



## RESTORATION OF PATHOLOGICAL CHANGES IN KIDNEY TISSUES FROM DIET INDUCED OBESE RATS TREATED WITH *RHINACANTHUS NASUTUS* (LINN) KURZ LEAF EXTRACT

D. Sajeda\*, L. Lakshaman Kumar, K. Peera and K. Thyaga Raju

Dept. of Biochemistry, SV University, Tirupathi, India.

Article Received on  
01 Feb. 2018,

Revised on 22 Feb. 2018,  
Accepted on 14 March 2018,

DOI: 10.20959/wjpps20184-11205

### \*Corresponding Author

D. Sajeda

Dept. of Biochemistry, SV  
University, Tirupathi, India.

### ABSTRACT

Obesity is an important cause of kidney diseases and it has close association with diabetes and hypertension and excess weight. The present study was designed to identify the kidney damage like vacuolisation in obesity induced rats and restoration of normalcy after treatment with methanolic leaf extract of *Rhinacanthus nasutus* (*R. nasutus*). The methanolic leaf extract of *R.nasutus* (RNME) was orally administered to rats, 200mg/kg/day, while orlistat was administered of 25 mg/kg/day for 6 weeks before being sacrificed. The fat induced

vacuolization, glomeruli atrophy of epithelial cells and dark pyknotic nuclei were restored to almost normal upon the treatment of rats with RNME.

**KEYWORDS:** Obesity, vacuolisation, RNME, glomeruli atrophy, pyknotic nuclei, orlistat.

### INTRODUCTION

Obesity has become a serious global health issue affecting both adults and children. The prevalence of overweight, obesity is approximately 66% in the United States.<sup>[1]</sup> Over the last two decades, a worldwide rise in obesity has resulted in 1.46 billion over weight (Body Mass Index (BMI) >25) and 502 million obese (BMI >30) adults.<sup>[2]</sup> Obesity leads to a decrease in overall life expectancy<sup>[3]</sup> and metabolic syndrome.<sup>[4]</sup> Moreover, metabolic changes found in obesity such as insulin resistance, hyperinsulinemia, hyperleptinemia and abnormal lipid metabolism, can also promote podocyte damage leading to proteinuria and renal injury. Early progressive podocyte damages and macrophage infiltration is associated with hyperlipidemia and type II diabetes mellitus in Zucker rats and precedes both the development of glomerulosclerosis and tubule interstitial lesions.<sup>[5]</sup>

However, renal risks induced by obesity, especially its role in initiation and progression of renal diseases, have only been recently recognized.<sup>[6]</sup> Overweight and obesity due to fat diet of rat are associated with hemodynamic, structural and histopathological alterations in the kidney, as well as metabolic and biochemical alterations that predispose to kidney disease, even when renal function is normal in the conventional tests.<sup>[7,8]</sup> The identification of plants is useful to human beings from natural strands commenced in prehistoric studies. Experiments and trails are the two main ways through which humans have learnt various uses of the plants.<sup>[9]</sup>

*Rhinacanthus nasutus* is widely distributed in some parts of the subcontinent India and in the region of Southeast Asia and China. Various parts of this plant have been also used for the treatment in various other diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension, high blood pressure and various skin diseases, and the active components of this plant have been widely investigated.<sup>[10-20]</sup>

Therefore the studies conducted on kidney of rat is the presence of high fat diet and high fat diet treated with *Rhinacanthus nasutus* were discussed as follows.

## MATERIALS AND METHODS

### Animals and their maintenance

Male Wistar rats (110 - 130 gm) obtained from Sri Venkateswara Animal Agency, Bangalore, India, were fed with pellet diet and water *ad libitum*, and maintained under controlled laboratory conditions (12 h light/dark; temp.  $26 \pm 2$  °C; relative humidity  $60 \pm 10\%$ ).

### Experimental design

To study this experimentation the animals were divided into five groups of six rats each.

