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Combating effects of *Azadirachta indica* leaves extract on biochemical and neuropsychological decline observed in diabetes

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Abstract: Diabetes is a group of metabolic disorder effecting health of wide number of population and cause neuropsychological decline. In the present study, effect of AI leaves extract on neuropsychological behaviors was observed in diabetic rat's model. Rats were divided into 4 groups as control (saline treated healthy rats), positive control (pioglitazone treated diabetic rats), diabetic control (untreated diabetic rats) and AI leaves extract treated diabetic rats. Diabetes was induced by giving 35% fructose for 6 weeks and a single dose of Streptozotocin (40 mg/kg). After 3 weeks of treatment behavioral and biochemical analysis were done. Behavioral results revealed that induction of type 2 diabetes produced anxiety, depression, decreased motor activity and impaired recognition memory in rats. Treatment with AI leaves extract in diabetic rats significantly decreased anxiety, depression, increased motor activity, enhanced recognition memory. Biochemical investigation revealed that AI leaves extract treat diabetes via improving the levels of fasting insulin and HbA1c and a significant decrease in CK and SGPT levels were observed in AI leaves treated diabetic rats. So, AI besides treating diabetes, helps in lowering the risk of co-occurring diabetic diseases and found effective in lowering neuropsychological decline observed in type 2 diabetes.

Keywords: Anxiety, memory, motor activity, *Azadirachta indica*, creatine kinase, SGPT, triglyceride, HbA1C, fasting insulin, Type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is one of the major health issues around the globe (Guariguata *et al*., 2014). Affecting both adults as well as infants (Ziegler *et al*., 2011) causing dysfunction of many organs of living system. Cardiac complications arises due to diabetes in previous years was increased numerous folds causing deaths (Ansley and Wang, 2013). Diabetes represents the damaging set of biopsychological challenges not only for patients but their families irrespective of its type either type 1 or type 2 (American Diabetes Association [ADA], 2015). Depressive anxious like behaviors are multifactorial disorders affecting immune response and neurotransmission and it is corelated to disruption in metabolic system (Felger & Lotrich, 2013; Hernandez *et al*., 2013), which is somehow related to the co-morbidity between type 2 diabetes mellitus (T2DM) and anxio-depressive disorders (Lustman *et al*., 1992). Previous studies suggested that mood fluctuation and anxiogenic behaviors were commonly observed in diabetic peoples than people without diabetes (Engum 2007). Recent researches also revealed that cognitive decline was observed in T2DM (Jellinger 2015). The person with T2DM is at higher risk of developing different types of dementia and comorbidity, patient with high blood pressure and high cholesterol levels are at high risk (Jellinger 2015b; Haroon *et al*. 2015). Cognitive impairment caused due to insulin resistance in medial temporal hyper metabolism results in dementia (Willette *et al*. 2015).

Pioglitazone is thiazolidinediones derivative identified as synthetic agonist of peroxisome proliferator-activated receptor gamma (PPARγ) used to treat T2DM by improving insulin sensitivity via acting on adipocytes results in decreasing blood glucose levels (Lehmann *et al*., 1995). Meta-analysis revealed the facts that besides the beneficial effect treatment with pioglitazone increases the risk of developing cardiac complications (+32%), edema (+63%), osteoporosis (+52%) and obesity (+60%) (Liao *et al*., 2017).

Neem botanically known as *Azadirachta indica* (AI) belongs to family Meliaceae, well known for its potential therapeutic effects (Silva *et al*., 2004; Siswomihardjo *et al*., 2007; Chandrabhatla *et al*., 2012; Asif, 2012). Polyphenolic compounds are highly associated with oxidative radicals quenching and cytotoxic activities are found in AI leaves revealed in recent study (Shikha Agrawal *et al*., 2020). Anti-diabetic activity of neem plant extract has a strong relationship with their anti-oxidant

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property and polyphenolic contents enabling the extract to combat against diabetes, depression, infections and cardiovascular disorders (Sabu, Kuttan, 2002; Andrade-Cetto, Heinrich, 2005). Previously neem extract has also proven to possess neuroprotective effects because of its anti-oxidant properties (Bamidele *et al*., 2013). The present piece of work was planned to find out the effect of neem leaves extract on neuropsychological decline observed in diabetes.

MATERIALS AND METHODS

Extract preparation

Fresh neem leaves were taken from local plants identified and shade dried for three days. They were powdered in an electric blender and 1 kg powdered leaves were dipped in ethanol for 7 days. It was then filtered with the help of Whatman's No. 1 filter paper and then extract was prepared from the filtrate at reduced pressure with the help of rotary evaporator.

