerides, LDL and VLDL and decrease in the levels of HDL as compared to the NPD fed rats. Administration of Ethyl pyruvate (10 mg/kg, 50 mg/kg and 100 mg/kg) and its combination with the metformin (100 mg/kg) decreased (P<0.05, P<0.01, P<0.01 and P<0.01 respectively) the triglycerides and LDL levels (p<0.05, p<0.01, p<0.01 and p<0.01 respectively). No significant change was found after the administration of ethyl pyruvate (10 mg/kg) on VLDL levels. While Ethyl pyruvate (50 mg/kg and 100 mg/kg) and its combination with Metformin (100 mg/kg) showed significant decrease (p<0.05, p<0.05and p<0.05 respectively) in the VLDL levels as compared to the diabetic rats. Ethyl pyruvate (10 mg/kg) did not show significant change in the HDL levels. On the other hand administration of Ethyl pyruvate (50 mg/kg and 100mg/kg) and its combination with the metformin (100 mg/kg) showed significant increase (p<0.05, p<0.05 and p<0.01 respectively) in the levels of HDL as compared to the type 2 diabetic rats.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as

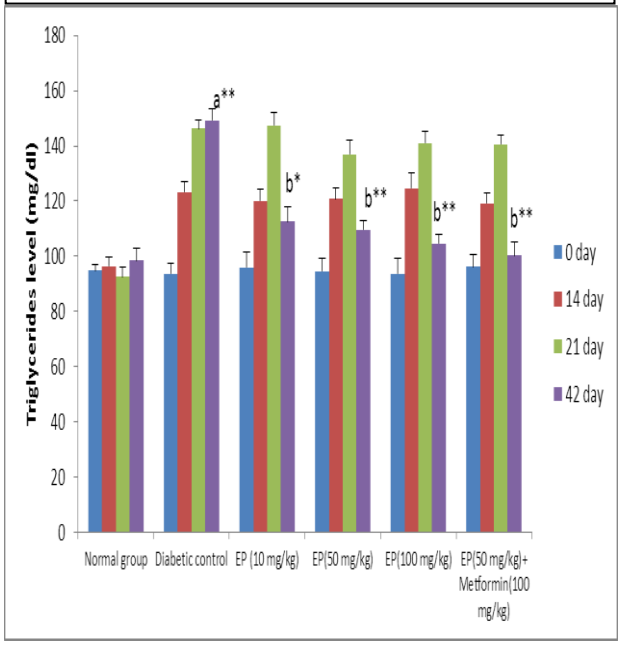

Fig. 4: Effect of Ethyl pyruvate on triglycerides levels of rats.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

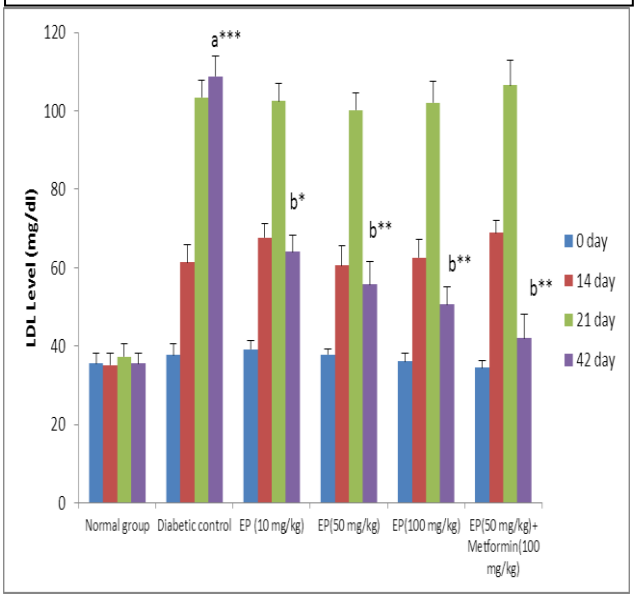

Fig. 5: Effect of Ethyl pyruvate on LDL levels of rats.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