**Group I:** The Control group rats were fed with normal diet pellet and *ad libitum* with water,

**Group II:** The experiment Control group rats were fed with normal diet pellet and RNM fraction,

**Group III:** Experiment animals were fed with High Fat Diet,

**Group IV:** Experiment animals were fed with High Fat Diet and with standard drug Orlistat and.

**Group V:** Rats were fed with high fat diet along with RNM fraction.

The orlistat at 25 mg/kg body weight and RNM fraction extract 200mg/kg body weight were administered for 6 weeks, once a day, to the respective treatment groups. The dose was suspended in distilled water and given orally using a gastric gavage.

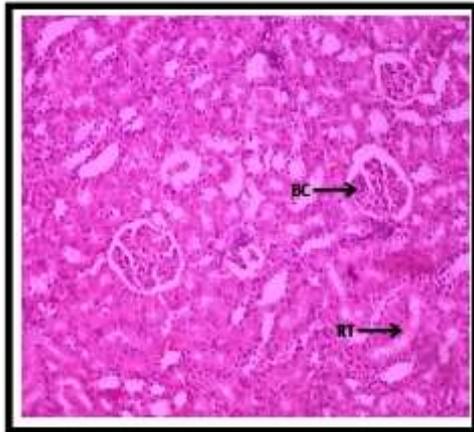
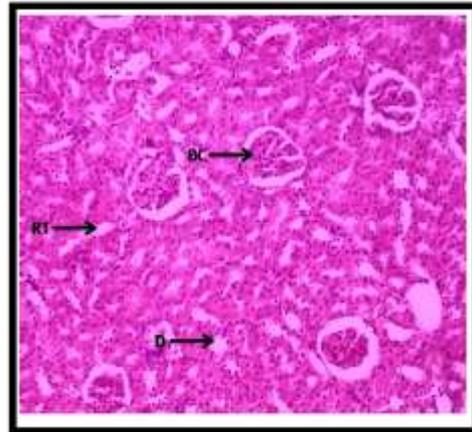
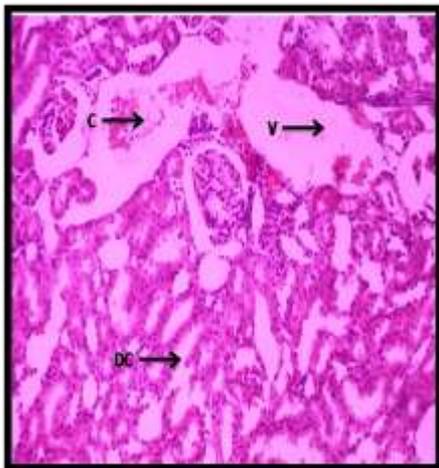
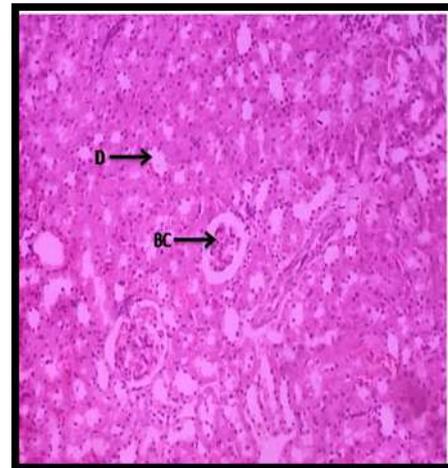
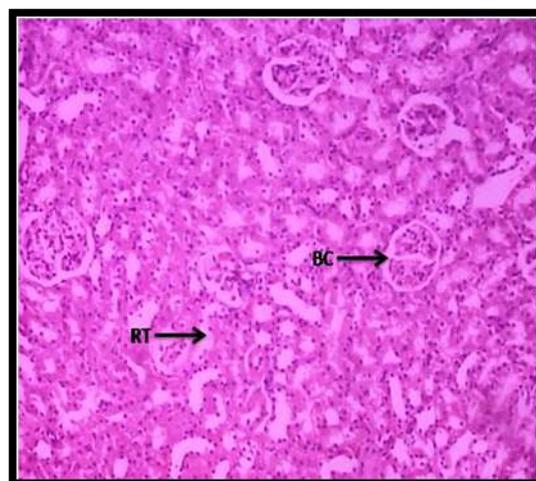
### Histological analysis

- The kidney were isolated from control and treated rats after decapitation and were gently rinsed with physiological saline to remove blood and fat debris adhering to them.
- They were fixed in Bouin's solution until processing. The tissues were washed with running tap water for maledhyde followed by to remove Bouin's solution.
- After dehydrating through a graded series of alcohols, the tissues were cleaned in methyl benzoate and embedded in paraffin wax.
- Sections were cut to 6 $\mu$  thickness using microtome and stained with haematoxylin (Harris, 1900) and counter stained with Eosin dissolved in 95% alcohol.
- After dehydration and cleaning with alcohol sections were mounted in Canada balsam. Histological examinations of the tissues were followed according to Humason, 1972 and the specimens were observed under the light microscope.