Animals and treatment

Wistar rats weight (150±180) kept individually at room temperature (22±2ºC) for 3 to 4 days before the start of the experiment. All experiments were conducted according to a protocol approved by IBR of Federal Urdu University of Arts, Science and Technology, Karachi.

Induction of diabetes

Rats were fed with 35% fructose solution for 6 weeks and after that a single low dose of Streptozotocin (40 mg/kg) dissolved in 0.1 M citrate buffer of pH 6.3 was given intraperitonially to stab the diabetic condition. Hence, the combination of fructose-feeding and a lower dose of STZ injection may induce all major pathogeneses of type 2 diabetes in rats (Wilson and Islam, 2012). Next day after that animal with fasting blood glucose level > 200 mg/dl were considered as diabetic.

Grouping

Rats were divided into four groups (n=6). Control (healthy rats received saline), Diabetic control (untreated diabetic rats), positive control (Pioglitazone at the dose of 15 mg/kg treated diabetic rats) and test (AI at the dose of 100 mg/kg treated diabetic rats). Behaviors were monitored after 3 weeks of treatment.

Behavioral analysis

Elevated plus maze

The Elevated Plus Maze (EPM) test was done according to the procedure described by Pellow for the assessment of anxiety in rodent strain (Pellow *et al*., 1985). It could be used to screen for putative anxiolytic compounds.

Open field test

This test was performed to evaluate locomotor activity and exploration for 5 minutes. The dimensions of the apparatus are 76 cm x 76 cm with surrounding opaque 42 cm tall walls and 25 identical squares being drawn on the base of the apparatus. Rodents were placed in center square and numbers of square crossed were counted.

Forced swimming test

It is used to evaluate depression like symptoms in rodents by calculating immobility time. It is done as per procedure described by Siddiqui (Siddiqui *et al*., 2017).

Novel object recognition task

The cognitive ability of animals is evaluated by the novel object recognition test. Done as described by Ennaceur and Delacour in 1988 (Ennaceur and Delacour, 1988).

Biochemical investigation

After decapitation of animals' blood plasma was collected for evaluation of biochemical parameters by commercially available kits.

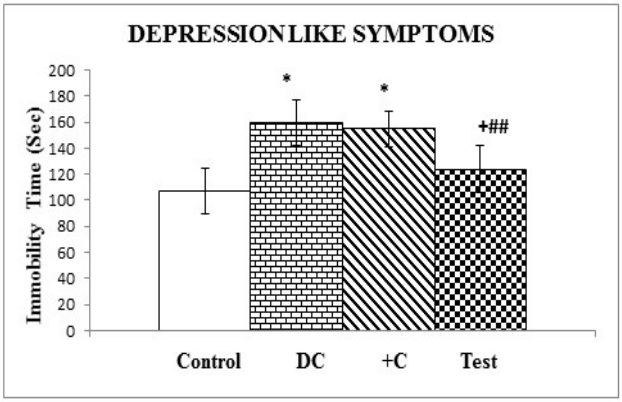
STATISTICAL ANALYSIS

Results are represented as means ± SD. SPSS software version 20 is used for analysis. Data were analyzed by one-way ANOVA. Tukey's HSD test is used for post-hoc analysis P<0.01 and P<0.05 was considered significant.

RESULTS

Azadirachta indica leaves extract effect on depression like symptoms in diabetes

Fig. 1 shows that diabetic control and positive control increased P<0.01 depression like symptoms when compared with control. Azadirachta indica leaves extract treated diabetic group decrease depression like symptoms as compared to diabetic control P<0.01 and positive control P<0.05.

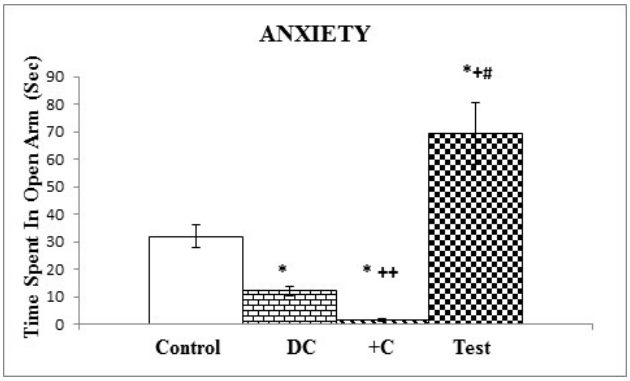


Statistical significant difference: *P<0.01 vs control, +P<0.01 vs Diabetic control and ##P<0.05 vs Positive control.