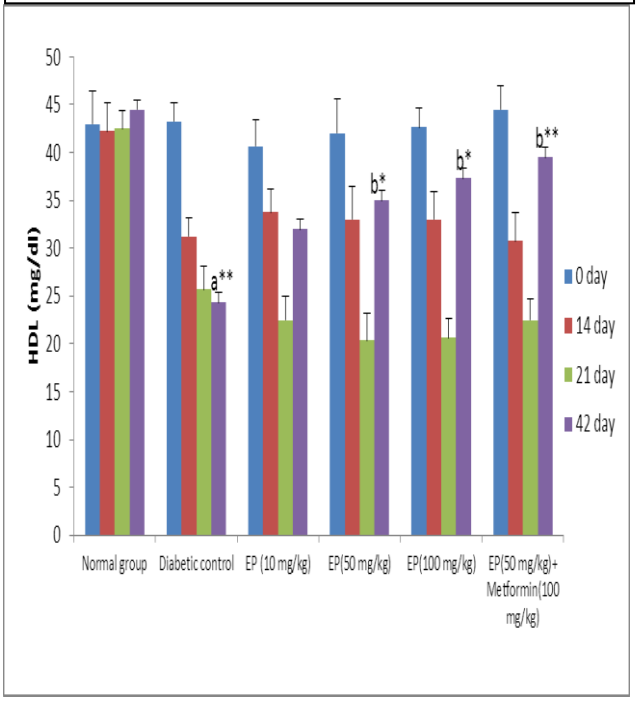

Fig. 6: Effect of Ethyl pyruvate on HDL levels of rats.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

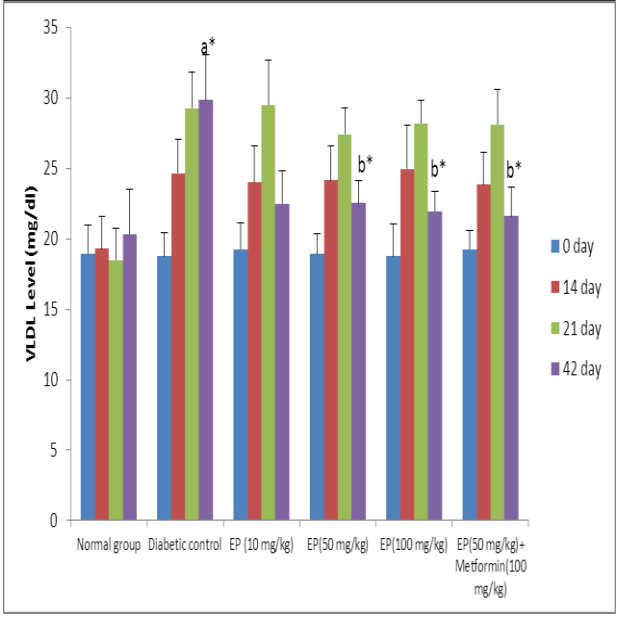

Fig. 7: Effect of Ethyl pyruvate on VLDL levels of rats.

Effect of Ethyl pyruvate on behavioral parameters

1 Elevated plus maze model

In the elevated plus maze model, rats with type 2 diabetes showed decrease in the number of entries to open arms and time spend in open arms as compared to the normal rats. Significant increase in the number of entries to the close arms was shown by type 2 diabetic rats as compared to the normal rats. Treatment with Ethyl pyruvte (10 mg/kg) did not show any significant change in the number of entries to open arms and time spend in the open arms. On the other hand Ethyl pyruvate (50 mg/kg and 100 mg/kg) and combination of ethyl pyruvate (50 mg/kg) with the metformin (100mg/kg) increased the number of entries to open arms and time spend in the open arms. Treatment with the Ethyl pyruvate (10 mg/kg, 50 mg/kg and 100 mg/kg) and combination of ethyl pyruvate (50 mg/kg) with the metformin (100mg/kg) also showed decrease in the number of the close arms of the type 2 diabetic rats.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

