Evaluating the Therapeutic Potential of Ethyl Acetate Extract from *Dregea volubilis* Leaves in Ameliorating High Fructose-Induced Insulin Resistance in Experimental Models

A.V.Yalsangi 1 , D.D.Bandawane*, P.K.Hajare 2 , R.P.Babhulkar 3
Department of Pharmacology, P. E. S. Modern College of Pharmacy, Nigdi, Pune-44
Maharashtra, India.

Dr. Deepti.D.Bandawane
Professor,
Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44
Maharashtra, India.

1. Arpita.V.Yalsangi
Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44
Maharashtra, India.

2. Pratiksha.K.Hajare
Assistant Professor
Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44
Maharashtra, India.

3.Rasika.P.Babhulkar
Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44
Maharashtra, India.

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Abstract

Introduction: Insulin resistance is a metabolic disorder where the body's cells become less responsive to insulin, leading to elevated blood sugar levels and various metabolic disturbances. Diets high in fructose are particularly problematic, as they can induce insulin resistance by decreasing insulin secretion and increasing fat breakdown, resulting in higher triglycerides and glucose levels.

Method: The study involved male Wistar rats divided into groups receiving high treatment with Dregea volubilis extract (EADV) improved metabolic parameters, including blood glucose and lipid profiles.

Results: Treatment with Dregea volubilis extract (EADV) improved metabolic parameters, including blood glucose and lipid profiles, although it was less effective than the standard drug pioglitazone.

Conclusion: However, EADV was proven effective in managing hyperglycemia and hyperinsulinemia, highlighting the need for further research on Dregea volubilis as a potential therapeutic agent for insulin resistance and related metabolic disorders.

Key words: Insulin resistance, Pioglitazone, Hyperinsulinaemia, Dregea volubilis

Introduction

Insulin resistance is a metabolic condition where the body's cells become less responsive to the actions of insulin, a crucial hormone that regulates blood sugar levels. This leads to a range of metabolic disturbances, including Impaired Insulin-Stimulated Glucose Transport and Metabolism the transport and metabolism of glucose in fat tissue and skeletal muscle are impaired, resulting in higher blood sugar levels[1].

Insulin normally suppresses the breakdown of fat and the production of glucose by the liver. In insulin resistance, this regulatory function is impaired, leading to increased fat breakdown and excessive glucose production[2].

Insulin resistance is not limited to just glucose metabolism; it can also affect the metabolism of amino acids, lipids, and other metabolites, indicating a widespread metabolic dysregulation.

Recognizing and addressing insulin resistance is crucial, as it is a key contributor to the development of various metabolic disorders, such as type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular disease[3]. Understanding the underlying mechanisms of insulin resistance can help healthcare professionals develop targeted interventions to improve metabolic health and prevent the progression of these related conditions[4].

Dregea volubilis is a large, twining shrub from the Apocynaceae family, widely used in Indian traditional medicine. Native to regions like the Himalayas, Sri Lanka, China, Burma, Indonesia, and Thailand[5], its leaves are valued for treating rheumatic pain, cough, fever, and cold[6]. It is commonly known as cotton milk plant and locally referred as Harandodi in Marathi and Hemjivanti in siddha medicine. The leaves contains diverse compounds, including oleanolic acid, ursolic acid, triterpenoids, phenolics, steroids, and more[7]. And has shown its significant in vitro and in vivo anti-inflammatory, antioxidant, and antidiabetic activities[8].

Diets high in fructose can induce insulin resistance in rodents through a complex interplay of metabolic disturbances[9]. Fructose consumption leads to decreased insulin secretion, reduced leptin production, and elevated nonesterified fatty acids (NEFA). These changes contribute to increased body weight and further exacerbate insulin resistance[10].

Fructose also promotes the development of hypertriglyceridemia by increasing the production of very-low-density lipoprotein (VLDL) triglycerides in the liver. This excess of circulating triglycerides is a hallmark of insulin resistance[11].