Fig. 1: Effect of AI leaves extract on depression like symptoms in diabetes.

Azadirachta indica leaves extract effect on anxiety in diabetes

Fig. 2 shows that diabetic group and positive control group increased anxiety P<0.01 compared to healthy group whereas diabetic group treated with Azadirachta indica leaves extract show decreased in anxiety P<0.01 as compared to control, diabetic control and positive control.

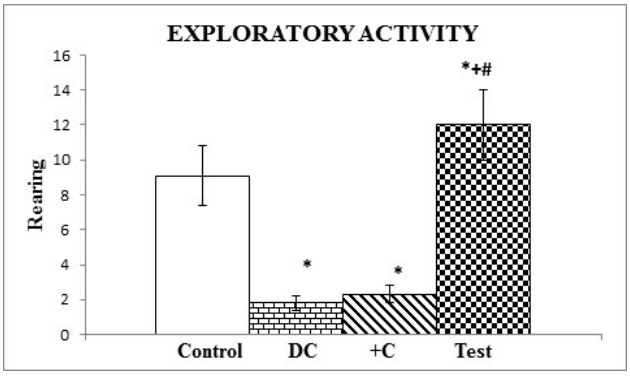


Statistical significant difference: *P<0.01 vs control, +P<0.01, ++P<0.05 vs Diabetic control and #P<0.01 vs Positive control.

Fig. 2: Effect of AI leaves extract on anxiety in diabetes.

Azadirachta indica leaves extract effect on exploratory behavior in diabetes

Fig. 3 shows decreased exploratory behavior p<0.01 in diabetic group and Pioglitazone treated diabetic group as compared to control. Azadirachta indica leaves extract treated diabetic group shows increased P<0.01 exploratory behavior when compared with rest of the groups.



Statistical significant difference: *P<0.01 vs control, +P<0.01 vs Diabetic control and #P<0.01 vs Positive control.

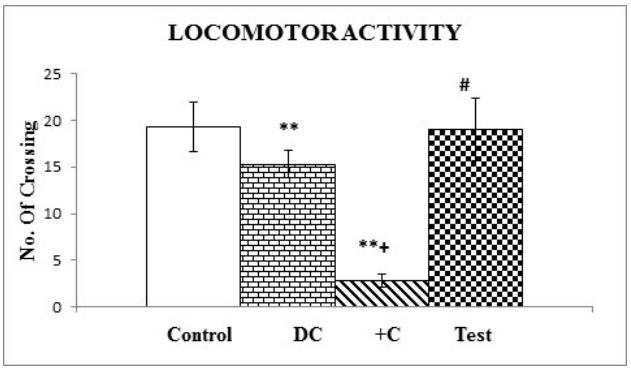
Fig. 3: Effect of AI leaves extract on exploratory behavior in diabetes.

Azadirachta indica leaves extract effect on locomotor activity

Fig. 4 shows decreased p<0.05 locomotor activity by diabetic group compared to healthy group. Pioglitazone treated diabetic group decreased locomotor activity compared with control P<0.05 and diabetic control P<0.01 where as Azadirachta indica leaves extract treated diabetic group shows increase P<0.01 in locomotor activity as compared to diabetic control.

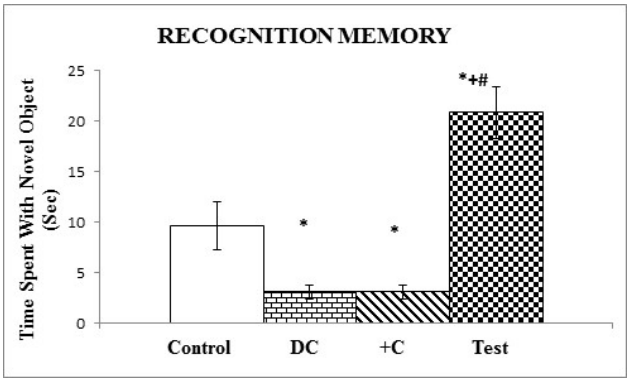
Azadirachta indica leaves extract effect on recognition memory

Fig. 5 shows impaired recognition memory P<0.01 in diabetic and Pioglitazone treated diabetic group as compared to control. Azadirachta indica leaves extract treated diabetic group enhance memory P<0.01 as compared to other groups.



