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HISTOLOGICAL STUDIES ON ETHANOLIC EXTRACT OF ANNONA SQUAMOSA LEAVES TREATED STZ-INDUCED DIBETIC ALBINO RAT KIDNEY

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Abstract: The present study has been conducted to identify the Histological variations in ethanolic extract of Annona squamosa leaves treated stz-induced dibetic albino rat kidney. The histopathological observations were made to elucidate the relationship between lipid peroxidation and tissue damage in diabetic condition. The pathological changes in kidney might be due to increased free radical production and elevated lipid peroxidation. Whereas, in *Annona* treated, Glibenclamid and *Annona* treated diabetic rats the tubules and glomeruli damages caused by diabetes were recovered. In conclusion, it revealed that one month treatment with selected dosage of *Annona* is beneficial in countering the alterations in antioxidant enzyme system, oxidative enzymes and lipid metabolic profiles in Wistar strain rats. The changes in markers of oxidative stress which include triglycerides, phospholipids MDA content and total cholesterol indicate efficient adaptative machinery of oxygen species that was operated in the renal tissue in detoxification of antioxidant system that are produced due to diabetes. The study concluded that *Annona* treatment in diabetic rats improved the metabolic efficiency as well as the health status.

IndexTerms: **Diabetic, annona squamosa, histopathological.**

I.INTRODUCTION

In normal conditions glucose is an essential fuel for the body. In diabetic condition glucose levels were increased in the blood called hyperglycemic condition, which effects the metabolic activities in the body. Blood glucose levels are not constant; they rise and fall depending on the body's needs, regulated by hormones. Oral hypoglycemic drugs were used as a part of treatment to control diabetes.

Kidneys filter wastes and toxins out of the blood and keep it balanced. Complications of prolonged excess blood glucose can affect small blood vessels throughout the body including kidney in which the kidneys fail to filter the blood. Waste that would ordinarily be excreted remains in the blood causing severe problems. Oxidative stress has been shown to play a role in the causation of diabetes mellitus. Antioxidants have been shown to have a role in the alleviation of diabetes mellitus (Oberley,1988). In diabetes mellitus, Oxygen Free Radicals (OFRs) are generated by stimulating H_2O_2 in-vitro, as well as in-vivo, in pancreatic *ß*-cells (Halliwell & Gutteridge, 1989). OFR-scavenging enzymes can respond to conditions of oxidative stress with a compensatory mechanism that increases the enzyme activity in diabetic rats (Yam *et al.*, 1978).

The therapeutic efficacy of many indigenous plants, for various diseases has been described by traditional herbal medicinal practitioners. In Ayurveda number of medicinal plants has been described for diabetes among these *Annona squamosa* is one of the antidiabetic medicinal plant.

Annona Squamosa:

Figure-1: *Annona squamosa* plant

Annona squamosa, also called **sugar-apple** or **sweetsop** is a species of *Annona* native to the tropical Americas and widely grown in El Salvador, India, Pakistan and Philippines. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has antifertility, antitumor and abortifacient properties.

The previous studies demonstrated the antidiabetic effect of *A. squamosa* in streptozotocin (STZ)-induced diabetes mellitus in rats (Kaleem, 2006). Therefore, the present study aimed to examine the oral administration of *A. squamosa* ethanolic extract of leaves on the STZ induced diabetic rats.

Blood glucose levels were measured to know the state of regulation of diabetes under treated and untreated conditions. Histopathological studies were carried out in all the experimental groups to assess pathological situation of the kidney.

II. Material and methods:

2.1 Preparation of Extracts

The leaf powder of *Annona squamosa* was extracted according to Trease and Evans (1994). The leaf powder was extracted with 5 volumes of 95% Ethyl alcohol, then distilled and concentrated under reduced pressure at temp (40^0C) in the rota evaporator. A dark green, semi solid residue was obtained which was stored at 4^0C and used for further studies.

2.2 Induction of Diabetes in rats

Diabetes was induced in healthy male Wistar Albino rats aged 3 – 4 months, with body weights ranging from 160 $\pm$ 20g, by single intra peritoneal injection of freshly prepared streptozotocin (50 mg/kg b.w.) dissolved in ice cold 0.1 M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12-15 hours (Rakieten *et al.*, 1963). Since STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release due to destruction of βcells, 8 hrs after STZ administration the rats were kept for next 24 hours on 15% glucose solution to prevent hypoglycemia. Diabetes was assessed by determining the fasting blood glucose after 48 hrs of injection of STZ. The blood glucose levels in STZ rats were increased markedly higher levels than normal. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (blood glucose level $\geq$ 250g) were selected and used for the study.