The combination of decreased insulin sensitivity, impaired glucose metabolism, and elevated triglycerides creates a vicious cycle that perpetuates the development of insulin resistance[12].

With its traditional use, unique phytochemistry, and proven therapeutic potential, Dregea volubilis is a promising subject for anti-insulin resistance effect. And to investigate whether management with DV has any ameliorative effect on plasma glucose, insulin, triglycerides, cholesterol, HDL, anti-inflammatory and antioxidant status in fructose fed rat model of insulin resistance.

Materials and Methods

Preparation of fructose diet

The control diet for the rats contained 66% starch, 10% casein, 8% lard, 0.004% Zinc carbonate 15% cellulose, 5% of each mineral and vitamin mix. The fructose diet contained 66% of fructose instead of starch. Both the diets were obtained from VRK Nutrition Solution, Sangli , Maharashtra, india[13].

Chemicals

Pioglitazone was purchased from a nearby medical store. Other solvents and chemicals used for the study are of analytical grade and were purchased from local vendors and suppliers.

Collection and authentication of plant

The leaves of *Dregea volubilis (L.f) Benth* were collected from Kalakatu, Tirunelveli District ,India. The plant was identified and authenticated by Dr. Randive S.D & Dr. Jagtap M.N, Botanist, herbarium & e herbarium, Department of Botany & Research centre Solapur, Maharashtra having a specimen no.1: 214 (1783) wfo-0000245853.

Plant extract

The leaves of *Dregea volubilis (L.f) Benth.* were dried in the shade and subjected to size reduction to a coarse powder. The 4 kg leaf powder was macerated into 15 l ethyl acetate for two days and this process was repeated twice. The solvent was filtered using absorbent cotton wool and filter paper (Whatman No A-1). Filtrate was collected and evaporated on a rota evaporator at 40^0 C to obtain a yield of 11.36% w/w. The obtained ethyl acetate extract of *Dregea volubilis* (EADV) was preserved in the refrigerator till further use. Acute toxicity study was performed according to OECD 423 guideline. And 2000 mg/kg was considered as toxic dose[14].

Animals

Male Albino Wistar rats (160–180 g) used for the present study were procured from Crystal Biological Solutions, Pune, India. The animals were acclimatized for 7 days in our animal house (Regd. No. 884/PO/Re/S/05/CPCSEA) before dietary manipulation. They were housed six per cage in an air-conditioned room (22 ± 2 ^{0}C) with 12 h light/dark cycle and had free access to standard pellet diet and water. All the procedures were performed in accordance with the Institutional Animal Ethics Committee.

Experimental design

The rats were divided into five groups with six animals in each.
Normal: Control rats received normal diet daily.
Diseased: Rats received 66 % high fructose diet for 8 weeks.
Std-Pioglitazone(10mg/kg): Rats received 66% high fructose diet for 6 weeks and 10 mg pioglitazone/kg. b. wt. orally for last 2 weeks along with diet.
EADV(100mg/kg): Rats received 66% high fructose diet for 6 weeks and EADV 100mg/kg. b. wt. orally for last 2 weeks along with diet.
EADV(200mg/kg): Rats received 66% high fructose diet for 6 weeks and EADV 200 mg/kg. b. wt. orally for last 2 weeks along with diet.

Animals were maintained in their respective groups for 56 days. The dose of EADV used in the current study was based on the earlier report on the hypolipidemic effect of this plant in experimental diabetic rats.

Parameters studied

Body weight (g), blood glucose levels (mg/dl), serum cholesterol levels (mg/dl), serum triglyceride (mg/dl), HDL (mg/dl), LDL (mg/dl),VLDL(mg/dl) were measured on initial, 42 and 42th day of experiment. The Serum insulin levels (mU/L), antioxidants such as catalase (CAT), super oxide dismutase (SOD) were determined, Serum level of TNF-a was evaluated by ELISA kits, histopathology of liver were detected in samples taken on 56th day of experiment. The serum glucose was estimated using commercially available glucometer (Accu check, Germany). Serum cholesterol, serum triglyceride, HDL, LDL,VLDL were determined by autoanalyser (Erba Mannheim test kits).