Statistical significant difference: **P<0.05 vs control, +P<0.01 vs Diabetic control and #P<0.01 vs Positive control.

Fig. 4: Effect of AI leaves extract on locomotor activity in diabetes.



Statistical significant difference: *P<0.01 vs control, +P<0.01 vs Diabetic control and #P<0.01 vs Positive control.

Fig. 5: Effect of AI leaves extract on recognition memory in diabetes.

Azadirachta indica leaves extract effect on HbA1C level

Fig. 6 shows a significant increase P<0.01 in HbA1C level in diabetic control and positive control when compared with control group whereas it is decreased in Azadirachta indica leaves extract treated diabetic group P<0.01 when compared with diabetic control and positive control.

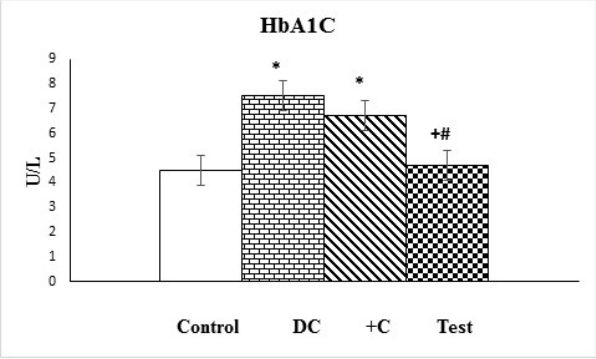
Azadirachta indica leaves extract effect on fasting insulin levels

Fig. 7 shows fasting insulin levels were decreased P<0.01 in diabetic group treated with Azadirachta indica leaves extract compared with healthy, diabetic and positive

control. And were increase P<0.01 in diabetic and positive control when compared with control.

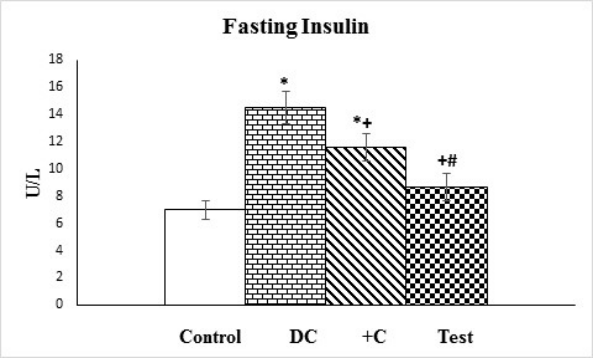
Azadirachta indica leaves extract effect on Triglyceride levels

Fig. 8 shows high TG levels P<0.01 in positive and diabetic control groups vs healthy group. And decreased levels P<0.01 in Azadirachta indica leaves extract treated diabetic group when compared with diabetic control and diabetic group treated with Pioglitazone.



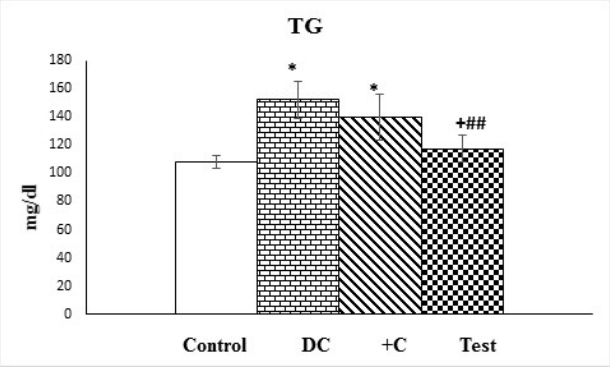
Statistical significant difference: *P<0.01 vs control, +P<0.01 vs Diabetic control and #P<0.01 vs Positive control.

Fig. 6: Effect of AI leaves extract on HbA1C levels in diabetes.



Statistical significant difference: *P<0.01 vs control, +P<0.01 vs Diabetic control and #P<0.01 vs Positive control.

Fig 7: Effect of AI leaves extract on fasting insulin levels in diabetes.



Statistical significant difference: *P<0.01 vs control, +P<0.01 vs Diabetic control and ##P<0.05 vs Positive control.

