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# THE EFFICACY OF AESCIN ON DIABETIC NEPHROPATHY: A STREPTOZOTOCIN-INDUCED DIABETIC RAT MODEL

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**Abstract:**

***Introduction***: *Hyperglycemia triggered by diabetes can cause damage to the kidney making the renal filters leaky. Cytokines like transforming growth factor $\beta_1$ and raised oxidative stress in the body are believed to be accountable for the generation of Diabetic Nephropathy.*

***Objective:*** *To observe the ameliorative effect of Aesculus hippocastanum (AH) drug in streptozocin induced diabetic nephropathy in albino wister rats.*

***Materials And Methods:*** *Twenty one albino wister rats were included and divided in to three groups, control, diabetes and diabetes + Aescin. The control group received normal diet. Both the diabetes groups were induced diabetes by administration of streptozocin injections intraperitoneally. No drug was given to diabetes group while diabetes + Aescin group received drugs for 4 weeks. At the end of experiment, the glomerular area was assessed, severity of sclerosis was observed and levels of malondialdehyde, TGF- $\beta_1$, blood urea and sugar levels and creatinine were analyzed.*

***Results:*** *The results showed that glomerular regions, sclerosis intensity, levels of malondialdehyde, TGF- $\beta_1$, blood sugar, urea, and creatinine were found to be reduced in the diabetes + Aescin group. It is believed that raised blood sugar levels induce diabetic nephropathy. Aesculus hippocastanum extract improved diabetic nephropathy without lowering blood sugar level.*

***Conclusion:*** *Aesin is found to have positive role on the functional status of kidney and histological betterment in diabetic nephropathy.*

***Keywords:*** *Aesin, kidneys, diabetic nephropathy, rats, oxidative stres*

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## INTRODUCTION:

Diabetic nephropathy is a frequent and serious manifestation of diabetes, occurring in 20 to 40 percent of diabetic patients.[1] Diabetic Nephropathy is represented by disseminated glomerulosclerosis and nephrotic syndrome, resulting due to micro vascular disease of capillaries in the renal glomeruli.[2]

Various pathways have been suggested for the progress of diabetic nephropathy, one of which is glomerular hyper-filtration and hyper-perfusion associated with increased blood glucose levels.[3] Sodium depletion is caused by hyper filtration of glomerular glucose which leads to diminished sodium reception to the macula densa. Angiotensin 2 level is raised due to feedback mechanisms activated by macula densa cells, which results in sclerosis and fibrosis in glomerulus owing to activation of transforming growth factor $\beta_1$.[4] Additional hypothesized mechanism for diabetic nephropathy is non-enzymatic glycosylation of tissue proteins caused by long term elevated blood glucose levels. The glomerular basement membrane is severely damaged by the production and degradation of progressive glycation output products.[5]

Moreover, such progressive compounds can adjust signal transduction, which are responsible for the disease progression of diabetic nephropathy through altering signaling compounds like hormones, cytokines and free radicles.[1]

Disease progression of Diabetic nephropathy has also been attributed to oxidative stress in the latest research studies. Hyperglycemia apparently causes oxidative stress prior to the appearance of the clinical features of Diabetes. [6] The initial episode leading to Kidney deterioration is the raised generation of mitochondrial reactive oxygen species. [7] It occurs due to increased provision of pyruvic acid in Kreb's cycle, which is due to rapid glucose entry into cell. The aforementioned steps occur in endothelial cells of kidneys, which do not have ability to downgrade the transporter-1 of glucose even after raised sugar levels.[8] These steps lead to the generation of chains of detrimental steps like protein kinase C activation, formation of advanced glycation output compounds, TGF-β upregulation and utilization of cytosolic NAPDPH.[8] In recent research studies, the compounds of lipid oxidation in the intra-glomerular mesangial meshwork were identified through microscopic observation of renal biopsy tissue. [9]

Aescin (Aesculus hippocastanum, AH), the active ingredient found in horse chestnut has been found to exhibit at least three pharmacological actions: anti-inflammatory, anti-edematous and venotonic.[10] AH has been shown to have a positive effect in the treatment of various diseases such as arthritis and tendonitis etc.[11] Recent studies have also shown Aescin can reduce the level of oxidative stress and inflammation by decreasing plasma levels of inflammatory cytokines such as like tumor necrosis factor (TNF-a) and interleukin (IL-1b) in various tissues.[12]

The objective of this study was to assess the anti-oxidative and protective effects of Aescin in a streptozocin induced diabetic rat model.