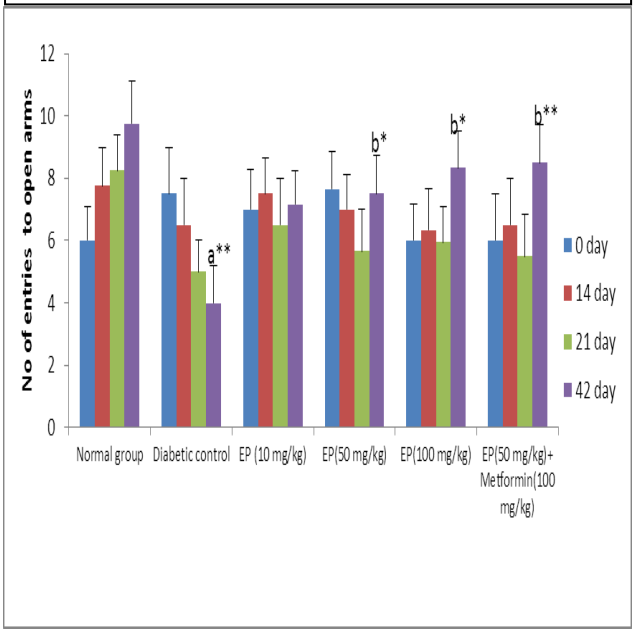

Fig. 8: Effect of Ethyl pyruvate on Number of entries to open arm.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

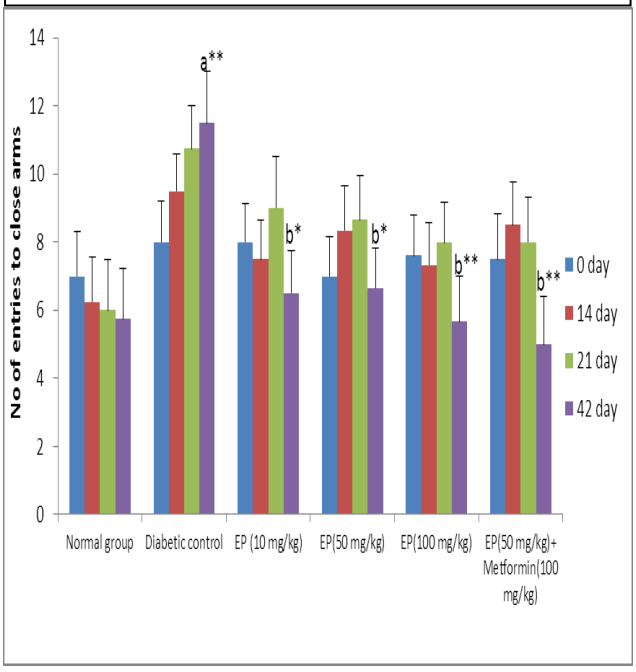

Fig. 9: Effect of Ethyl pyruvate on Number of entries to close arm.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

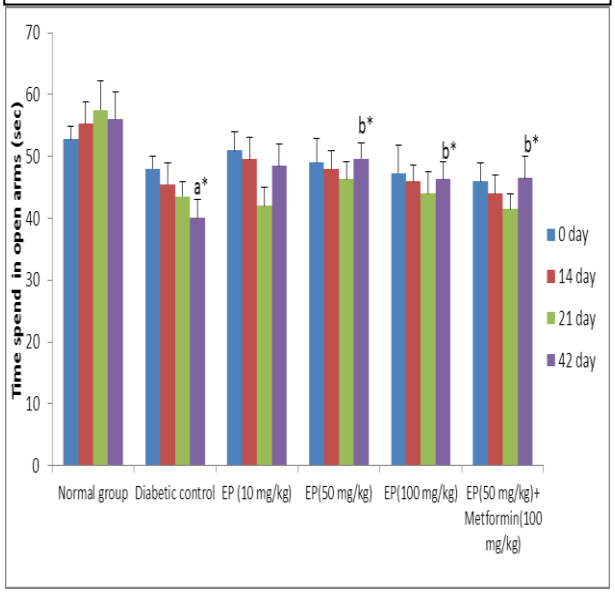

Fig. 10: Effect of Ethyl pyruvate on time spends in open arms.

