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Streptozotocin-Induced Diabetic Retinopathy in Swiss Albino Mice: Anatomical, Behavioral, and Histopathological Evaluation of the Colostrum and Astaxanthin in the Retinas

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Key Words:
Albino Mice, Deoxyribonucleic Acid, Streptozotocin, Retinopathy.

Abstract:
Insulin-secreting pancreatic beta cells are damaged and insulin receptors become resistant in people with diabetes mellitus, causing an increase in blood sugar. Changing dietary and exercise habits are the primary cause of diabetes mellitus's development. When left untreated, diabetes mellitus may cause a number of secondary problems that diminish quality of life over time. DR, DKN, DN, and DCM are the four most common secondary consequences of diabetes. Glycated proteins and AGE are formed when glucose interferes with proteins, which is a major cause of the aforementioned difficulties. Furthermore, AGE are known to produce cellular oxidative stress, inflammation, mitochondrial dysfunction, modification of ion channel states, and functional alterations of DNA & mRNA. In this publication, the effects of streptozotocin on the retinas of swiss albino mice are examined.

1. Introduction

Elevated blood sugar levels are caused by diabetes mellitus, a metabolic illness characterised by the destruction of insulin-secreting pancreatic beta cells and the subsequent resistance of the body's insulin receptors. Changes in lifestyle have a major role in the development of diabetes mellitus and its subsequent progression. The secondary problems that develop as a result of diabetes mellitus in a chronic state also have an impact on the patient's quality of life. Diabetic cardiomyopathy, kidney, neuropathy, and retinopathy DR are the primary secondary consequences. The formation of glycated proteins and AGE is largely to blame for these issues. In addition, AGEs are known to trigger cellular oxidative stress, inflammation, mitochondrial dysfunction, changes in ion channel states, and functional alterations of DNA and mRNA.[1]

Heart disease, rheumatoid arthritis, osteoporosis, stroke, and the ageing process may all be made worse by abnormalities at the cellular and molecular level, such as changes in cholesterol control, barrier function, and the activity of metabolic enzymes. These secondary problems may be divided into two groups based on their effects on the blood vessels. Too many intricate systems are involved in DR, making therapy difficult. Dexamethasone and intravitreal triamcinolone are two medicines used to lessen the severity of DR progression.[2-3]

Diabetic neuropathy comes in many forms, including those listed above as well as thoracoabdominal neuropathy, diabetic autonomic neuropathy, and third nerve palsy. The incidence rate is same for males and females. Male patients with type 2 diabetes are more likely to develop diabetic peripheral neuropathy,

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whereas female patients are more likely to develop morbidity from neuropathic pain. Although diabetic neuropathy may occur at any age, it is more common in the elderly and in those with severe, long-term diabetes. Some hypotheses have been put up as to what causes diabetic neuropathy, but no one knows for sure. [5-6]

It's common knowledge that this is a complicated undertaking. Overall hyperglycemic sensitivity, as well as risk factors like high lipids, blood pressure, smoking, and increased exposure to other potentially neurotoxic substances including ethanol 3, have a role in the development of symptoms in DPN. Natural fluxes, such as variations in muscular activity in response to gravity, occur during stability, which may be thought of as the warmth of a dynamic system to various anxieties, and local constancy is the sensitivity of the structure to internal distresses. In order to evaluate various indicators of postural influence, the effects of such natural changes were looked at. Multiple research on diabetic patients with neuropathic postural instability suggest that these patients have a worse capacity for maintaining posture than non-diabetic patients, are more unstable than non-diabetic individuals, and have positive relationships between falling and postural instability.[7-8]

2. Material And Methods

Animal

In this study, healthy 12-month-old Swiss albino mice (weighing 20-30 g) were employed. The animals were fed a regular laboratory diet and cared after by the central animal house at AIMST University. The pet could drink as much water as it wanted. Light and dark cycles of around 12 hours each were preserved. The ambient conditions in the animal housing facilities were designed to be 25 degrees Celsius and 50 percent relative humidity. Protocol for the use of animals in research at AIMST University was authorised. In accordance with AUAEC regulations, we provided animal care..