## RESULT AND DISCUSSION

### Kidney

**Fig.1**, the photomicrographs (10x), of the kidney of a normal rat showing normal architecture with normal glomeruli and tubular epithelial cells. **Fig. 2**, photomicrographs (10x) of the kidney of RNME treated rats showing normal architecture as that of control. **Fig.3**, photomicrographs (10x) of the Kidney of High fat diet untreated rats showing vacuolisation atrophy of the glomeruli, necrotic tubular epithelial cells and dark pyknotic nuclei. **Fig.4**, photomicrographs (10x) of the kidney of High fat diet rats treated with RNME showing almost of normal glomeruli, normal intertubular vessels and tubular epithelial cells. **Fig.5**, photomicrographs (10x) of the Orlistat rats treated with high fat diet showing similar changes as in those treated with RNME.

**HISTOLOGICAL MORPHOLOGY OF KIDNEY USING HEMOTOXYLIN AND EOSIN STAINING****Fig 1: Normal control rats (10X)****Fig 2: RNME + Pellet(10X)****Fig 3: High fat diet control rats (10X).****Fig 4: RNME +High fat diet rats (10X).****Fig 5: Orlistat + High fat diet (10X).**

BC-Bowman's Capsule; RT-Renal Tubules, G-Glomeruli, D-Distal, C-Congestion; V-Vacuolization; DC-Degenerative Changes, EC-Epithelial Cell.

Histology is the study of tissues and it gives the insight into the functioning of tissues and organs. In a precise sense it is the study of changes in cell environment which envisage the cell anatomy. Histological studies pave a way to understand the pathological conditions of the animal and gives a clear picture to understand how the drugs cause injury to the tissue. In this study kidney was selected since, kidney is the primary organ which play a role in the excretion of minerals and water. During fat diet intake of an animal kidney shall overload in the excretion of water and many of macromolecules which may include minerals to maintain acid base balance of the kidney.

In the present study, an obesity model was performed in rats by feeding with animal tallow, and the effects of this obesity on rat kidneys were studied. In kidney histological study, dilatation of glomerular capillaries large blood vessels, sub capsular adipocyte accumulation, tubular deformations, glomerular atrophy and necrosis were observed. The increase in the volume of the kidneys of HFD-fed animals may have resulted from edema due to mononuclear cell infiltrations among the tubules, and is clear that dilatation may lead to a volumetric increase in the kidney. Early reports have listed obesity as a risk factor for mortality from 'chronic nephritis, and the subsequent recognition of more association of obesity with diabetes, hypertension and heart disease, and questioned its being as risk factor for kidney disease that showed a high-caloric saturated fat intake induced diabetes, in hamsters. This model of study has revealed association with the development of a range of pathologies characteristic of human diabetes, including nephropathy and defects in vasculature.

Based on the results on kidney tissues, our observations seem to suggest that certain medicinal plants might have renal protective ability and prevent kidney dysfunction by accelerating regeneration. This may occur due to two possible effects of various herbs on kidneys, harmful or beneficial. Harmful effects include: polyuria, causing dehydration, acute renal failure, chronic renal insufficiency and stone formation. Possible beneficial effects include: diuresis, protection of the kidney from nephrotoxic agents, prevention or amelioration of renal lithiasis, and amelioration of kidney failure.<sup>[21]</sup>

## CONCLUSION

The microscopic examination of rat groups has revealed the disturbed glomerular architecture and kidney degeneration. So, the results of the present experiment clearly indicate that obesity causes significant structural changes of kidney.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the contribution and enthusiasm of our coworkers in the present studies.

