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Effect of methanolic leaf extract of Coccinia *grandis* on histopathology of Liver, Kidney and Intestine of STZ-induced diabetic Wistar rats.

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ABSTRACT

Coccinia *grandis* has been widely used in folk medicine for a number of therapeutic purposes. The aim of the study is to evaluate the methanolic leaf extraction of Coccinia *grandis* on pathology of kidney, liver and intestine in STZ induced diabetic rats. Diabetes was induced using i.p injection of STZ of 50 mg/kg body weight. The treatment schedule with Coccinia (200mg/kg body wt,) of diabetic rats was set for 21 days. Metformin (150mg/kg body wt.,) was used as standard reference drug. Pathology of all vital organs such as kidney, liver and intestine were studied. Hepatic tissue of treated diabetic rats showed significant improvement compared to untreated diabetic rats. Intestine histology of diabetic revealed structural changes in height and thickness of microvilli, space between microvilli with excessive proliferation of epithelium within microvilli which was improved by treating diabetic rats. Histopathological analysis of kidney confirms the protective effect of Coccinia. In conclusion the present study indicates a significant protective effect of Coccinia *grandis* on histology of liver, kidney and intestine.

Keywords: Coccinia *grandis*, Kidney, Liver, Intestine, Pathology.

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INTRODUCTION

Diabetes mellitus is known as a common public health problem and the global prevalence of it is on rise. Diabetes-induced oxidative stress has been known to affect liver, pancreas, kidney and reproductive organs pathologically [1].Various studies in both human and animal models confirm that diabetes is source of oxidant stress. Free radicals are generated by hyperglycemia in diabetes mellitus by various mechanisms that are thought to involve metal-catalyzed oxidation of glucose, oxidative degeneration and protein glycation [2].

Liver is major target of insulin action directly or indirectly. Major biochemical and functional abnormalities in the liver are accompanied by onset of diabetes. Apart from these various biochemical and functional abnormalities, a change in antioxidant status is a major determent in abnormality of liver. Liver is also damage as a result of kidney ischemia reperfusion [3]. One of the most important causes of renal disease in the world is diabetic nephropathy. The characteristics features of diabetic nephropathy include microalbuminuria, glomerular and renal hypertrophy, mesangial expansion with glomerular basement membrane thickening, and global glomerular sclerosis which ultimately cause in renal failure. The basis of diabetic nephropathy is as a result of establishing early lesions resulting from accumulation of the extra cellular matrix components in the mesangium and tubulointerstitiumis [4,5]. In development of the lesions, hyperglycemia plays a vital role [6, 7]. Intestine is a vital organ performing functions of digestion and absorption. In diabetic state intestine not only exposed to hyperglycemia but also toxicity of different chemicals drugs make it most vulnerable organ. Histopathological data of the intestine in diabetes is scarce. There are few studies showing remarkable ultra-structural changes in height and thickness of microvilli, space between microvilli and thickness of tight junctions under TEM [8].

There is scope for alternative drugs because the available drugs have side effects. Therefore, there is need to search for option in herbal medicine for diabetes. Coccinia *grandis* has been used in Ayurvedic medicine in India to treat diabetes from ancient times. The hydromethanolic extract of the leaves of *C. grandis* showed strong antioxidant activity, reducing power ability, free radical scavenging activity, metal chelating ability and inhibition of β-carotene bleaching. As the various fractions of *C. grandis* exhibited different reactive oxygen species scavenging activities, there may be different percentages of phytochemical constituents present in the fractions [9]. The present study was designed to evaluate the effect of Coccinia *grandis* leaf extract on histopathology of kidney, liver and intestine.

MATERIAL AND METHODS

Animals

The animals used were Wistar strain rats (Weighing 160±20gms, aged 11-12 weeks). Animals were acclimatized to laboratory conditions prior to experimentation. The animals were kept under standard conditions of light and dark cycle with food and water ad libitum in groups of single in plastic cages with corn cob as bedding. All institutional guidelines of the Institutional Animal Ethics Committee were strictly adhered to in the care and treatment of the animals used throughout the study (CPCSEA No, 383/01/a/CPCSE).

Chemicals used: STZ was obtained Sigma Chemical (USA).Metformin drug procured from Hetero drugs, INDIA. Other essential chemicals were obtained from SRL biochemical, INDIA.