Induction of DR

In all, 7 sets of male adult mice (n = 8 each set) were used. Group-I was the typical comparison group. Group II was the DR group, which stood for diabetic retinopathy. STZ (35 mg/kg) was injected intraperitoneally, which marked the beginning of day 1 of the diabetes experiment. On day 3, the diagnosis of diabetes was confirmed with blood glucose testing. On day 7, 20 l of a 7% w/v STZ stock solution was injected intravitreally, hastening the development of retinopathy. CLSdosages of 125 and 250 mg/kg were administered orally for 21 days in a row as test compound treatments in Groups III and IV. Test compound groups V and VI received oral administration of either 10 or 20 milligrammes per kilogramme of body weight of AST for 21 days. Reference medication therapy was provided by group VII, which received DEX 10 mg/kg orally for 21 days. On days 3 and 21, the blood sugar level was calculated. On days 8 and 28, the participants' weight was recorded. various tests were used to evaluate the animals' behaviour at various intervals, including the visual cue function test, the optomotor response , the penta-maze , and the radial arm maze. TBARS, GSH, AR, and NSE activity were estimated in retinal tissue to corroborate the molecular pathways. To evaluate cholinergic neurochemical change in the CNS, the amount of AChE in brain tissue was calculated. Microscopic alterations in the retina were studied by means of histopathology.

Examining the microscopic structure of the retina in mice

After evaluating the mice's behaviour over the course of 28 days, their eyes were removed for study. Ten percent paraformaldehyde in a neutral buffered solution was used to preserve the tissues of the eyes. Paraffin wax served as the preparatory medium for the tissue block. The semi-automatic cryo-microtome was utilised to cut sagittal sections through blocks of implanted mouse ocular tissue. Tissues were sectioned at 5 m and then stained with Hematoxylin and Eosin. “Under 400x magnification, we studied the stained slides to look for microscopic anatomical alterations in the retinal layers of mouse eyeballs. The deviations were recorded and compared to findings in slices of tissue from normal and control animals. Vacuolations, retinal detachment, and the growth of blood vessels are not part of the typical arrangement of rod and cone ganglionic layers in mice.”

2.1 Biochemical Estimations

All the animals were given diethyl ether anaesthesia on day 28. Blood was drawn by puncturing the

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patient's tail vein, and glucose levels were assessed using a commercial Accu-Chek glucometer. After the animals had been put down, their retinas and brains were removed to determine the amounts of tissue biomarkers such as TBARS, GSH, AR, NSE, AChE, and total protein.

Assessment of lipid peroxidation through TBARS estimation:

The following equation was used to put a numerical value on the TBARS concentration**:**

$$\mathbf{MDA\ (nmol/ml)} = \frac{\delta\ \mathbf{O.D.sample}}{\varepsilon\ \mathbf{x\ PL}}\ \mathbf{x\ DF}\ \ldots$$

Absorbance variations over time are denoted by " O.D.," the extinction coefficient is "," and the route length and dilution factor are denoted by "PL" and "DF," respectively. MDA (in nmol/ml) was then multiplied by protein (in milligrammes) for an overall result. Amounts of malondialdehyde per unit of protein were reported in units of nanomoles.

2.2 Tbars Quantification as a Measure of Lipid Peroxidation

$$\mathbf{GSH} = \frac{\delta\ \mathbf{O.D.Standard} - (\mathbf{y} - \mathbf{intercept})}{\mathbf{Slope}}\ \mathbf{x}$$

This equation describes the transformation of one mole of oxidised glutathione into two moles of reduced glutathione, where " O.D." “Stands for the standard's absorbance changes, "y-intercept" stands for the y-intercept of the linear curve value from the standard plot, "slope" stands for the value obtained from the standard plot, "DF" stands for the dilution factor, and "2" stands for the number two.” GSH concentration was converted to a millimoles per litre (mmol/l) value and then combined with protein milligrammes. The overall amount of GSH was calculated as micromoles per milligramme of protein.

Estimation of total proteins:

The principle of in-vitro total protein estimation is described belowCalculating the Sum of All Proteins:

This article explains how in-vitro total protein concentration is determined.

$$\text{Protein} + Cu^{2+} \rightarrow \text{Protein} - Cu^{1+}\ \text{complex} \ldots\ldots\ldots\ldots\ldots \text{Step (1)}$$

$$\text{Protein} - Cu^{1+}\ \text{complex} + \text{Folin} - \text{Ciocalteu} \rightarrow \text{Purple colured complex} \ldots\ldots\ldots \text{Step (2)}$$

At the first stage, protein reacts quickly with Cu 2+ ions, forming the proteinCu1+ complex. The second stage involves treating the protein-Cu 1+ combination with Foiln-Ciocalteu reagents to produce the purple chromogen. A spectrophotometer was used to record the variations in absorbance of purple colour chromogen at 750 nm. The absorbance value (y-axis) was plotted against the concentration of the reference standard (x-axis), which was calculated by adding 0.2 to 2.4 milligrammes of bovine serum albumin (BSA) per millilitre of supernatant (mg/ml). The following equation was used to determine the concentration of total protein:

$$\mathbf{Total\ protein} = \frac{\delta\ \mathbf{O.D.test}}{\mathbf{Slope}}$$

The formula is as follows: where'O.D.' is the absorbance variation throughout the test and'slope' is the value derived from the standard plot. Protein content was reported in milligrammes per millilitre.