Statistical analysis

Values are given as mean ± SEM. The results were analyzed by one-way ANOVA test using, GraphPad Prism Version 10.2.3(403) software.

Results

Body weight

The body weights of five groups of animals during experimental period are represented in Fig. 1A. No significant variation in body weights of Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) was observed when compared with Diseased upto 42 days. but a significant reduction was observed from 42 days onwards till 56 day when compared with Diseased.

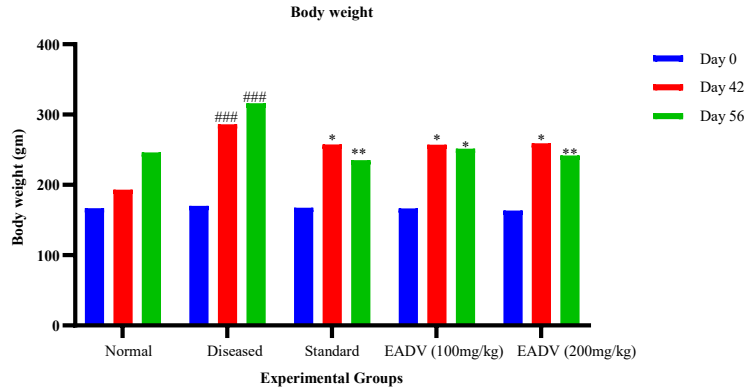

Fig. 1A: Body weight

Blood glucose

There was no significant variation in the glucose concentrations of Diseased, Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) groups throughout the experimental period (Fig. 1B). Normal showed a no gradual and significant increase in glucose levels till the end of experimental period. The glucose concentrations of Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) showed reduction in glucose from day 42 till the end of experimental period.

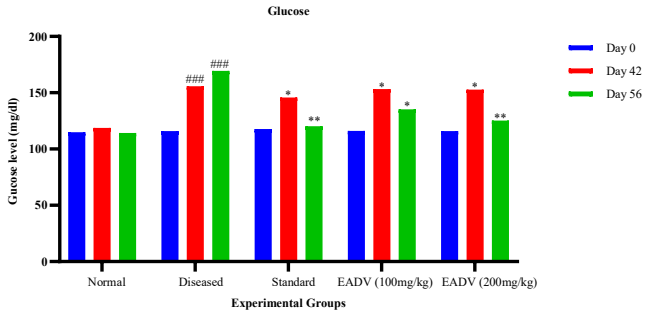

Fig. 1B: Blood glucose

Fasting serum insulin

Diseased showed a gradual increase in plasma insulin during the experimental period as compared to Std-Pioglitazone(10mg/kg) (Fig. 1C). The insulin levels of Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) at the end of experimental period were significantly lower than Diseased but still significantly higher than Normal.

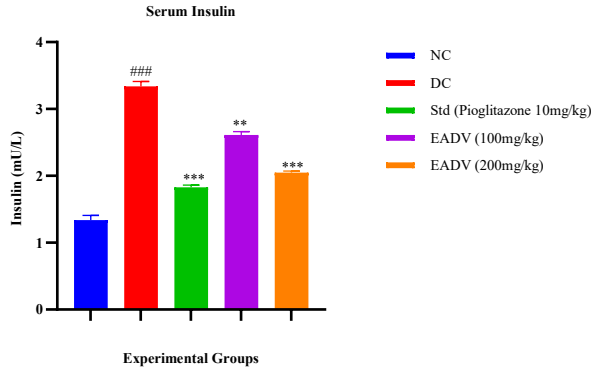

Fig. 1C: Fasting serum insulin

HOMA-IR

HOMA-IR is assessed by formula Blood glucose *Serum insulin / 22.5. It is the parameters that show how much are the cells resistant to insulin. As shown in Fig. 1D Diseased group shows more insulin resistance than Normal. And Std-Pioglitazone (10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) groups shows less insulin resistance than diseased group.