Plant material and extraction- The fresh leaves of Coccinia *grandis* were collected locally. A voucher specimen (No.018) was deposited at Department of Botany, University College of Science, Osmania University, Hyderabad-500007. Leaves were then shade dried at room temperature. Dry material was coarsely pulverized to powdered form. The powder was extracted with boiling water and methanol using rotary evaporator and the crude extracted was used for experiment.

Experimental design: The animals were randomized into 5 groups: The control group, untreated diabetic group, Metformin treated diabetic group, Coccinia treated diabetic group and Coccinia treated control group. Control group are normal untreated control and received physiological saline (Control); Untreated diabetic group rats are STZ (Streptozocin 50 mg/Kg body weight in 100mM Citrate buffer pH 4.5) induced diabetic rats (Diabetic); Metformin treated diabetic group rats are STZ induced diabetic rats given daily Metformin(150mg/kg body weight in RO water) (Met); Coccinia treated diabetic group rats are STZ induced diabetic animals treated with Coccinia leaf extract (200mg/kg body weight in RO water) (Coc+D); Coccinia treat

control group rats are normal control animals which were treated with Coccinia leaf extract (200mg/kg body weight in RO water) (Coc+C). After the last treatment, the rats were fasted overnight and killed by cervical dislocation. The kidney, liver and intestine was dissected and transferred in to 10% formalin solution for histological studies.

Histological studies:

For histopathological studies, all experimental rats were sacrificed. Liver, kidney and Intestine were removed immediately from rats; paraffin sections of 5µm thickness were made and stained by hematoxylin-eosin (H&E) stain. After staining the sections were observed under light microscope and photographs were taken.

RESULTS

Intestine: There was no histological significance of transverse section of intestine of control rats on 21st day. But STZ-induced diabetic rats showed drastic histological alterations such as excess proliferation of epithelium in villi, villi edema, lymphocyte infiltration, goblet cells hyperplasia Fig.1. The metformin treated rat's also similar results such as diabetic rats but not drastic. But animals treated with Coccinia *grandis* were recovered from damage done by diabetes. Control animals treated with Coccinia *grandis* did not show any histology differences.

Kidney: T.S of kidney of all experimental groups is shown in Fig.2. The normal histological structure of the kidney was seen in control groups (Fig.A). The outer cortical region of the kidney is recognized by the presence of renal corpuscles, enclosed in Bowman's capsule. Many renal tubules are seen in the cortex. Whereas histological study of kidney of diabetic rats showed severe degeneration, vacuolar degeneration in some tubular epithelial cells and cell debris scattered in tubular Lumen, increase in thickness of tubular epithelial cells with narrowing of lumen, Massive cellular infiltration, areas of haemorrhage in interstitial tissue and deformed renal tissue architecture were seen. Some glomeruli showed complete degeneration with thickening of Bowman's capsule (Diabetic glomeropathy), while others showed lobulation with wide urinary space (Fig.B). The T.S of kidney after 21st day of metformin treated diabetic rats is shown, degeneration of glomeruli is seen but number of glomeruli damaged compared to diabetic rats is very few (Fig.C). After simultaneous treatment with Coccinia of STZ-induced diabetic rats, the T.S of kidney shown single glomeruli being damaged (Fig.D). There were no other histological changes which were shown in diabetic animals. The T.S. of kidney of control rats treated with Coccinia has normal histological features (Fig.E).

Liver: The histological analysis of T.S. of liver of control rat has normal histological appearance. The histopathological examinations of diabetic rats showed necrosis of hepatic cells, granular cytoplasm, dilatation in the sinusoids. Diabetic rats treated with metformin also shown normal histology. The T.S. of liver of STZ induced diabetic rats treated with Coccinia has shown mild sinusoid dilation, with few shrunken nuclei with granular cytoplasm. Control rats treated with Coccinia did not shown any histological changes.

DISCUSSION

The present study assessed protective effects of Coccinia *grandis* on hyperglycemia induced toxicity on different organs. Different studies have shown that hyperglycemia toxicity is largely by free radical mediated damage [11]. This damage is responsible for adverse effects, especially in kidney, liver and intestine. Most of the studies have shown to modify antioxidant defense, altering the activities of detoxifying enzymes in all tissues [10]. Cell death may result due to oxidative damage or inflammation, resulting releasing products of protein breakdown, including transition metals, there by effecting generation and regeneration of reactive oxygen species. Hence in diabetes conditions with severe oxidative stress antioxidant therapy plays a vital role in amelioration of diabetic complications. Hence Coccinia *grandis* which was proved to purport with antioxidant property was tested on different organs of diabetic rat.