Statistical analysis

Mean and SD were used to summarise all data. Using Graph Pad Prism version 5.0, we ran two-way ANOVA and Bonferroni's post hoc test on the behavioural data, and one-way ANOVA and Tukey's multiple range test on the tissue biomarker data (TBARS, GSH, AR, NSE, and AChE levels). For statistical significance, a p-value of less than 0.05 was used..

3. Results

Gross anatomical alterations caused by DR and the role of AST

Significant vasculogenesis with enlarged blood vessels in the retina was seen after STZ treatment. When AST was compared to the DR-associated ocular alterations, a substantial reduction in vascular proliferation was seen. These outcomes were on par with those seen with the standard of care medication.

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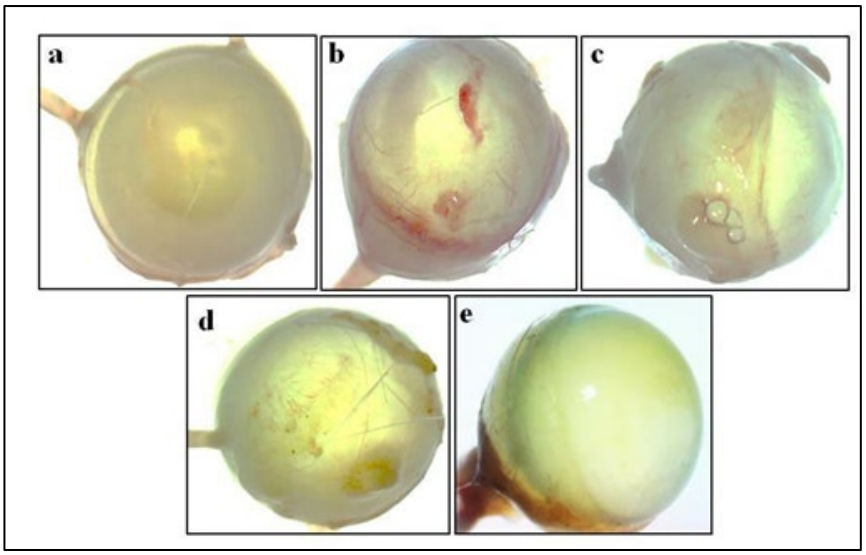

Figure1: Gross anatomical alterations due to DR and the role of AST. Gross structural differences between the control, DR, AST, and DEX groups are shown. Blood vessel growth was not seen in the retinal layer of mice, as shown. Vasculogenesis, or the growth of new blood vessels, was clearly visible in the retina. Vein proliferation decreased. An important reversal of DR-associated blood vessel growth. Displayed the possible protection of retinal layers from the DR.

Impact of CLS and AST on the Outward Manifestations of Disability

Eyeball opacities and axial length (diameter) in mice were not significantly altered after STZ treatment. In addition, there were no discernible differences between the abnormal and normal groups in terms of retinal colour, eye-to-body weight ratio, or water content. No differences were seen in the aforementioned mouse ocular gross anatomy when CLS (125 and 250 mg/kg; p.o.), AST (10 and 20 mg/kg; p.o.), and DEX (10 mg/kg; p.o.) were compared.

Micro-anatomical alterations in the retina caused by DR and the role of CLS and AST

Potential DR induction was seen after STZ treatment. The micro-anatomical change in the retina caused by DR is suppressed when mice are given either CLS Or AST. CLS therapy has been demonstrated to be more effective than AST treatment. CLS and AST have been demonstrated to have an ameliorative effect that is comparable to that of the reference medication administration, which is DEX.

Changes in vision and behavior due to DR and central auditory stimulation

Changes in visual behaviour and DR-associated retinal micro-anatomy were statistically significant (p 0.05) after STZ treatment. When compared to the DR group, the visual behavioural alterations measured by the VCFT, OMR, PM, and TM tests were considerably alleviated after administration of CLS and AST. CLS and AST had comparable effects to those seen in the DEX treated reference group. The next section will go into further depth about.