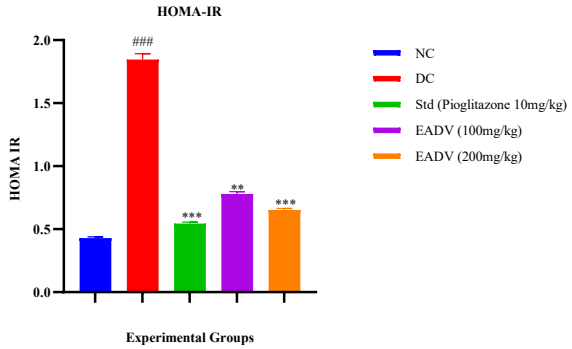

Fig. 1D HOMA-IR

Lipid Profile

Serum cholesterol

Feeding of fructose-enriched diet caused hypercholesterolemia as indicated by higher cholesterol values on the 14th as well as 42th day of the experiment (Fig. 1E). However, on 56th day serum cholesterol level of Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) was significantly lower than the Diseased.

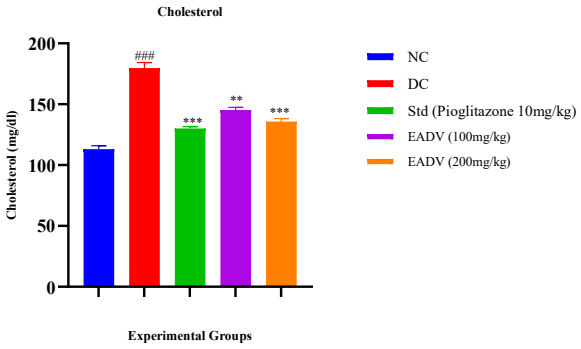

Fig. 1E: Serum cholesterol

Serum triglycerides

There was no significant variation in the serum triglyceride concentrations of Diseased, Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) throughout the experimental period (Fig. 1F). Normal showed a no gradual and significant increase in serum triglycerides levels till the end of experimental period. The serum triglycerides concentrations of Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) showed reduction in serum triglycerides from day 42 till the end of experimental period .

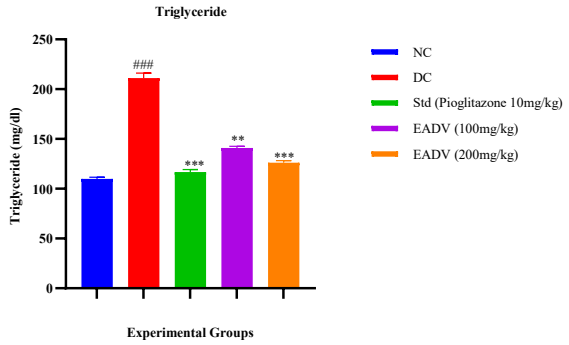

Fig. 1F : Serum triglycerides

HDL

The HDL levels of five groups of animals during experimental period are represented in (Fig. 1G). No significant variation in hdl levels of Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) was observed when compared with Diseased upto 42 days. but a significant increase was observed from 42 days onwards till 56 when compared with Diseased. hdl levels were similar throught the experiment for Normal.

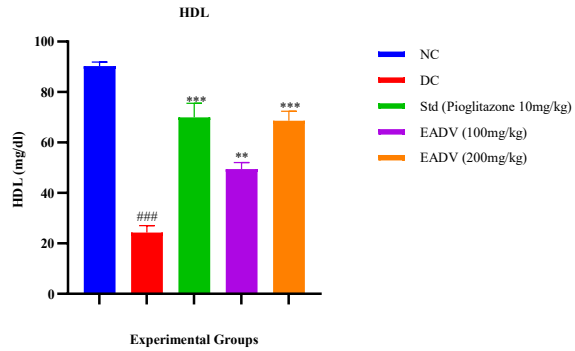

Fig. 1G: HDL

LDL and VLDL

The LDL and VLDL levels of five groups of animals during experimental period are represented in (Fig. 1H and 1I). No significant variation in ldl and vldl levels of Std-Pioglitazone(10mg/kg), EADV(100mg/kg) and EADV(200mg/kg) was observed when compared with Diseased upto 42 days. but a significant decrease was observed from 42 days onwards till 56 when compared with Diseased. ldl and vldl levels were similar throught the experiment for Normal