## MATERIALS AND METHODS:

### ANIMAL PROTOCOL:

The animal ethical approval was granted by the Isra University animal research ethical committee. 21 male albino wistar rats with an average weight of 200 to 220g were included in the study. The animals were allowed free access to normal chow diet in the form of pellets ad libitum and all rats were kept in stainless steel cages. The environmental conditions of the laboratory were kept optimum for the proper behavior and living of the rats. The rats with blood sugar levels more than 120 mg/ml were excluded from the study

### EXPERIMENTAL PROTOCOL

The rats were divided into two groups, control group having 7 rats, and experimental group having 14 rats. The control group rats were given normal chow diet. The experimental group received 60 mg/kg of streptozocin intravenously. [13] As single dose of streptozocin causes type 1 diabetes After 24 hours, blood sugar levels were noted with the help of glucometer. Rats were fasted for 8 hours and blood sugar levels more than 250 mg/dl were labelled as diabetic rats. The experimental rats were divided into two groups: the diabetic group (n=7) and the diabetic + Aescin group (n=7). The diabetic group received no drugs, while the diabetic + Aescin group were administered 50 mg/kg/day aescin by oral gavage for 4 weeks. The control and diabetic group rats were only administered 20 ml/kg/day of clean water via oral gavage.

After 4 weeks, the rats were anesthetized by intraperitoneal injection of 80 mg/kg of ketamine and 7mg/kg of xylazine. Blood samples were obtained by cardiac puncture for biochemical analysis. Urine samples were collected by the use of stick and for histopathological analysis kidneys were removed.

### HISTOPATHOLOGICAL EXAMINATION OF RENAL TISSUE:

Kidneys were placed in 4% formaldehyde. 5 micrometer sections were obtained from paraffin embedded blocks and were stained with hematoxylin and eosin stains and were examined under light microscope for histopathological analysis. About 50 glomeruli from every single rat were included for observation.

**BIOCHEMICAL ANALYSIS:**

The blood samples from the control and experimental rats were centrifuged at 3000 rpm for 10 minutes at optimum temperature and were allowed to store at 20 centigrade. Cytokines levels in plasma were calculated by Enzyme linked immunosorbent assay (ELISA) via TGF-β. Lipid peroxidation was assessed in blood by calculating Malondialdehyde (MDA) being served as a marker for oxidative stress. Levels of blood sugar, urea and creatinine were calculated by BioAssay technology ELISA kits.

**STATISTICAL ANALYSIS:**

The data was analyzed using SPSS (Statistical Package for Social Sciences) version 22.20. One way analysis of variance (ANOVA) was applied to compare the means between control and experimental groups. Statistical significance was taken at $p \leq 0.05$.

**RESULTS:**

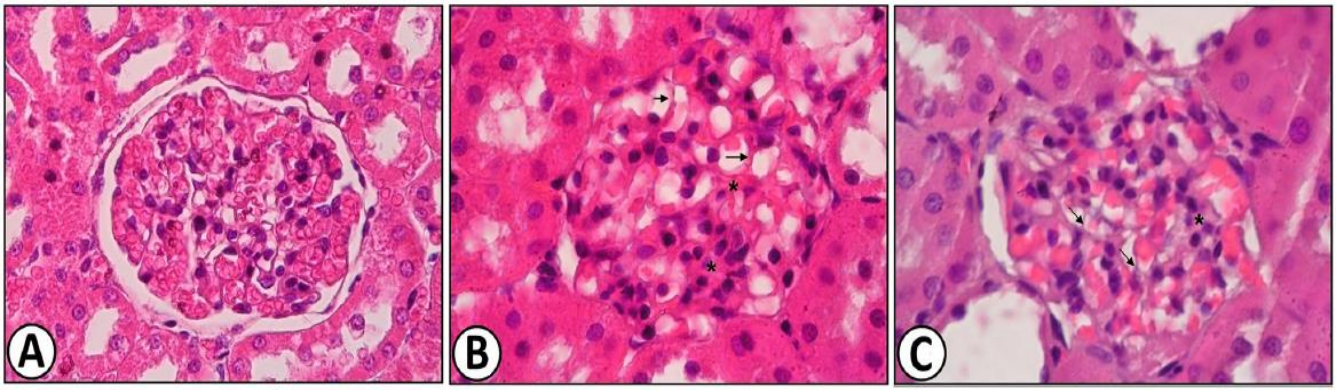
Regarding the histopathological observations, there was marked thickening in the diameter of the glomerular basement membrane. The intra glomerular mesangial matrix was also increased. Such changes were observed in the diabetes group as compared to the control group. However, in the experimental diabetes + Aescin group, thickening of the basement membrane of the glomerulus was reduced and the mesangial matrix was lowered as compared to diabetes group. The histopathological observations are mentioned in Fig. 1.