CLS and AST's impact on DR-caused VCFT

Visual behaviour in VCFT was significantly (p 0.05) impaired after STZ treatment, as shown by a longer latency to RVCP and lower TSTQ values compared to the control group. When compared to the DR group, the aforementioned VCFT responses were dramatically reduced when CLS and AST were administered. These ameliorative effects were found to be comparable to those seen in the DEX-treated reference group.

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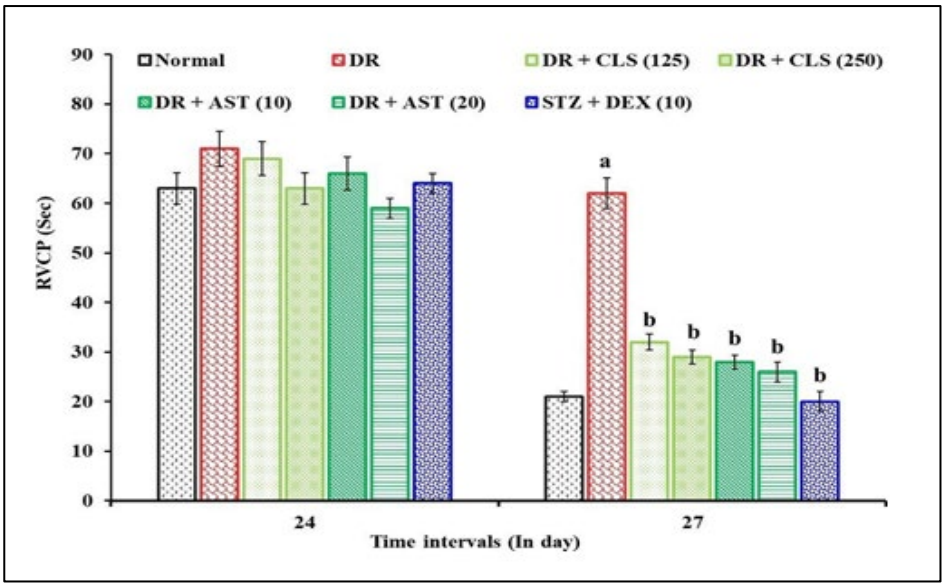

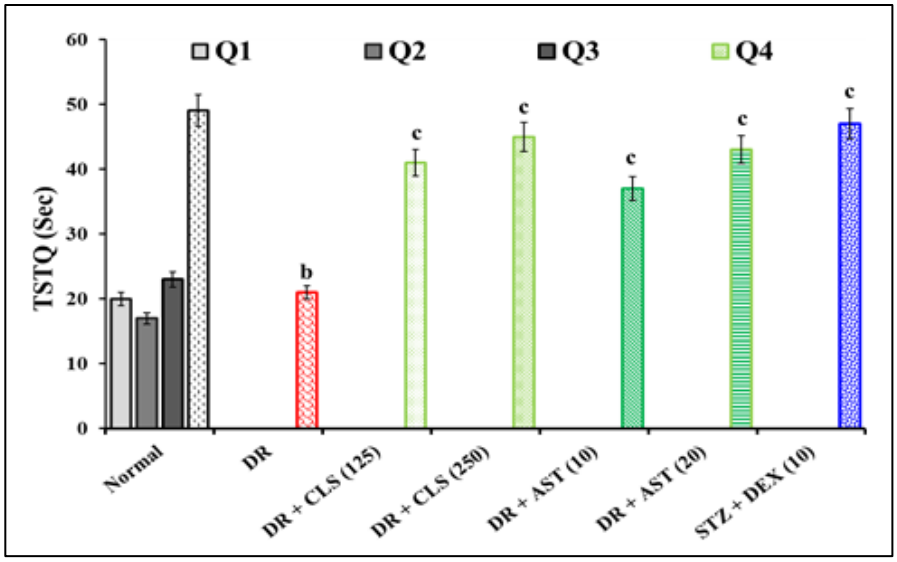

CLS and AST's impact on OMR test results after DR

The number of rearings and SFT values in the OMR test decreased significantly (p 0.05) after STZ administration. This was in comparison to the normal control group. When compared to the DR group, the responses shown in the aforementioned OMR test were markedly improved after administration of CLS and AST. These ameliorative effects were found to be comparable to those seen in a group administered with the reference medication DEX.