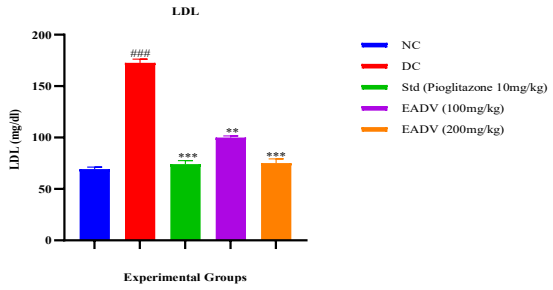

Fig. 1H: LDL

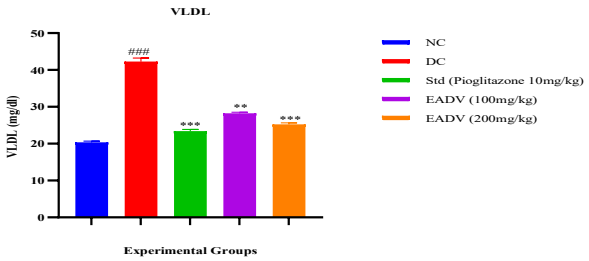

Fig. 1I: VLDL

Oxidative stress markers

Table 1 shows the levels of antioxidants SOD, CAT in the liver of animals. Diseased showed significantly lower levels of SOD and CAT as compared to Normal rats. Std-Pioglitazone(10mg/kg), EADV(100mg/kg), EADV(200mg/kg) showed significantly higher levels of SOD and CAT when compared with Diseased but still significantly lower than Normal (27% and 4%, respectively). The activities of enzymatic antioxidants SOD, CAT were significantly lower (12%, 14%, respectively) in Diseased rats than in Normal rats.

Anti-inflammatory marker (TNF-α level)

levels of anti-inflammatory TNF-α in the liver of animals. Diseased showed significantly lower levels of TNF-α as compared to Normal rats (Table 1). Std-Pioglitazone(10mg/kg), EADV(100mg/kg), EADV(200mg/kg) showed significantly higher levels of TNF-α when compared with Diseased but still significantly lower than Normal.

Parameters	Normal	Diseased	Std-Pioglitazone (10mg/kg)	EADV (100mg/kg)	EADV (200mg/kg)
SOD (Units/mg of protein)	950.35	415.76	876.43	513.89	813.95
CAT (μM of H_2O_2 / gm of tissue)	289.76	51.97	161.18	85.39	143.62
TNF- α (pg/ml)	109.53	173.46	113.86	131.49	116.89