The protocol of this study was submitted to Institutional Animal Ethics committee and approved in its resolution: (Resolution Number438/01/a/CPCSCA/IAEC/SVU/KSR-1 dt: 11-9-2008)

2.3 Care and Maintenance of Experimental Animals:

Pathogen free, Wister strain male albino rats of young age 3 months age group and body weight 160 $\pm$ 20g considered as 'Young age' as per the life span of Wister strain, Albino rats were used in the present study. The rats were housed in clean polypropylene cages under hygienic condition with photo period of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan Lever Ltd., Mumbai) and water *ad libitum.*

2.4 Grouping of Animals:

Group I Normal Control (NC): Six rats were received 0.9% Nacl/kg b.w. via orogastiric tube for a period of one month.

Group II Ethanol extract of As (Annona treated-AT) Six rats were received the ethanolic extract of *Annona squamosa* (As), 300 mg/kg. b.w via orogastric tube for a period of one month.

Group III Diabetic Control (DC): Six rats were used as diabetic control rats by the induction of STZ (50mg/kg) intraperitonial injection after 18 hours of fasting.

Group IV Diabetic + Glibenclamide (Di+Glbt): Six rats were received the *Glibenclamide* 20 mg/kg b.w. via orogastric tube for a period of one month.

Group V Diabetic + Ethanol extract of As (Di+Annona treated-At): Six rats were received the ethanolic extract of *Annona squamosa* (As), 300mg/kg, b.w. via orogastric tube for a period of one month.

The animals were sacrificed after 24 hrs of the last treatment by cervical dislocation and the kidney tissue

was excised at 4^0C. The tissue was washed with ice cold saline and immediately stored in deep freezer at -80^0C for biochemical analysis.

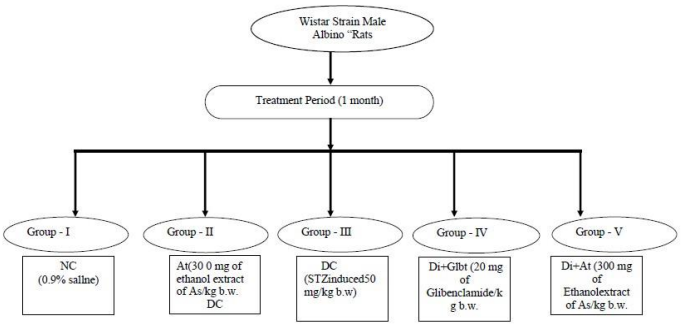


Table 1: Experimental Design Chart

2.5 Procurement of chemicals:

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qyakugebs (Mumbai, India).

2.6 Preparation of the histological samples:

Kidneys from Normal control, Annona treated, diabetic control, diabetic plus glibenclaimade, and diabetic plus Annona treated rats were collected after sacrificing the rats with cervical dislocation. The specimens were fixed in neutralized formalin, dehydrated with ethanol and embedded in paraffin wax (56^0). Serial sections (5 µm) were taken and stained with haematoxylin and eosin. The stained sections were observed under microscope and the histological changes were recorded with the helpof a pathologist.

III. Results:

3.1 Histological Changes:

Histological examinations of the kidneys by light microscope are figured in plate 1, 2, 3, 4, 5, 6. In *Annona* treated rats, Degeneration of tubular epithelium, Dilatation of renal tubules and Degeneration of glomeruli (Focal degeneration). Congestion of blood vessels were observed

In Diabetic rats (STZ) Severe tubular degeneration, greater cystic dilatation of tubules and degeneration of glomuruli were observed. Focal necrosis of tubules. Severe damage compared to Glibenclimad treatment.

In *Annona* treated diabetic rats Degeneration of glomuruli (Hyaline castis), degeneration of tubules and Dilatation of Bowmen's capsule were observed. Congestion of blood vessels were also observed.