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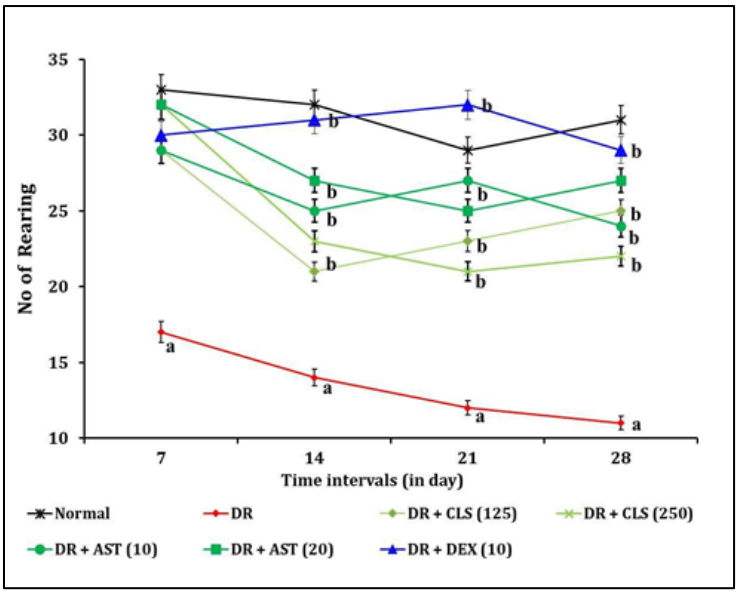

Figure 4: The role of CLS and AST in the OMR test for DR-induced rearing. In parentheses are the milligrammes per kilogramme of body weight.

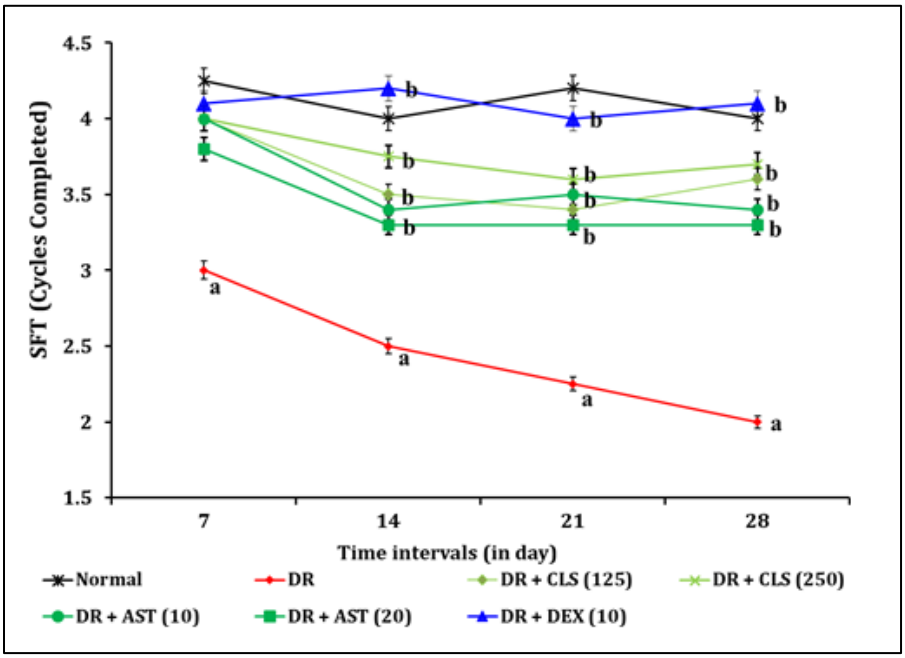

Figure5: The role of CLS and AST in the SFT triggered by DR in the OMR exam. The numbers within the brackets represent the dosage in milligrammes per kilogramme. With a total of eight mice in each group, data were presented as a mean SD.

"Significant (p 0.05) impairment of spatial imagery transformations was seen in the PM test after STZ administration, as shown by a drop in PR and an increase in the number of erroneous values compared to the normal control group." The PM test responses were considerably improved by the administration of CLS and AST compared to the DR group. Similar beneficial effects were seen between the DEX treated group and the reference medication group.