Effects of fructose on liver histology

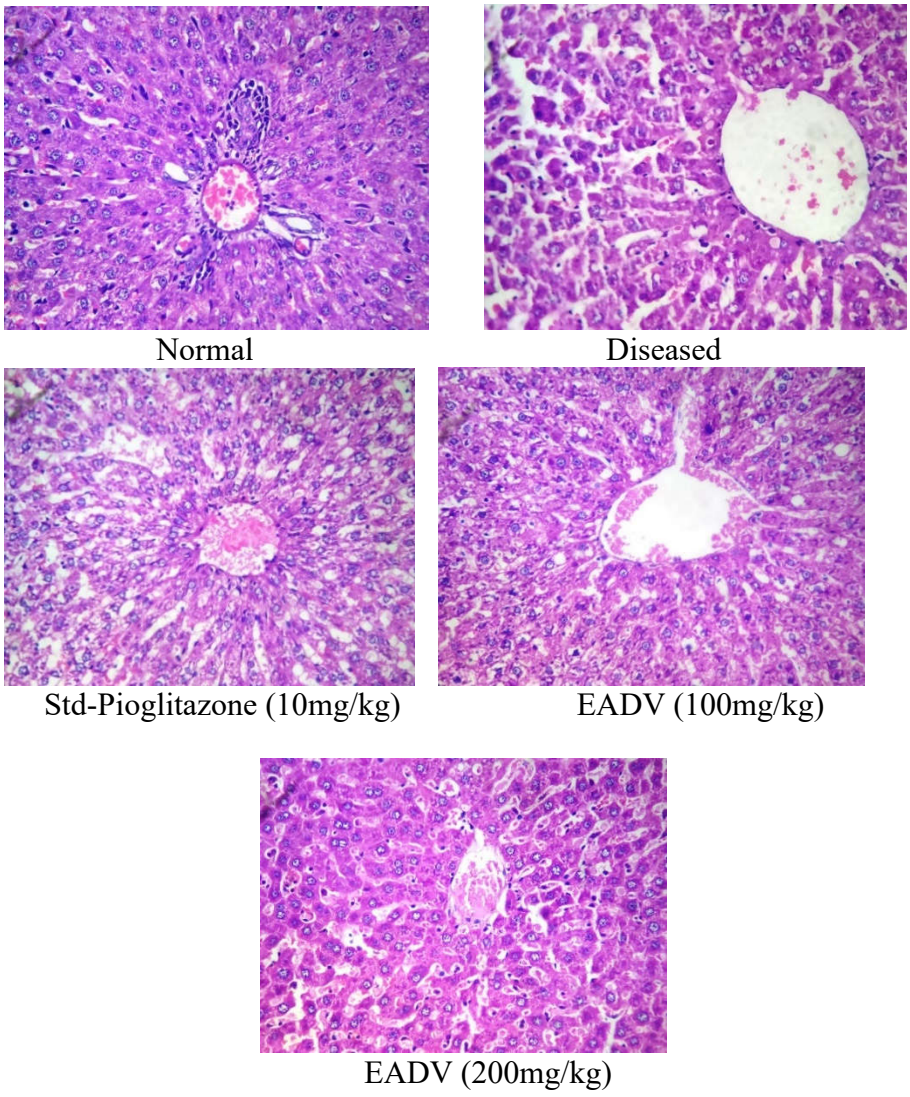

Fig 2 Liver histological examination

Liver histological examination (Fig. 2) shows that livers from the Diseased displayed a Centrilobular Degeneration of Hepatocyte, Mild Focal infiltration of Inflammatory cells with disorganization of Parenchymatous Sinusoidal Structure than Normal. Then in Std-Pioglitazone(10mg/kg) where there is Normal Branching and Anastomoising cords of hepatocytes radiating from Central Vein, Mild to Minimal Degeneration EADV(200mg/kg) shows more resembalance than EADV(100mg/kg) when compared with Std-Pioglitazone(10mg/kg).

Discussion

Insulin resistance is increasingly recognized as a common underlying factor in various atherogenic diseases, significantly impacting individuals' health. This condition predisposes people to a range of metabolic disorders, including hypertension, dyslipidemia, obesity, cardiovascular diseases, and type 2 diabetes mellitus. Research indicates that managing insulin levels is crucial for effectively treating these related diseases[15].

Insulin plays a vital role in the body's response to nutrient intake, particularly carbohydrates. When insulin functions properly, it helps regulate blood sugar levels and facilitates the uptake of glucose into cells for energy. However, in individuals with insulin resistance, the body's cells become less responsive to insulin, leading to elevated blood sugar levels and increased insulin production, a state known as hyperinsulinemia. This dysfunction can result in a cascade of

metabolic issues, including obesity and dyslipidemia, characterized by abnormal lipid profiles that heighten cardiovascular disease risk[16].