2. Light and dark box model

In the light and dark box model, decrease in the time spend in the light area in the diabetic rats was found as compared to the normal rats. No significant change was found after the administration of Ethyl pyruvte (10mg/kg) in time spend in light arena. On the other hand significant increase in the time spend in the light arena was found after the administration of Ethyl pyruvate (50 mg/kg, and 100 mg/kg) and its combination with Metformin (100 mg/kg) in time spend in light arena. Rats with type 2 diabetes showed decrease in the number of crossings to light arena as compared to the normal rats. No significant change was found after the administration of Ethyl pyruvte (10mg/kg) did not show significant effect on number of crossings. On the other hand administration of Ethyl pyruvate (50 mg/kg, and 100 mg/kg) and its combination with Metformin (100 mg/kg) showed a significant increase in the time spend in the light arena.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

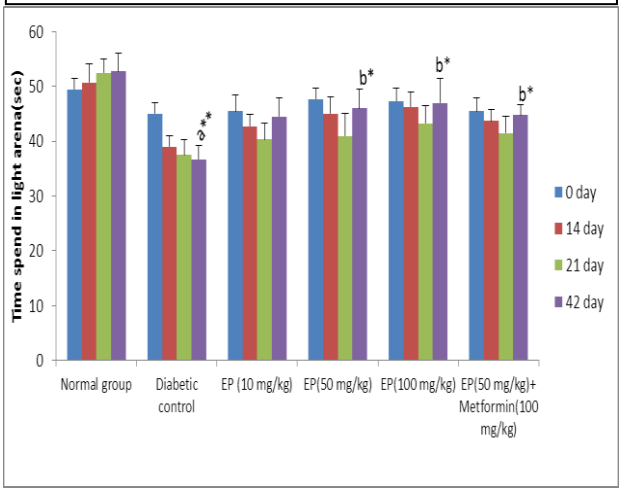

Fig. 11: Effect of Ethyl pyruvate on time spend in light arena.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

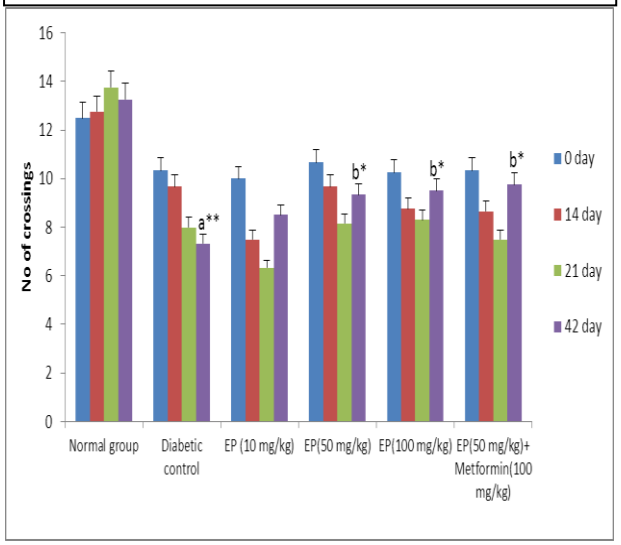

Fig. 12: Effect of Ethyl pyruvate on number of crossings.

3. Open field model

Rats with type 2 diabetes showed a significant decrease in the number of the square crossed and number of grooming as compared to the normal rats. Administration of Ethyl pyruvte (10mg/kg) did not show significant change in the number of grooming. On the other hand after the treatment with the Ethyl pyruvate (50 mg/kg and 100 mg/kg) and combination of ethyl pyruvate (50

mg/kg) with the metformin (100mg/kg) significant increase in the number of the square crossed and number of grooming was found.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

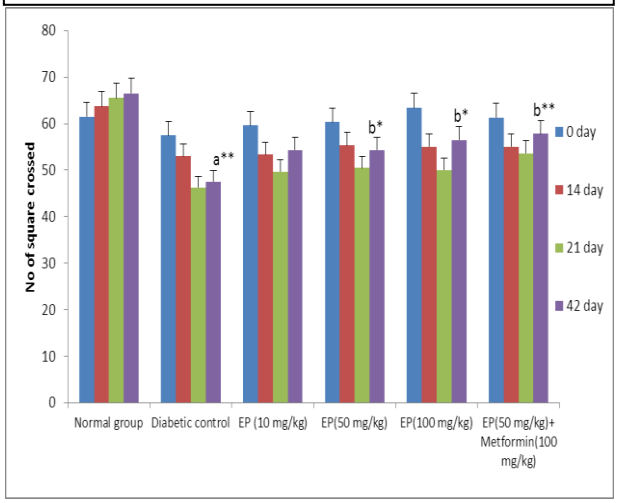

Fig. 13: Effect of Ethyl pyruvate on number of square crossed.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.001. a as compared with normal group, b as compared with diabetic group.