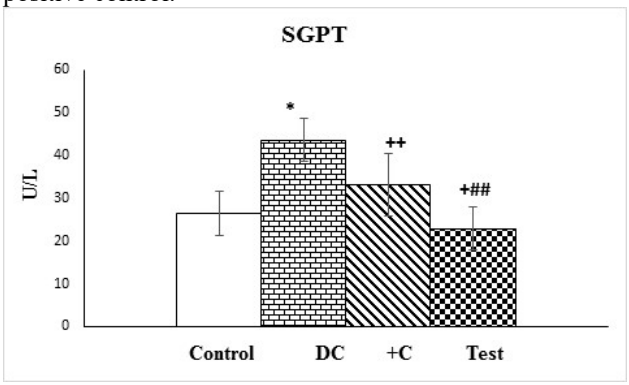
Fig. 8: Effect of AI leaves extract on TG levels in diabetes.

Azadirachta indica leaves extract effect on SGPT levels

Fig. 9 shows increased level P<0.01 levels of SGPT in diabetes untreated group compared with control whereas decrease P<0.05 in positive control compared with diabetic group. Azadirachta indica leaves extract treated diabetic group decreased SGPT levels compared with diabetic control P<0.01 and positive control P<0.05.

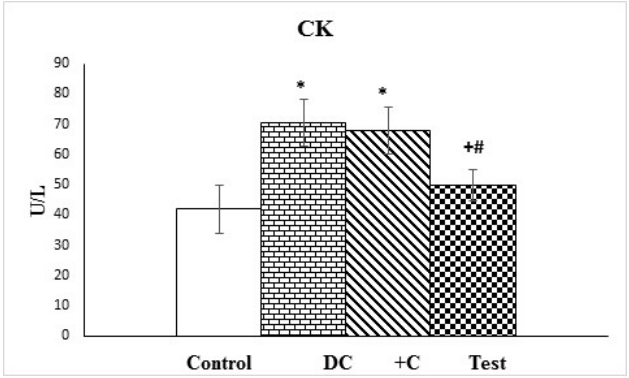
Azadirachta indica leaves extract effect on CK levels

Fig. 10 shows increase in CK levels P<0.01 in diabetic control and positive control compared to control group. Azadirachta indica leaves extract treated diabetic group decrease CK levels P<0.01 compared to diabetic and positive control.



Statistical significant difference: *P<0.01 vs control, +P<0.01, ++P<0.05 vs Diabetic control and ##P<0.05 vs Positive control.

Fig. 9: Effect of AI leaves extract on SGPT levels in diabetes.



Statistical significant difference: *P<0.01 vs control, +P<0.01 vs Diabetic control and #P<0.01 vs Positive control.

Fig. 10: Effect of AI leaves extract on CK levels in diabetes.

DISCUSSION

Diabetes is deadly disorder that caused various complications including neuropathies (Rota and Morelli, 2016), cognitive dysfunctions, dementia, depression and anxiety (Ho *et al*., 2013). Neuropsychological decline in diabetes destroy quality of life and it also increased mortality rate. In present study induction of T2DM by fructose and streptozotocin significantly impaired

memory function significant decrease in exploratory activity and increase in depression like symptoms was exhibited by diabetic rats as compared to controls. Increase in anxiety was also seen in diabetic rats. All the above stated neuropsychological alteration exhibited by diabetic rats was significantly reversed to normal by the treatment with AI leaves extract. Increase in the levels of CK, SGPT, HbA1c, fasting insulin and TG were also observed in diabetic control as compared to control. These elevated biochemical parameters were also significantly reversed to normal by the treatment of AI leaves extract.

There are different allopathic medicines offering therapeutic treatment for diabetes but, they have bulging adverse effects and are less efficacious in the treatment of emerging complications of diabetes (Rang and Dale, 1991). Due to the adverse effect of these conventional drugs people have driven towards natural products. Among such natural products the extract of AI leaves has been used in treatment of diabetes. However, treatment of neuropsychological decline observed in diabetes through AI leaves extract was not investigated before. Previously it has been reported that T2DM cause atrophy of hippocampus and amygdala by oxidative stress and decreasing protective antioxidant affecting learning and memory which was similar as observed in dementia (den Heijer *et al*., 2003). It has been reported previously that impaired memory function in diabetes may be caused due to defects in neuronal insulin signaling (Costello *et al*., 2012). The present study showed induction of T2DM significantly impaired recognition memory. Impairment in recognition memory exhibited by diabetic rat was unaltered by positive control whereas AI leaves administration significantly reversed this impairment in recognition memory exhibited by diabetic rats. AI treated diabetic rats also improved recognition memory as compared to healthy control rats. AI leaves extract has been known to possess antioxidants and anticarcinogenic properties due to the presence of β-carotene, nimbin, nimbidin, azadirachtin, quercetin, nimbidiol and nimbatiktan (Subripriya and Nagini, 2003). Among these antioxidant components of AI leaves β-carotene was reported as potent in decreasing LPO by trapping peroxyl radicals (Blasiak *et al*., 2002). Previously it was reported that antioxidant property of AI leaves extract is mainly due to its major component azadirachtin (Manikandan *et al*., 2008). It was previously reported that AI leaves reverse oxidative damage in mice (Dkhil *et al*., 2013) and exerted neuroprotective effect (Balasenthil *et al*., 1999) by decreasing LPO and increasing GSH. This may be the reason for enhancement in recognition memory in AI leaves treated diabetic rats. It is suggested in the present study that AI mediate neuroprotective effects by modulation of LPO and enhancing antioxidant enzymes by azadirachtin or β-carotene.