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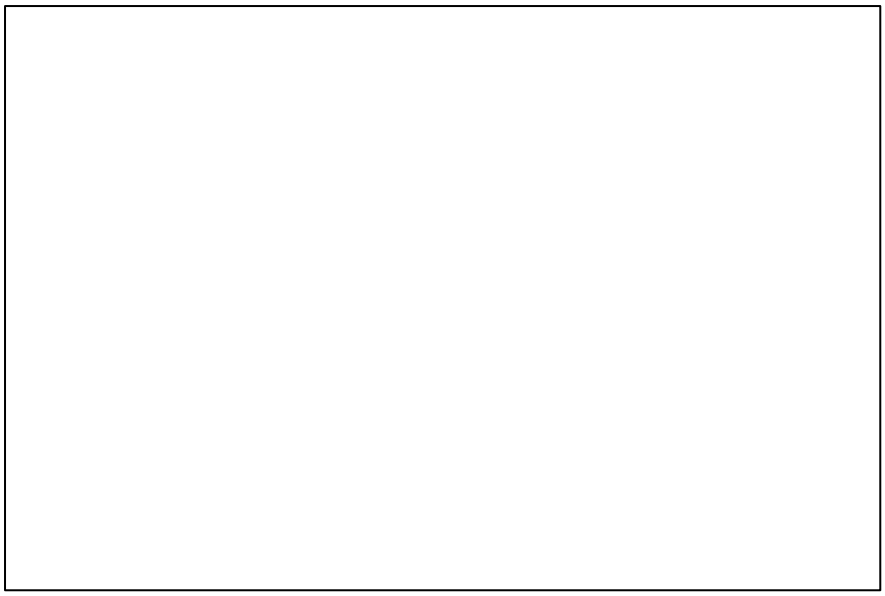
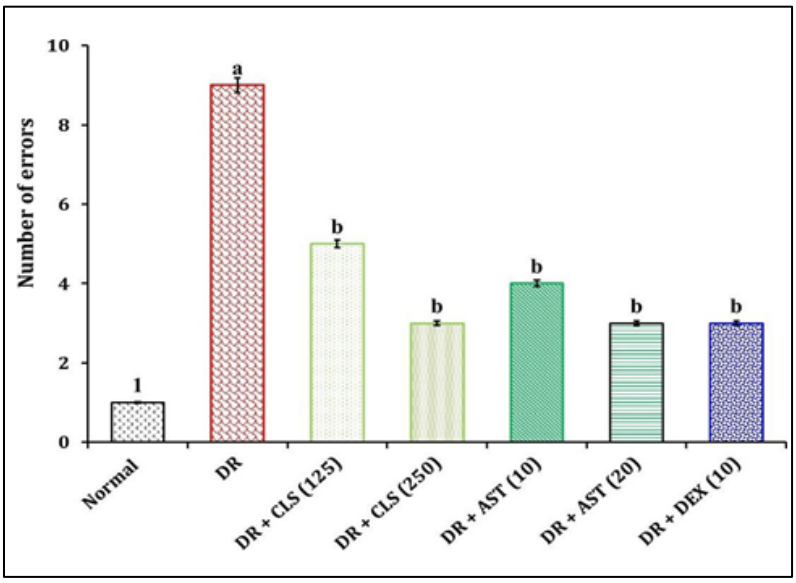


Figure 6: The role of CLS and AST in the PM test for DR-induced PR. Dosage is shown in milligrams per kilogram in parentheses.



There was a statistically significant (p 0.05) drop in PA and an increase in TL values in the TM test after STZ administration (35 mg/kg; i.p. and 20 l of 7% w/v; i.vit.). These results were compared to those of a normal control group. When compared to the DR group, the responses shown in the aforementioned TM test were considerably improved after administration of CLS (125 and 250 mg/kg; p.o.) and AST (10 and 20 mg/kg). These ameliorative effects were found to be comparable to those seen in a group administered with the reference medication DEX (10 mg/kg; p.o.). Figures 5.16 and 5.17 show the outcomes.

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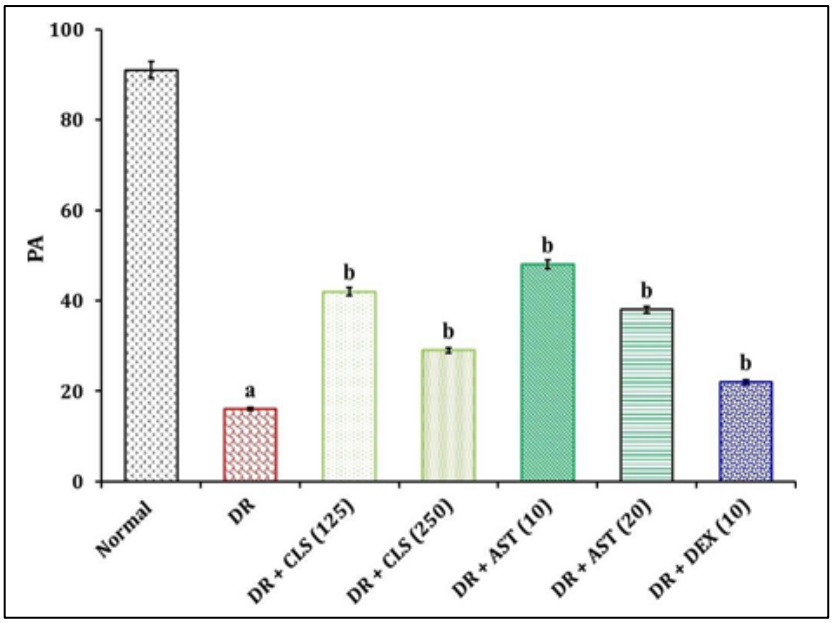