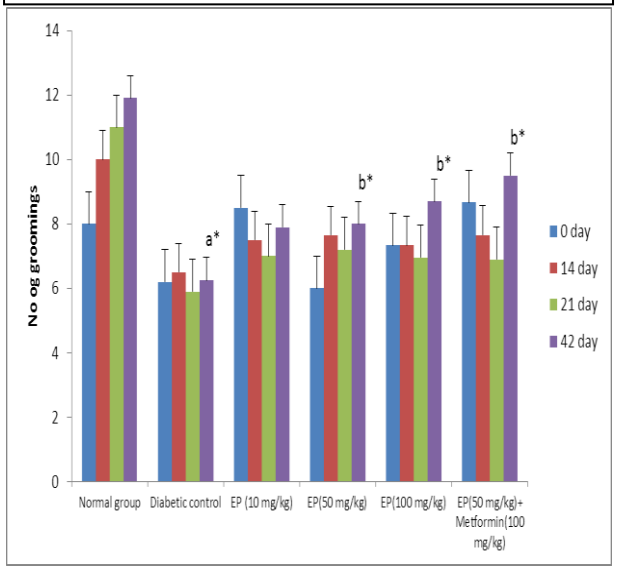

Fig. 14: Effect of Ethyl pyruvate on number of groomings.

Effect of Ethyl pyruvate on lipid peroxidation

Combination of HFD/STZ showed a significant increase in the malondialdehyde levels as compared to the NPD fed rats. Administration of Ethyl pyruvate (10 mg/kg) did not produced the significant difference in the TBABS levels while the administration of Ethyl pyruvate (50 mg/kg and 100mg/kg) and its combination with the metformin (100 mg/kg) also showed a significant decrease in the TBARS levels as compared to the type 2 diabetic rats.

Values are expressed as Mean ± SEM, n= 8. *p<0.05, **p<0.01, ***p<0.01. a as compared with normal group, b as compared with diabetic group.

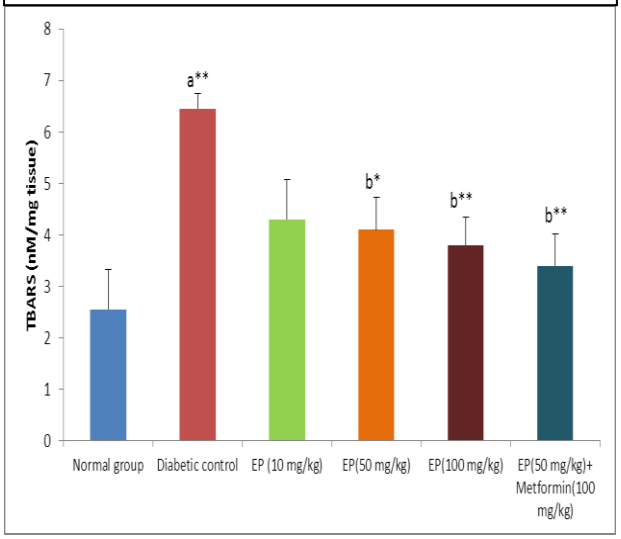

Fig. 15: Effect of Ethyl pyruvate on lipid peroxidation.

Effect of Ethyl pyruvate on glutathione levels

The level of glutathione was found to be decreased in the diabetic rats as compared to the normal rats. Administration of Ethyl pyruvate (10 mg/kg, 50 mg/kg and 100mg/kg) and its combination with the metformin (100 mg/kg) caused a significant increase in the glutathione levels as compared to the diabetic animals.

Values are expressed as Mean ± SEM, n= 8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. a as compared with normal group, b as compared with diabetic group.