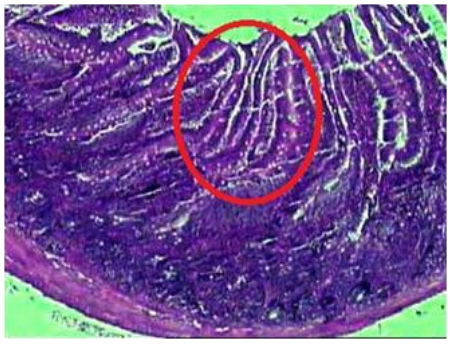
In our present intestine of STZ-induced diabetic rats showed drastic histological alterations such as excess proliferation of epithelium in villi, villi edema, lymphocyte infiltration, goblet cells hyperplasia, which may be contributed to free radical damage. The results of this study are in correlation with other studies such as Noor *et al.,* 2008 [12]. There was a drastic difference between intestine of diabetic rat and that of diabetic

rat treated with Coccinia *grandis* leaf extract. This may be implicated to the substantial antioxidant property of Coccinia *grandis*. When same extract was given to control animal there was no histological significant changes indicating its safety without any side effects. Liver plays a vital role in glucose and lipid homeostasis. In diabetes, several mechanism that are implicated in the pathogenesis of the functional and morphological alteration of the liver [13] as it is also an insulin-dependent organ. The histopathology of liver of STZ induced diabetic showed more drastic changes when compared to control group. Damage may be attributed to STZ induced free radical generation. The increased production of highly reactive free radicals can deplete GSH store, allowing the reactive intermediate to react with and destroy hepatic cells [14]. When the STZ induced diabetic rats were treated with Coccinia *grandis* leaf extract have shown to be protecting the tissue against STZ action. The results obtained reveal that methanolic extract of Coccinia has more potent antioxidant activity which quenches reactive oxygen species.

Diabetic nephropathy is one the microvascular damage of kidney due to long standing diabetes. There is much evidence indicating oxidative stress will induce pathological alternations in kidneys. Most of the researchers have reported that increased generation of reactive oxygen species and decreased antioxidant enzymes resulting in oxidative stress in kidneys [15]. In our study Coccinia leaf extract had showed slight protection of the kidney, which may be attributed to strong antioxidant property of Coccinia and also hypoglycemic effect.

CONCLUSION

In conclusion, the pathological changes in all the tissues reflect how kidney, liver and intestine are susceptible to increased oxidant stress. It may be concluded that the possible mechanism by which Coccinia exerts its protection against STZ induced diabetes may be due to its strong antioxidant effect and also decreasing generation of free radical by hypoglycemic property.



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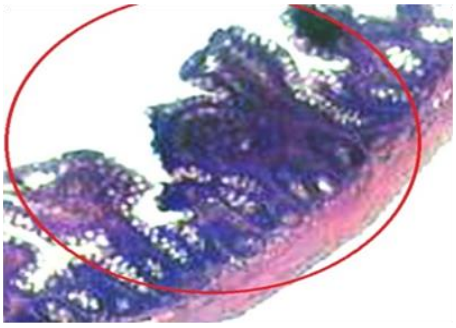

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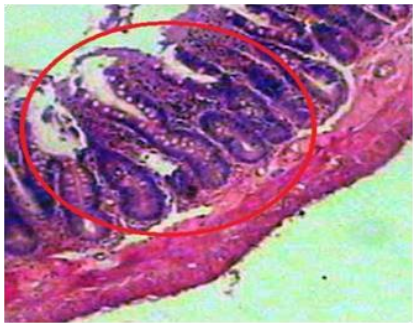

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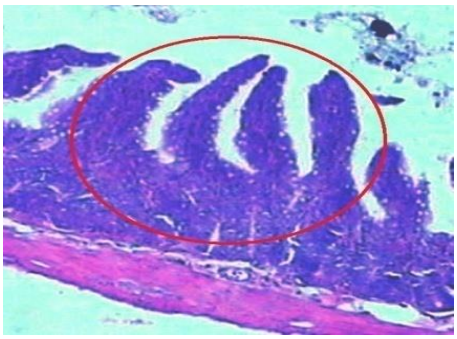