The anxiolytic effects of AI are also documented in the previous studies along with increase in the locomotion and stimulation of the test subjects (Thaxter *et al*., 2010). Results of the present study showed that anxiety was produced in T2DM that were worsen when treated with conventional drug pioglitazone. However, it was observed that treatment with AI leaves extract for three weeks significantly produced anxiolytic effect in diabetic rats as compared to diabetic control, positive control and healthy control. Present study showed depression was developed in T2DM that were unaffected when treated with conventional drug pioglitazone. However, it was completely reversed to normal by treatment with AI leaves extract for three weeks. Thus, AI leaves extract produced antidepressant effects that was evident by decrease in immobility time exhibited by AI leaves treated diabetic rats as compared to all other groups. Previously it was reported that depression and anxiety develop during metabolic diseases like T2DM (Felger and Lotrich, 2013; Hernandez *et al*., 2013) and it is often associated with increased HbA1c levels and poor glycemic control (Lustman *et al*., 2000). Chronic hyperglycemic condition cause insulin resistance (He *et al*., 2014; Kulkarni *et al*., 2014) which can cause psychological disorders (Shen and Bergquist-Beringer, 2013; Sharma *et al*., 2014). Several oxidative stress pathways are involved in the development of insulin resistance (Ye, 2011; Fabbrini *et al*., 2014) which is often associated with impaired fasting glucose (Kulkarni *et al*., 2014; Meigs, 2007). In the present study HbA1c and fasting insulin levels were significantly reversed to normal in AI leaves extract treated diabetic rats. It was therefore reported in this study that neurophysiological changes observed in diabetes was brought back to normal by AI leaves extract treatment in diabetic rats.

Present study reported that AI leaves extract increased exploratory activity in diabetic rats. This increase in exploratory activity points towards the positive and fruitful stimulation of the animals as Ho *et al* reported that induction of diabetes decreased these behaviors in rats (Ho *et al*., 2013). AI leaves were previously reported to normalize biochemical parameters and decreasing oxidative stress in diabetic rat's model (El-Hawary and Kholeif. 1990; Shukia *et al*., 2000; Mahdi *et al*., 2003). The present study also indicated cardioprotective role of AI leaves extract in the treatment of diabetes as significant decline in triglycerides and CK levels were observed in AI treated diabetic rats which were elevated in diabetic rats and no effect on these parameters was observed by treatment with pioglitazone. Normal levels of SGPT in AI leaves extract treated diabetic rats revealed hepatoprotective activity of neem leaves extract. These findings indicate that treatment of diabetes with AI leaves extract reduced the risk of cardio-hepatic complications develop during diabetes and enlighten the potency of AI

leaves extract for the treatment of neuropsychological decline observed in T2DM.

CONCLUSION

The results of this study revealed that Induction of T2DM by streptozotocin plus fructose has been reported to produced anxiety, reduced motor coordination, impaired recognition memory and produced depression like symptoms in diabetic rats. Treatment with conventional medicine has been reported to exaggerate adverse effects on these behavior. AI leaves extract administration in diabetic rats significantly decreased anxiety, increased motor coordination, enhanced recognition memory and produced anti-depressant effects. The results of current study showed that treatment of T2DM with AI leaves extract improved hyperglycemia, hyper insulinemia and HbA1c levels in rats. Thus, the current study concluded that AI leaves extract can be implicated as a therapeutic agent in the cure of T2DM. AI leaves extract reduce the risk of complications related with cardio-hepatic system as well as fight against neuropsychological decline appeared in diabetes. Further studies are needed to determine molecular mechanism of action of AI leaves extract in type 2 diabetes.

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