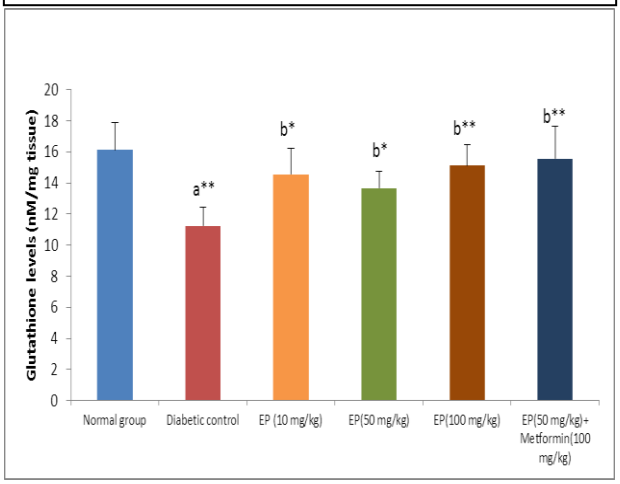

Fig. 16 Effect of Ethyl pyruvate on glutathione levels

Effect of Ethyl pyruvate on brain serotonin levels of rats

We found a significant decrease in the brain serotonin levels in the diabetic rats as compared to the normal rats. Lower dose of Ethyl pyruvate (10 mg/kg) did not show any significant change in the serotonin levels. On the other hand Administration of Ethyl pyruvate (50 mg/kg and 100mg/kg) increased the serotonin levels as compared to the diabetic rats. Combination of ethyl pyruvate (50 mg/kg) with metformin (100 mg/kg) was also effective in increasing the serotonin levels.

Values are expressed as Mean ± SEM, n= 8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.01$. a as compared with normal group, b as compared with diabetic group.

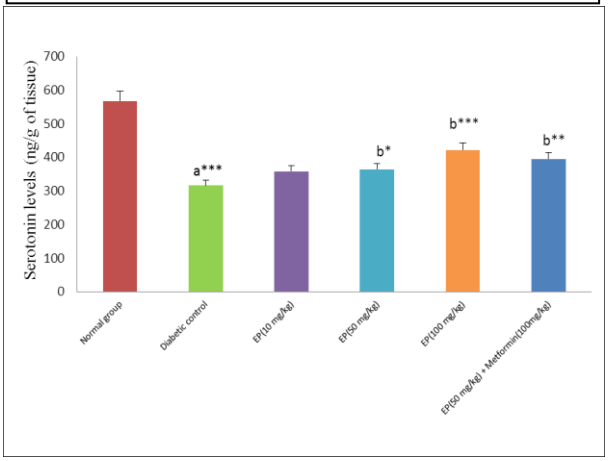

Fig. 17 Effect of Ethyl pyruvate on brain serotonin levels of rats.

DISCUSSION

Diabetic people are more vulnerable to anxiety as compared to the normal population (Mikaliukstiene *et al.*, 2014, Ganasegeran *et al.*, 2014, Smith *et al.*, 2012). Co morbidity of anxiety in diabetic population also represent the most common health problems which have impact on quality of life, health outcomes, health care expenditures etc (Masmoudi *et al.*, 2013). Anxiety itself is a major contributor in the worsening of glycemic control (Grigsby *et al.*, 2002, Adl *et al.*, 2013). In diabetes induced anxiety several biochemical and behavioral changes are involved like alteration in HPA axis (Edwards and Mezuk 2012, Zarate *et al.*, 2012), neurotransmitter (Miyata *et al.*, 2007, Abraham *et al.*, 2010, Goddard *et al.*, 2010), obesity, oxidative stress etc. Currently available antianxiety drugs have several side effects which limits their use for type 2 diabetes.

HFD/STZ model was selected for inducing type 2 diabetes in rats. This model mimics the metabolic features as well as the natural history of the human type 2 diabetes. Apart from that this model is less expensive as compared to the other available models for developing type 2 diabetes and this model is more useful for testing various compounds for the treatment of type 2 diabetes (Srinivasan *et al.*, 2005).

In the present study, rats that were on HFD showed a significant increase in the body weight, glucose levels, triglycerides and total cholesterol as compared to the NPD fed control group. After administration of the STZ, HFD fed rats showed increase in the glucose, triglycerides and total cholesterol. HFD also caused insulin resistance syndrome by various mechanisms but mainly through Randle or glucose fatty acid cycle. Increase in the triglyceride levels due to consumption of HFD could also lead to increase in the fatty acid availability and oxidation.