Group	Intact/ Normal Glomerulus	Inflammatory changes	Degenerative/ Necrotic renal tubules	Regenerative renal tubule	Damage in Bowmen's capsules
G-I	+	-	-	-	-
G-II	-	+	+	-	-
G-III	-	-	+	-	+
G-IV	+	+	-	-	-
G-V	+	+	-	+	-

(+) = Presence

(-) =Absence

Table 2: The effect of *Annona* extract on kidney in non-diabetic rats, untreatedand treated diabetic rats

PLATE – 1

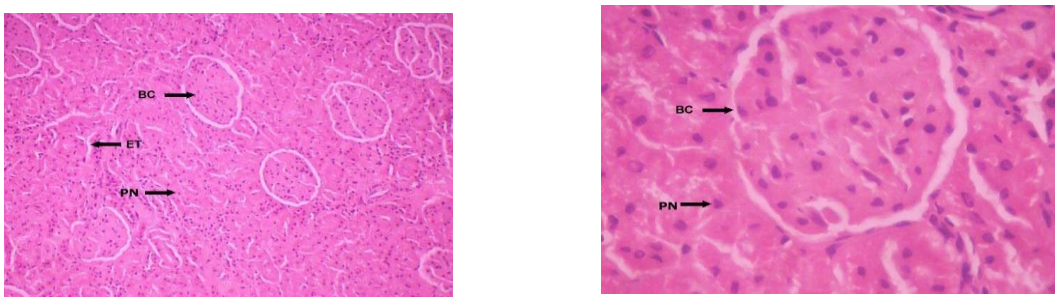


Figure 2: Histopathological Changes in Kidney of Normal Control and experimental rats with lower magnification (10X); And Higher magnification (40X). Normal rat kidney architecture shows glomeruli (GM) and proximal convoluted tubules. BC-Bowman's capsule; ET -Renal tubule; PN-Pycnotic nuclei

PLATE – 2

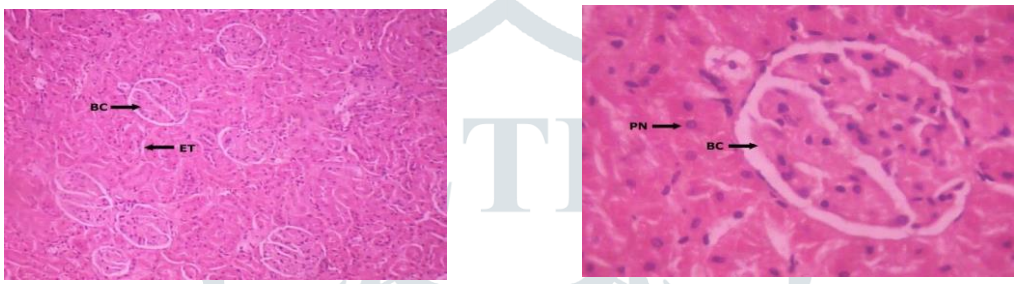


Figure 3: Histopathological Changes in Kidney of Plant extract Control (A.S) and experimental rats with lower magnification (10X); And Higher magnification (40X).. Plant extract control rat kidney architecture shows mild glomerular (GM) inflammation with lymphocytes and mild degenerative changes in renal tubules. BC-Bowman's capsule; ET -Renal tubule; PN- Pycnotic nuclei

PLATE-3

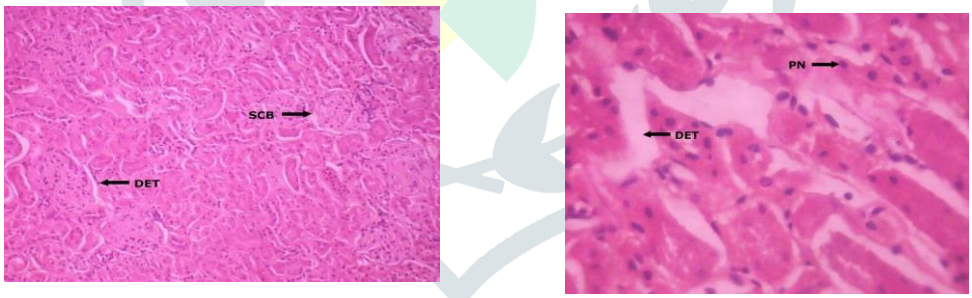


Figure 4: Histopathological Changes in Kidney of Diabetic Control (STZ Induced) and experimental rats with lower magnification (10X); And Higher magnification (40X)... STZ Induced rat kidney shows Glomeruli architecture is damaged within folding of the Bowman's capsules and mild necrotic changesin renal parenchyma. SCB-structural changes in bowman's capsule; DET- Degenerative changes in renal tubules; PN-Pycnotic nuclei