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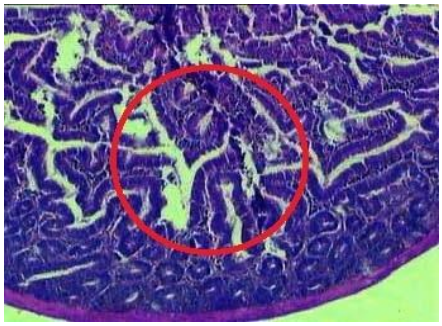

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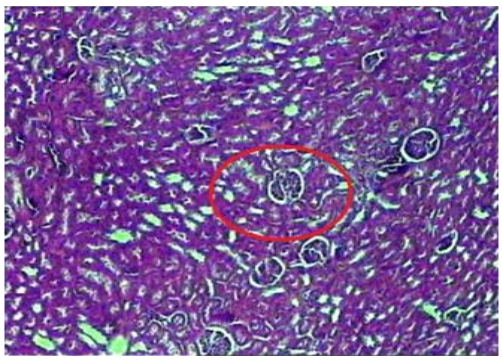
Fig.1 Photographs showing Transverse section of the intestine Fig.A is of Control rat showing normal histological features of Intestine. Fig.B is that of STZ induced diabetic rat showed drastic histological alterations such as excess proliferation of epithelium in villi, villi edema, lymphocyte infiltration, goblet cells hyperplasia. In Fig.C STZ-induced diabetic rat treated with metformin show similar results such as diabetic rats but not so drastic. STZ-induced diabetic rats treated with Coccinia are shown in Fig.D were recovered from damage induced by diabetes. Control animals treated with Coccinia grandis did not show any histology differences Fig.E.



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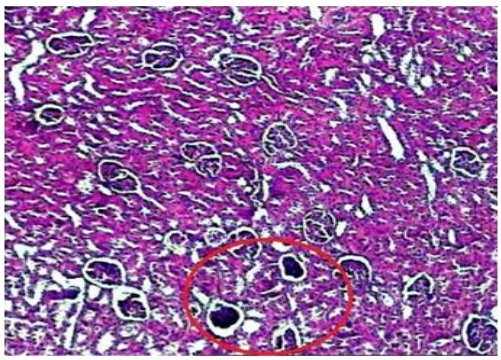

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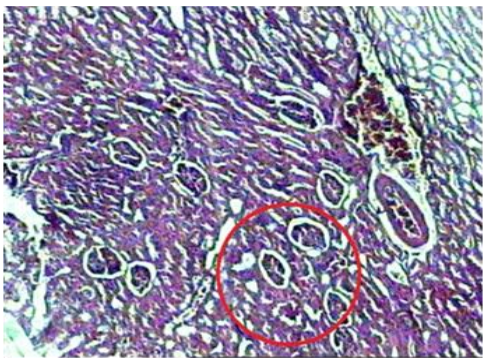

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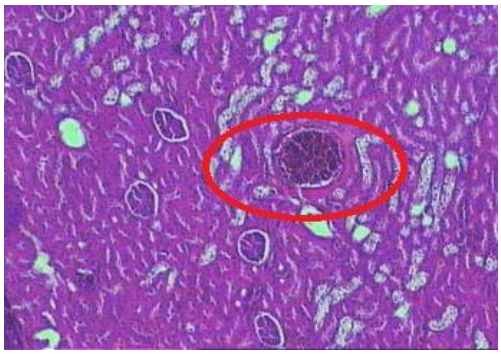

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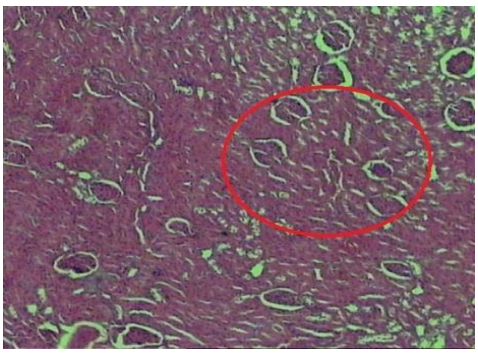

E

Fig.2 Histopathological changes in kidney (H&E stain) A: Control rat kidney section (day 21) showing normal histology. B: Kidney of STZ induced diabetic rat showing degenerated glomerulus. C: Kidney of STZ induced diabetic rat treated with metformin showing normal histology except edema in some regions. D: Kidney treated with Coccinia showing necrosis in only one glomeruli and remaining tissue is showing normal histology. E: Kidney of control rat treated with Coccinia is showing normal histoarchitecture.