Figure 8: The role of CLS and AST in the PM test for DR-induced PA. Dosage is shown in milligrams per kilogram in parentheses.

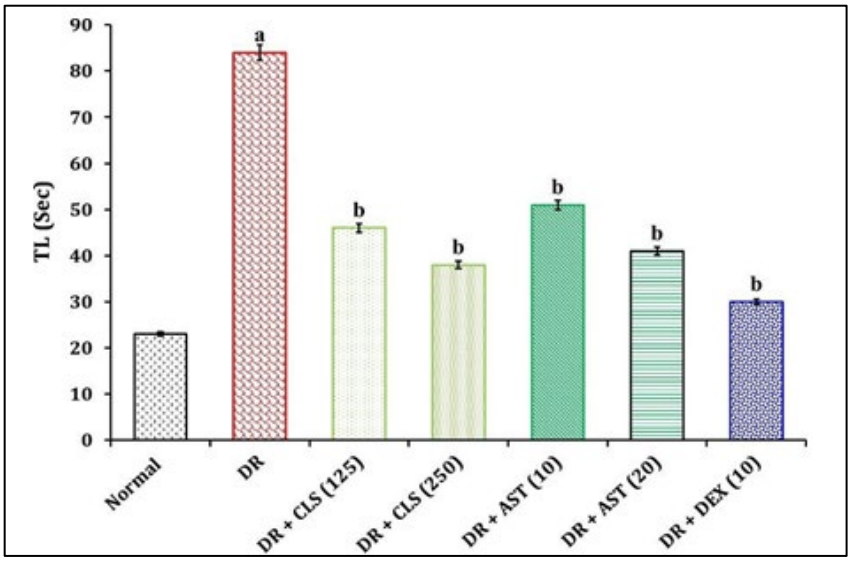

Figure 9: The role of CLS and AST in the PM test for DR-induced TL. Dosage is shown in milligrams per kilogram in parentheses.

All the animals were given diethyl ether anesthesia on day 28. Blood was drawn by puncturing the patient's tail vein, and glucose levels were assessed using a commercial Accu-Chek glucometer. After the animals were put down, their retinal tissues were harvested to measure variations in tissue biomarkers such as TBARS, GSH, AR, NSE, AChE, and total protein. The following sections go into depth on how we arrived at our estimates for each biomarker.

There was a substantial (p 0.05) increase in blood glucose level on days 3 and 28 after STZ treatment (35 mg/kg; i.p.; and 20 l of 7% w/v; i.vit.). It was a symptom of the worsening of diabetes-related problems associated with high blood sugar. When compared to the DR group, the STZ-induced high

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blood glucose level on day 28 is considerably reduced when CLS (125 and 250 mg/kg; p.o.) and AST (10 and 20 mg/kg; p.o.) are administered. In contrast, the raised blood glucose level caused by STX is somewhat attenuated by the administration of DEX (10 mg/kg; p.o.). Figure 5.18 shows the outcomes.