PLATE – 4

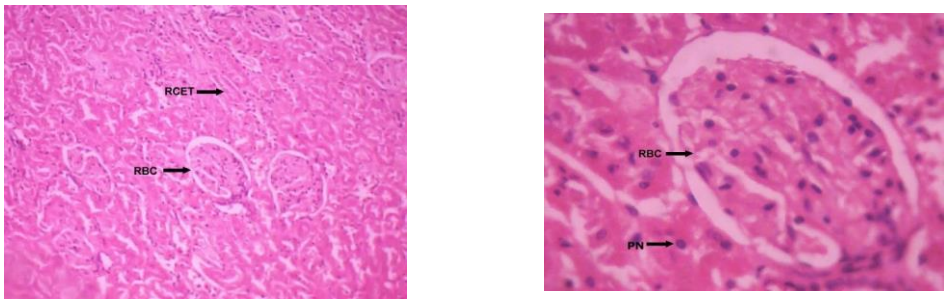
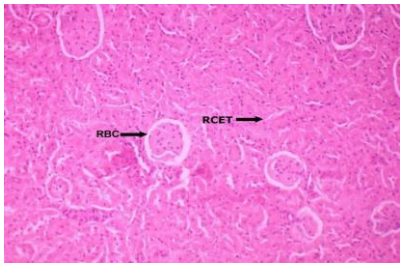


Figure 5: Histopathological Changes in Kidney of Diabetic Control (STZ Induced) +Treated with glybencliamade and experimental rats with lower magnification (10X); And Higher magnification (40X)..Glebenclamide treated STZ Induced rat kidney shows Intact Glomerules architecture of cell is normal and mild inflammatory changes. RCET-Regenerative changes in elongated renal tubules; RBC-Regenerative bowmans capsule; PN-Pycnotic nuclei

PLATE – 5



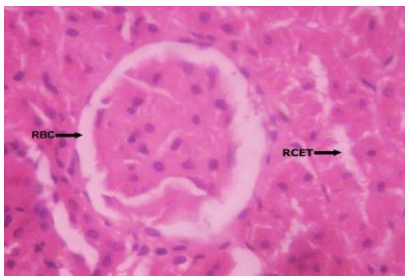


Figure 6: Histopathological Changes in Kidney of Diabetic Control (STZ Induced) +Treated with Plant extract (ethanolic extract Annona Squamosa leaves) and experimental rats with lower magnification (10X); And Higher magnification (40X). Plant extrct treated STZ Induced rat kidney shows normal, healthy and functional Glomeruli and regenerative renal tubules with minimal inflammation is seen.No evidence of any necrotic changes. RCET- Regenerative changes in elongated renal tubules; RBC-Regenerative bowmans capsule

IV. Discussion:

From the above observations it is evident that in STZ-induced diabetic rats marked histopathological changes in kidney tissue were observed. In diabetic rats severe tubular degeneration, greater cystic dilatation of tubules and degeneration of glomuruli were observed. Focal necrosis of tubules and severe damage compared to control group.

Since kidney is the major target organ in diabetic condition, degeneration of glomeruli H (Hyaline castis), degeneration of tubules and dilation of bowmens capsule and congestion of blood vessels was also observed.

In *Annona* treated diabetic rats there is less damage compared to diabetic rats. In this group there was mild to moderate Cystic dilatation, tubules and glomuruli appears to be restored.

Hence *Annona* has the capacity to reverse the damage caused by STZ. Therefore, in *Annona* treated diabetic rats there is less damage compared to diabetic rats.

Hence to conclude *Annona* treatment to diabetic rats has reversed the damagewhich was caused by STZ in the kidney tissue. Also, the antioxidant compounds of *Annona* has been played a major in repairing the tissue damage.

V. Conclusion:

The present findings revealed that one month treatment with selected dosage of *Annona* is beneficial in countering the alterations in antioxidant enzyme system, oxidative enzymes and lipid metabolic profiles in wistar strain rats. The antioxidant defense system which plays a major role in countering the free radicals in diabetic rats were reversed back to normal levels when *Annona* issupplemented. The oxidative enzymes also reversed back to normal control values. The changes in markers of oxidative stress which include triglycerides, phospholipids, MDA content and total cholesterol indicate efficient adaptative machinery of oxygen species that was operated in the renal tissue in detoxification of antioxidant system that are produced due to diabetes. *Annona* treatment to diabetic rats may be beneficial to improve the metabolic efficiency and thereby improve the health status. Thus, *Annona* may be useful in the formulation of herbal drugs which can be used in the treatment of diabetes. Hence, we recommend that further studies are mandatory to establish the precise nature of *Annona* active constituents as well as their mechanism of action.

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