Regarding the blood parameters, the diabetes group rats showed significant increase in the levels of plasma TGF-β, malondialdehyde, blood urea and creatinine as compared to the control group. In the diabetes + Aescin group, there was significant decrease in the plasma TGF-β, malondialdehyde, blood urea and creatinine levels. In the streptozocin-induced rats, the blood sugar levels were raised significantly. The blood sugar levels as compared between the diabetes and the diabetes +Aescin group were found to be nearly same. The results of plasma TGF-β, malondialdehyde, blood urea and creatinine levels are given in Table 1.

**Table-1. Analysis of Severity of glomerular sclerosis, levels of MDA, TGF- β, Urea, Creatinine and Blood glucose.**

| Parameters | Control Group | Diabetes Group | Diabetes + Aescin group |
|---|---|---|---|
| **Severity of glomerular sclerosis** | 0.37 ± 0.29 | 4.49 ± 0.38* | 1.49 ± 0.41** |
| **MDA** | 67.48 ± 5.51 | 381 ± 47.4* | 146.2 ± 19.68*** |
| **TGF-β (pg/ml)** | 5.31 ± 0.71 | 34.62 ± 7.43* | 14.88 ± 2.64* |
| **Urea (mg/dl)** | 24.60 ± 3.8 | 88.24 ± 8.7* | 48.62 ± 2.32* |
| **Creatinine (mg/dl)** | 0.41 ± 0.08 | 1.64 ± 0.24* | 0.74 ± 0.06*** |
| **Blood Glucose (mg/dl)** | 98.6 ± 4.1 | 402.12 ± 37.5* | 388.42 ± 28.65 |

*** = p < 0.0001, diabetes group compared with control;**
**** = p < 0.05 Diabetes+Aescin group compared with diabetes group;**
***** = p < 0.0001 Diabetes+Aescin group compared with diabetes group.**



## DISCUSSION:

In this study, effect of Aescin was assessed in streptozocin induced diabetic nephropathy in rats. The results showed that Aescin therapy prevented development and/or progression of diabetic nephropathy.

In previous scholarly articles, Aescin therapy has shown to increase the level of endogenous anti-oxidants in various organs like kidneys, intestine and liver. [14-16] In the present study, the anti-oxidant effects of Aescin were evident by MDA levels in the Aescin receiving animals as compared to the diabetic group. In addition, Aescin therapy also reduced the plasma levels of inflammatory cytokines TGF-β. These findings are similar to the findings of Onur elmas et al. (2016) who also reported that Aescin therapy was able to reduce the levels of TGF-β, a cytokine which has been held responsible for causing the development of diabetic nephropathy.[17] In the present study, Aescin therapy was also able to reduce glomerular sclerosis, halting the development of diabetic nephropathy; seemingly due to its effects on reducing oxidative stress and inflammatory cytokine levels.

Clinically, Aescin has shown to have a positive effect in conditions such as hemorrhoids, chronic venous insufficiency (CVI) and post-operative edema. [18] In addition, Aescin has also shown to inhibit the action of hyaluronidase, an important enzyme involved in proteoglycan degradation. [19] The buildup of leucocytes in CVI affected limbs leading to stimulation and discharge of such enzymes are considered a significant patho-physiological pathway of CVI.[19] In conditions of inflammation, the mitochondrial machinery responsible for the process of oxidative phosphorylation is compromised in endothelial cells leading to hypoxia-induced activation of endothelial cells. [20] This eventually leads to neutrophil adherence and activation as well as release of pro-inflammatory molecules like prostaglandins and platelet-activating factor. [21] Recent experimental studies have revealed anti-inflammatory actions of Aescin and suggested the fact that Aescin exhibits glucocorticoid like anti-inflammatory effects. [22] Similarly, in our study the prevention of diabetic nephropathy by Aescin therapy can be attributed to the glucocorticoid-like effects of Aescin, resulting in decreased TGF-β levels.

There were some limitation in our study. Renal levels of cytokines and anti-oxidants were not assessed. Moreover, urine parameters such as proteinuria and blood parameters such electrolyte imbalance were also not examined due to limited funds and resources.

It was concluded that Aescin therapy ameliorated diabetic nephropathy without decrease in blood glucose levels and can play a vital role in the treatment of this disease as an adjuvant therapy.

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