## REFERENCES

1. Wahba I, Mak R. Obesity and Obesity-Initiated Metabolic Syndrome: Mechanistic Links to Chronic Kidney Disease. *Clin J Am Soc Nephrol*, 2007; 2: 550-562.
2. Finucane M, Stevens G, Cowan M, Danaei G, Lin J, Paciorek C, et al. National, regional, and global trends in body-mass index since 1980, systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet*, 2011; 377: 557-567.
3. Olshansky S, Passaro D, Hershow R, Layden J, Carnes B, et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med*, 2005; 352: 1138-1145.
4. Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among U.S. adults. *Diabetes Care*, 2004; 27: 2444-2449.
5. COIMBRA TM, JANSSEN U, GRÖNE HJ, OSTENDORF T, KUNTER U, SCHMIDT H, BRABANT G, FLOEGE J: Early events leading to renal injury in obese Zucker (fatty) rats with type II diabetes. *Kidney Int*, 2000; 57: 167-182.
6. ABRASS, CK: Overview: Obesity: what does it have to do with kidney disease? *J Am Soc Nephrol*, 2004; 15: 2768-2772.
7. Kopple JD, Feroze U. The effect of obesity on chronic kidney disease. *J Ren Nutr*, 2011; 21: 66-71. DOI: <http://dx.doi.org/10.1053/j.jrn.2010.10.009>.
8. Declèves AE, Sharma K. Obesity and kidney disease: differential effects of obesity on adipose tissue and kidney inflammation and fibrosis. *Curr Opin Nephrol Hypertens*, 2015; 24: 28-36. DOI: <http://dx.doi.org/10.1097/MNH.0000000000000087>.
9. Bukke Suman, P.S. Raghu, G.Sailaja and Kedam Thyaga Raju, *Applied Pharmaceutical Science*, 2011; 1(8): 26-32.
10. Rojanapo W., Tepsuwan A., Siripong P., *Basic Life Sci*, 1990; 52: 447-452.
11. Wu T.-S., Tien H.-J., Yeh M.-Y., Lee K.-H., *Phytochemistry*, 1988; 27: 3787-3788.

12. Kodama O., Ichikawa H., Akatsuka T., Santisopasri V., Kato A., Hayashi Y., *J. Nat. Prod.*, 1993; 56: 292-294.
13. Awai N., Kuwahara S., Kodama O., Santisopasri V., *Biosci. Biotech. Biochem.*, 1995; 59: 1999-2000.
14. Wu T.-S., Yang C.-C., Wu P.-L., Liu L.-K., *Phytochemistry*, 1995; 40: 1247—1249.
15. Sendl A., Chen J. L., Jolad S. D., Stoddart C., Rozhon E., Kernan M., Nanakorn W., Balick M., *J. Nat. Prod.*, 1996; 59: 808—811.
16. Kernan M. R., Sendl A., Chen J. L., Jolad S. D., Blanc P., Murphy J. T., Stoddart C. A., Nanakorn W., Balick M. J., Rozhon E. J., *J. Nat. Prod.*, 1997; 60: 635-637.
17. Wu T.-S., Hsu H.-C., Wu P.-L., Leu Y.-L., Chan Y.-Y., Chern C.-Y., Yeh M.-Y., Tien H.-J., *Chem. Pharm. Bull.*, 1998; 46: 413-418.
18. Wu T.-S., Hsu H.-C., Wu P.-L., Teng C.-M., Wu Y.-C., *Phytochemistry*, 1998; 49: 2001—2003.
19. Gotoh A., Sakaeda T., Kimura T., Shirakawa T., Wada Y., Wada A., Kimachi T., Takemoto Y., Iida A., Iwakawa S., Hirai M., Tomita H., Okamura N., Nakamura T., Okumura K., *Biol. Pharm. Bull.*, 2004; 27: 1070-1074.
20. Thirumurugan R. S., Kavimani S., Srivastava R. S., *Biol. Pharm. Bull.*, 2000; 23: 1438-1440.
21. Myhre MJ. Herbal remedies, nephropathies, and renal disease. *Nephrol Nurs J.*, 2000; 27: 473-478.