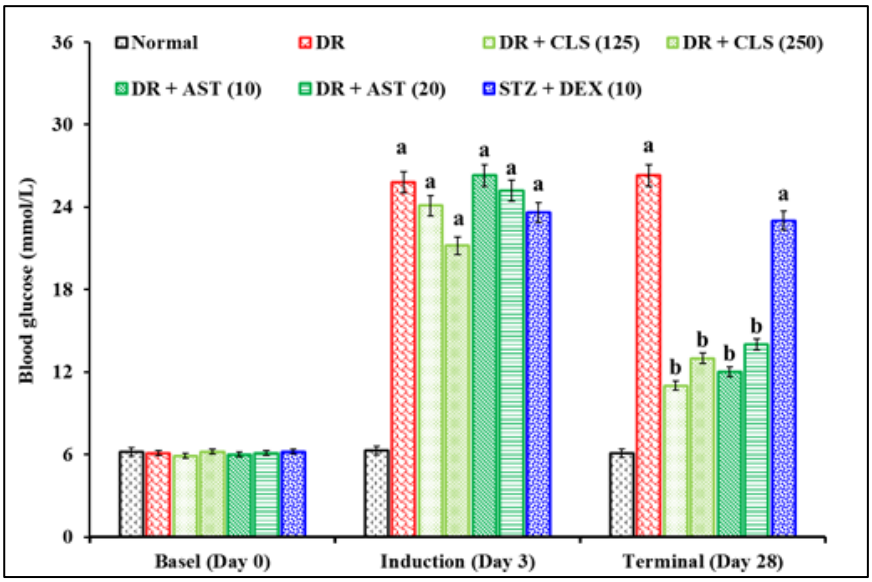

Figure10: Evaluation of glucose levels in blood among treatment groups. By the last day, the drop in groups 3, 4, 5, and 6 was statistically significant.

Changes in tissue biomarkers in response to streptozotocin:

When compared to the normal control group, STZ treatment resulted in statistically significant (p 0.05) changes in tissue biomarkers. When compared to the DR group, the STZ-induced alterations in tissue biomarkers were considerably mitigated by the administration of CLS and AST. In this model, CLS and AST have been shown to have a role in controlling blood sugar levels. These ameliorative effects were found to be comparable to those seen in a group treated with the reference medication, DEX.

Table1: Changes in tissue biomarkers in response to streptozotocin: impact of cyclosporine A and ascorbic acid

Groups	TBARS (nmol/mg of protein)	GSH (µmol/mg of protein)	AR (U/min/mg of protein)	NSE (pg/ml)	AChE (µmol/min/mg Of protein)
Normal	1.25±0.024	26.12±1.64	7.52±1.13	17.3±1.5	113.12±1.9
DR(35)	3.65±0.078[a]	7.94±1.32[a]	19.26±0.91[a]	85.7 ±2.9[a]	278.79±1.4[a]
DR+CLS(125)	2.01±0.045[b]	21.74±1.13[b]	11.08±0.71[b]	29.3±1.3[b]	155.92±1.4[b]
DR+CLS(25	1.46±0.016[b]	23.65±1.27[b]	9.84±1.26[b]	23.8±2.1[b]	142.17±1.6[b]

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DR + AST(10)	2.18±0.091[b]	19.49±1.06[b]	12.19±0.83[b]	32.2±1.2[b]	165.53±1.1[b]
DR + AST(20)	1.82±0.063[b]	25.82±1.17[b]	10.56±1.04[b]	23.4±1.6[b]	148.17±1.5[b]
DR+DEX(10)	1.31±0.082[b]	25.91±1.04[b]	8.93±0.78[b]	19.2±2.2[b]	135.93±2.2[b]

4. Discussion

The induction of DR by STZ was statistically significant (p 0.05). It manifests itself in the vitreous regions as dilated blood vessels, extravasation of blood, widespread vacuolations, and a disruption of the retinal cell layers. Visual behavioral tests, such as the VCFT, OMR, PM, and TM , were also administered to DR animals. Similarly, DR raised TBARS and reduced GSH in animal tissues. [9-10]

This was also the case for AR, NSE, and AChE. When CLS and AST were given to rats, the micro-anatomical alterations of the retina, the changes in visual behavior, and the biomarkers of tissue damage caused by DR were reduced. The effects were the same in the group given the reference medication, DEX.[11-12]

By altering gut flora, the more well-established version of ASX, ASX n-octanoic acid diester, has been shown in recent study to alleviate insulin resistance in mice fed a high-sucrose and high-fat diet. The research revealed that injections of Xanthophyllomyces dendrorhous ASX into the guts of mice given a high-fat diet were associated with changes in both total cholesterol plasma and triglyceride levels. In contrast, some studies did not find any significant impacts on insulin and glucose metabolism For instance, concluded that ASX did not significantly affect hypoglycemia. [13-15]

5. Conclusion

Microanatomical, visual-behavioral, and biochemical alterations caused by DR were alleviated by treatment with CLS and AST. These findings paralleled those of the DEX-treated group. Since CLS and AST have the ability to exert antioxidant, anti-inflammatory, and regulatory effects on neural, endocrine, and chemical systems, they should be considered for use as a natural medication in the treatment of DR.

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