Combination of the HFD/STZ produced a significant increase in the glucose levels in rats as compared to the NPD fed rats. STZ (35 mg/kg) produced high degree of glycemic difference in the HFD fed rats. Results were comparable with the previous studies (Srinivasan *et al.*, 2005). Reason for this may be that high fat diet already caused insulin resistance and compensatory hyperinsulinemia (so as to maintain glucose homeostasis). Thus low dose of STZ could compromise the beta cell function and thus leading to the hyperglycemic effect. As HFD caused insulin resistance in the rats, rats that were on HFD were already hyperglycemic and further increasing the susceptibility of diabetogenic effect of low dose of STZ (Luo *et al.*, 1998). Administration of Ethyl pyruvate (10 mg/kg, 50 mg/kg and 100mg/kg) decreased glucose levels in type 2 diabetic rats. Combination of Ethyl pyruvate with the metformin also showed a significant decrease in the glucose levels.

Combination of HFD/STZ showed abnormalities in the lipid metabolism as shown by significant increase in the levels of triglycerides, total cholesterol, LDL and VLDL and decrease in the levels of HDL as compared to the NPD fed rats. As there is increased absorption and formation of triglycerides due to consumption of diet rich in the fat, combination of HFD/STZ caused hypertriglyceridemia. HFD caused increase in the production of TG enriched hepatic VLDL and decrease in the TG uptake in peripheral tissues. Hypercholestesteromia may be attributed to increased dietary cholesterol absorption from small intestine following the intake of HFD in diabetic condition (Colca *et al.*, 1991).

Elevated plus maze is widely used behavior model to evaluate the anxiety (Walf and Frye, 2007, Pinherio *et al.*, 2007). In this model animal is exposed to its central area and number of entries to open and close arms and time spend in open arm is assessed which is indicative of its anxiety behavior. In the present study diabetic rats showed anxiety behaviour indicated by increase in the number of entries to close arms and decrease in the number of entries and time spend in the open arms. Ethyl pyruvate attenuated these behaviours of anxiety.

In light and dark box model in the diabetic rats decrease in the number of crossings to light arena and time spend in light arena was found. Ethyl pyruvate showed increase in the number of crossings and time spend in the light arena. In open field model decrease in the number of square crossed and number of grooming was found. But ethyl pyruvate attenuated these behaviour of animals.

Oxidative stress plays a major role in the development and progression of type 2 diabetes and its complications. Protective role of antioxidants has been proved in the type 2 diabetes in several studies. Several pathways including enzymatic, non-enzymatic and mitochondrial pathways are responsible for increase in oxidative stress in diabetic condition (Johansen *et al.*, 2005).

Serotonin (5-hydroxytryptamine) is an ubiquitary monoamine acting as one of the neurotransmitters at synapses of nerve cells (Murphy *et al.*, 1998). Serotonin is a major neurotransmitter involved in psychological problems including depression and anxiety (Curran and Chalasani, 2012). Diabetes mellitus causes decrease in the serotonin levels which further leads to psychological problems (Miyata *et al.*, 2007, Rajashree *et al.*, 2011). Decrease in serotonin levels was also found in the diabetic rats in previous studies (Sandrini *et al.*, 1997, Miyata *et al.*, 2007, Abraham *et al.*, 2010). In the present study type 2 diabetic rats showed a significant decrease in the brain serotonin levels as compared to the normal group. Administration of Lower dose of Ethyl pyruvate (10 mg/kg) did not produce the increase in the serotonin levels. But Administration of Ethyl pyruvate (50 mg/kg, 100 mg/kg) caused increase in the serotonin levels as compared to the diabetic rats.

CONCLUSION

All of the above findings have suggested that Ethyl pyruvate can be used to prevent the progression of diabetes induced anxiety. Ethyl pyruvate (10 mg/kg) did not show much significant effect in type 2 diabetes induced anxiety in rats. Ethyl pyruvate showed improvements in the glucose level and lipid profile of rats. Treatment with Ethyl pyruvate showed improvements in the anxiety behaviour of rats. Ethyl pyruvate is a known antioxidant and also known to scavenge the free radicles. So it is speculated that antioxidant property of Ethyl pyruvate might contribute in part to its antianxiety activity. Nevertheless further studies are needed to elucidate the mechanism of antianxiety action of Ethyl pyruvate.

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