Recent studies have demonstrated that high-fructose diets can exacerbate insulin resistance. for instance, a study involving rats fed a high-fructose diet for 56 days showed significant metabolic disturbances, including fasting hyperglycemia (high blood sugar), hypertriglyceridemia (high triglycerides), hyperinsulinemia (high insulin levels), and hypercholesterolemia (high cholesterol levels). These changes indicate a compromised antioxidant and anti-inflammatory response, further contributing to the development of insulin resistance[17].

The implications of insulin resistance extend beyond individual metabolic disorders. It is a central feature of metabolic syndrome, a cluster of conditions that significantly increases the risk of heart disease and diabetes. Factors such as obesity, particularly visceral fat accumulation, sedentary lifestyle, and genetic predisposition play critical roles in the development of insulin resistance and its associated health risks. Addressing insulin resistance through lifestyle modifications, such as diet and exercise, is essential for improving overall metabolic health and reducing the risk of serious complications[18]. This model has been recommended for assessing the therapeutic efficacy of insulin sensitizers and drugs that are likely to have effect on insulin sensitivity. Therefore, this model is selected to study efficacy of EADV in improving insulin resistance.

Chronic consumption of fructose has been shown to significantly impact body weight and metabolic health. In studies involving rats, it was observed that those fed a high-fructose diet experienced a steady increase in body weight from the first day up to the 42nd day. However, after treatment, this weight gain was prevented, highlighting the potential for intervention even in the face of increased energy intake.

The effects of fructose extend beyond just weight gain. The rats in these studies also exhibited elevated fasting blood glucose, triglyceride, and insulin levels throughout the experimental period. By the 28th day, these metabolic disturbances were evident, and they intensified by the 42nd and 56th days of fructose feeding. This pattern indicates that chronic fructose intake can lead to hyperglycemia (high blood sugar) and hyperinsulinemia (high insulin levels), primarily due to the development of insulin resistance.

The increase in triglycerides after fructose consumption is attributed to enhanced synthesis of very low-density lipoprotein (VLDL) triglycerides in the liver, coupled with a decrease in the clearance of triglycerides from the bloodstream. This metabolic shift can lead to further complications, such as fatty liver disease and cardiovascular issues[19].

These findings are not limited to animal models; they resonate with trends observed in human populations. Increased fructose consumption, particularly from sugar-sweetened beverages, has been linked to rising rates of obesity and metabolic disorders. As people consume more fructose, they may unknowingly increase their risk for serious health issues, including diabetes and heart disease[20].

Increased delivery of triglycerides to muscle tissue can significantly disrupt glucose utilization, a process explained by the Randle cycle. This cycle highlights how the competition between glucose and fatty acids for oxidation can impair insulin action, leading to conditions such as hyperglycemia and hyperinsulinemia. Individuals with high triglyceride levels often experience insulin resistance, suggesting that diets rich in fructose may contribute to this metabolic issue.

When triglycerides accumulate in the muscle, they can interfere with the body's ability to use glucose effectively. This interference is particularly pronounced in those consuming high-fructose diets, as the metabolism of fructose leads to increased triglyceride levels. Studies have shown that individuals with hypertriglyceridemia often exhibit reduced insulin binding, which could be a mechanism through which fructose promotes insulin resistance[21].

In experimental studies, treatment with Std-Pioglitazone (10 mg/kg) was found to be more effective in preventing fructose-induced hyperglycemia and hyperinsulinemia compared to EADV at doses of 100 mg/kg and 200 mg/kg. The positive effects of Std-Pioglitazone can be attributed to its ability to prevent hypertriglyceridemia, thereby improving insulin sensitivity and overall metabolic health.

These findings underscore the importance of understanding the relationship between dietary choices and metabolic health. As fructose consumption continues to rise, particularly through sweetened beverages and processed foods, the risk of developing insulin resistance and related metabolic disorders increases. Effective management of triglyceride levels and insulin sensitivity is crucial for preventing the adverse health outcomes associated with high-fructose diets[22].