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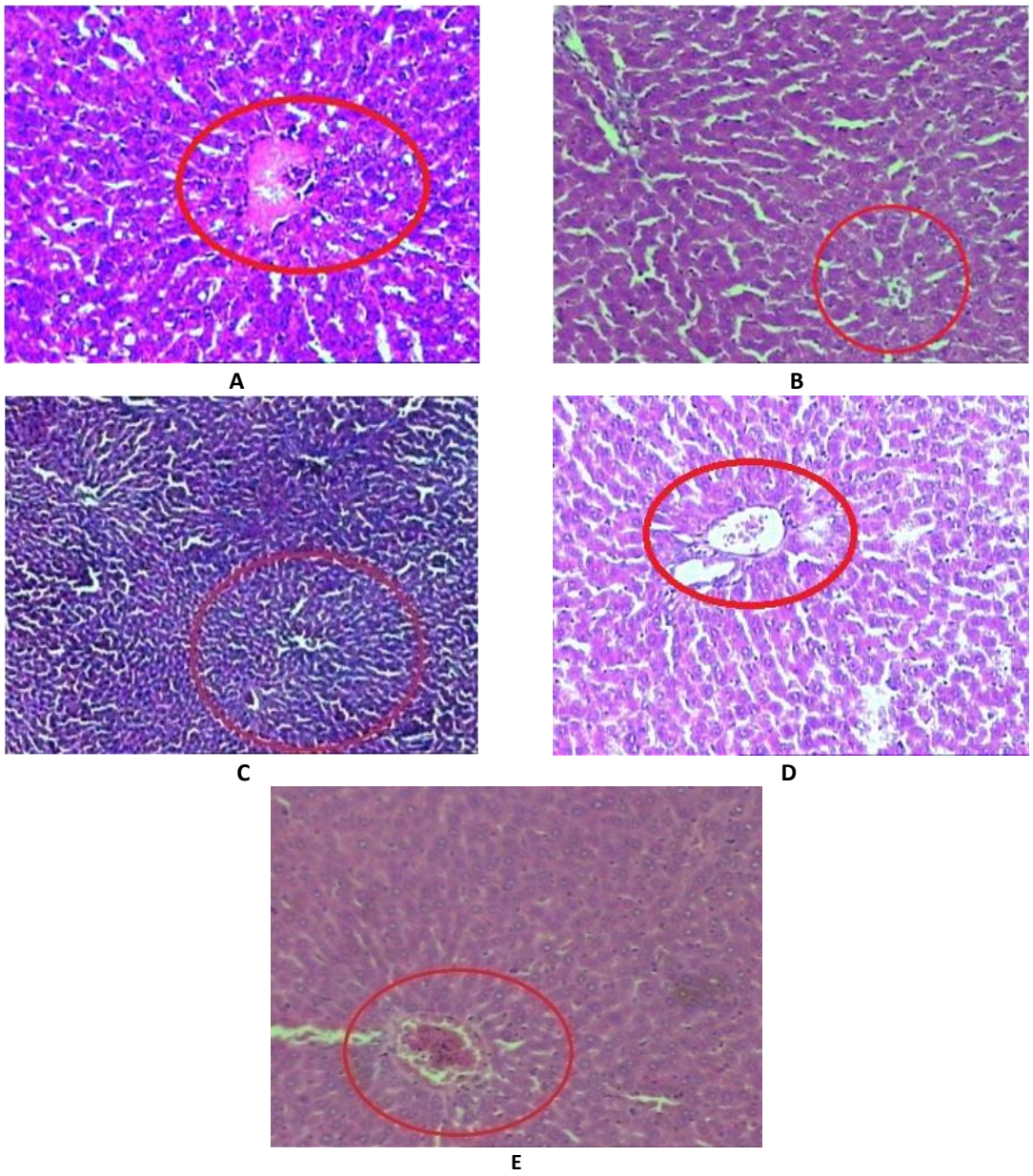

Fig 3. Histopathological changes in hepatic tissue (H&E stain). A: hepatic section of normal rat liver; B: hepatic section of diabetic rats treated with STZ; C: hepatic section from the diabetic rats treated with metformin drug; D: hepatic section of diabetic animals treated with Coccinia; E: hepatic section of control animals treated with Coccinia.

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