Fructose consumption can lead to increased production of free radicals, much like glucose. These reactive oxygen species (ROS) can diminish the effectiveness of antioxidant enzymes, such as catalase (CAT), which are crucial for protecting the body from oxidative stress. In studies involving rats fed a fructose-rich diet, a notable decrease in superoxide dismutase (SOD) activity was observed. This reduction may be linked to enhanced protein glycation caused by fructose, which is a more reactive reducing sugar compared to glucose and lactose[23].

Interestingly, the antioxidant potential of EADV (a treatment being studied) against oxidative stress induced by a fructose diet has shown promising results. When comparing the effects of EADV at different dosages (100 mg/kg and 200 mg/kg), there was a significant increase in the activities of antioxidant enzymes. This suggests that EADV may help mitigate the harmful effects of fructose on oxidative stress, supporting the body's natural defense mechanisms.

These findings are particularly relevant as they highlight the dual impact of high fructose diets: not only do they contribute to metabolic disturbances, but they also promote oxidative stress through increased free radical production. The ability of EADV to enhance antioxidant enzyme activity could provide a therapeutic avenue for addressing the oxidative damage associated with high fructose intake[24].

A high intake of fructose can lead to several negative health effects, including increased uric acid levels, elevated pro-inflammatory cytokines, and heightened intestinal permeability. These changes contribute to lipid accumulation in the liver and trigger inflammatory responses, exacerbating liver damage and metabolic disorders.

Excessive fructose consumption can initiate a cascade of inflammation. Pro-inflammatory cytokines promote widespread inflammation, especially in the liver. Additionally, heightened intestinal permeability allows toxins to enter the bloodstream, worsening the inflammatory response.

Fortunately, treatments have shown promise in reducing these inflammatory markers. In treated groups, inflammation levels decreased compared to untreated groups, suggesting that interventions can mitigate the harmful effects of high fructose intake and promote better liver health.

These findings emphasize the need to address high fructose consumption in our diets. Understanding the inflammatory mechanisms triggered by fructose can guide proactive measures to reduce its impact. Effective treatments that lower inflammation are crucial for preventing long-term complications associated with excessive fructose intake[25].

The histopathological examination of the livers from all rats revealed significant changes due to excessive fructose consumption. The diseased group exhibited notable damage, characterized by centrilobular degeneration of hepatocytes and mild focal infiltration of inflammatory cells. This led to a disorganized structure of the parenchymatous sinusoids, indicating serious liver dysfunction[26].

In contrast, the liver samples from rats treated with Std-Pioglitazone (10 mg/kg) displayed a more normal architecture, with healthy branching and anastomosing cords of hepatocytes radiating from the central vein and only mild to minimal degeneration. The EADV treatment at 200 mg/kg showed improvements in liver structure, resembling the Std-Pioglitazone-treated group more closely than the EADV at 100 mg/kg. This suggests that higher doses of EADV may be more effective in mitigating the damaging effects of a high-fructose diet.
These findings highlight the detrimental impact of a high-fructose diet on liver health and the potential for specific treatments to restore liver function and structure. By reducing inflammation and promoting healthier liver architecture, treatments like Std-Pioglitazone and EADV could play a crucial role in combating the adverse effects of excessive fructose intake[27].

Conclusion

The study demonstrates that ethyl acetate extract from Dregea volubilis leaves may help combat insulin resistance caused by high fructose intake. The extract effectively reduces hyperglycemia, hypertriglyceridemia, and hyperinsulinemia while improving insulin resistance in fructose-fed rats. The observed benefits likely arise from various active compounds in the extract, which may work both individually and together, contributing to its effectiveness in managing insulin resistance. However, further research is necessary to clarify the specific mechanisms behind these protective effects. These findings suggest that Dregea volubilis extract could be a valuable addition to dietary strategies aimed at preventing and managing insulin resistance.
To fully understand how the extract works, additional studies should focus on identifying its active components and their interactions, which could lead to new natural therapies for managing insulin resistance and metabolic disorders.

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