Biomedical & Pharmacology Journal, March 2019. Vol. **12**(1), p. 57-63

Effect of Methanolic Extract of *Phyllanthus niruri* on Leptin Level in Animal Model of Diabetes Mellitus

Anuj Kumar[2], Akhilesh Kumar Rana[2], Amit Singh[2] and Alok Singh[1]*

[1]Department of Pharmacology, All India Institute of Medical Sciences Raipur Chhattisgarh, India.
[2]Institute of Medical Sciences, Banaras Hindu University, Varanasi Uttar Pradesh India.
*Corresponding author E-mail: draloksingh@aiimsraipur.edu.in

http://dx.doi.org/10.13005/bpj/1613

(Received: 15 December 2018; accepted: 23 March 2019)

To study the effect of methanolic extract of *Phyllanthus niruri* on animal model of Diabetes Mellitus. Diabetes Mellitus was induced in rats by injecting Streptozotocin (60mg/kg) intraperitonealy. Blood glucose was measured on day 3by GOD-POD method. Rats having fasting blood glucose >250 mg/dl were further selected for study. Four groups were created i.e. Control, Control+Streptozotocin, Streptozotocin+ Metformin(75mg/kg) and Streptozotocin+ extract of *P. niruri* (250mg/kg). Each group was consisting of 6 rats of either sex. Metformin and experimentalextract were administered for 21 days. Blood Glucose was measured on day 7 and 21. Triglyceride, Cholesterol and Leptin level were also measure by commercially available kit. Anti-oxidant potential was assessed by estimating extent of Lipid peroxidation (LPO) by Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) and Glutathione (GSH) in four different tissues i.e. Liver, Kidney, Pancreas, Muscle on day 21. Unpaired and paired student's t-test were applied for statistical analysis using SPSS Software. The extract of *P. niruri* showed significant decrease in blood glucose level on day 21 (*p-0.04*). The treatment didn't show significant lowering of Leptin and Cholesterol level however Triglyceride level was significantly reduced (*p-0.05*). The treatment group showed improvement in oxidative stress by increasing SOD and GSH and decreasing LPO and NO activity. The study showed anti-hyperglycemic and anti-oxidative properties of methanolic extract of *P.niruri*.

Keywords: Anti-hyperglycemic, Anti-oxidant, *Phyllanthus niruri*.

Diabetes Mellitus (DM) is a major metabolic disorder involving glucose and lipid metabolism and is characterized by hyperglycemia. Diabetes Mellitus is among commonest non-communicable diseases and most of countries are facing its havoc. Latest data suggests that the worldwide prevalence of diabetes is on rise affecting 382 million people in 2013 and likely to affect 592 million people by year 2035[1] and India is also facing similar situation and over 62 million people living with diabetes[2]. Diabetic patients may have absolute or relative deficiency of insulin (Type1 DM) or tissue resistance to insulin (Type2 DM). Type 2 DM accounts for the maximum number of cases (90%)[3]and monogenic forms are responsible for 5% cases of DM[4].Diabetes causes secondary pathophysiologic changes in different organ systems leading to micro-vascular and macro vascular complications and increasing co-morbidities. There are seven different class of drugs which are currently being used in management of Diabetes Mellitus but effective

management of complications remains a problem[5]. Uncontrolled DM along with overproduction of reactive oxygen species (ROS), and decreased antioxidant enzymatic pathways are contributory to various complications of DM[6]. Important ROS are superoxide, hydrogen peroxide, hydroxyl radicals, and hypochlorous acid. These are generated through several metabolic processes and scavenged by efficient anti-oxidant system so that accumulation of ROS doesn't occur. Important antioxidant enzymes are Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), and reduced glutathione (GSH).Any mismatch in the action of these enzymes normally leads to faulty disposal of free radicals and its accumulation. The ROS are responsible for the oxidation of tissues leading to lipid peroxidation and damage to cell. Role of free radicals and oxidative stress in diabetes mellitus is well documented[7]. Free radicals damage cellular membranes by releasing intracellular components like lysosomal enzymes, which lead to further tissue damage. These radicals promote lipid peroxidation and membrane damage by cross-linking proteins, lipids and nucleic acids[8].

Currently the drugs available to manage DM are hypoglycemic agents which primarily reduce blood glucose level. They offer very less advantage to correct other pathological aspects of DM. Plants can be source of agents which not only can reduce blood glucose level but also show benefit in correction of pathological aspects of DM. Numerous plants have shown anti-hyperglycemic potential in animal model of DM[9].*Phyllanthus niruri*, commonly known as 'stone breaker', belongs to family Euphorbiaceae is an indigenous plant. The plant is of medicinal importance for numerous ailments like renal stones, dyspepsia, hepatotoxicity, antihyperlglycemic[10]. Systemic studies for anti-hyperglycemic and antioxidant potential are less, in the present study medicinal plant *Phyllanthus nirurui* is selected for hypoglycemic and anti-oxidative property.

Streptozotocin (STZ) can be used to induce both Type 1 and Type 2 DM[11]. It is taken up by beta cell of pancreas by GLUT2[12]. Damage to beta cell is mediated by alkylation of DNA. STZ is also an NO donor which also contribute to its cytotoxic action[13]. Free radicals generated by STZ is supposed to be ultimate cause of beta cell damage.

Leptin is primarily produced in adipose tissue and its level reflects energy status and it has got significant role in energy homeostasis, glucose and lipid metabolism[14]. Leptin is considered to act on central pathways and affect peripheral insulin sensitivity which are independent of its effects on food intake and weight[15].Effect of plant extracts on leptin level will also be assessed in present work.

MATERIAL AND METHODS

Plant Material and Extract Preparation

The dried aerial part of *Phyllanthusniruri* was procured from the botanical garden of Banaras Hindu University (BHU), Varanasi and identified with the department of Dravyaguna, Institute of Medical Sciences, BHU. The dried aerial part of *Phyllanthus niruri* of 500 grams was crushed into small pieces and prepared by adding sufficient amount of methanol in a glass jar for 72 hours and then filtered off. The methanolic extract of *Phyllanthus niruri* was vacuum dried and stored at -20°C until further use.

Animals

Inbred Charles-Foster (CF) albino rats (150-200 g) of either sex, were obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University,Varanasi. They were kept in the departmental animal house at 26 ± 2^0 C and relative humidity 44-56%, light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 18-24 h before the experiment though water was allowed ad libitum. Approval from the Institutional Animal Ethical Committee was taken prior to the experimental work.

Drug Treatment

A total of 32 rats were obtained from Central Animal House and 24 rats were chosen for study divided into 6 rats per group. Diabetes was induced in adult rats weighing between 150-200 g by a single intra-peritoneal injection of streptozocin (STZ, 60 mg/kg) dissolved in citrate buffer (pH 4.5) and fed normally thereafter[16]. On day 3 the fasting plasma glucose was assessed to validate the model. Details of groups are as under:

Group A-Control: The control rats received citrate buffer only as per their body weight.

Group B- Streptozotocin with no Treatment: In this group Streptozotocin was given and followed for 21 days without any other treatment.

Group C-Standard: This group received Metformin (75 mg/kg)suspended in 1% carboxymethyl cellulose (CMC) in distilled water.

Group D- Treatment Group: This group received extract of *Phyllanthus niruri* (250 mg/kg) orally once daily for 21 days.

Glycemic Study

Fasting plasma glucose levels were estimated on day7 and day 21 after extract administration. Blood samples were collected from the retro-orbital plexus. Rats showing fasting plasma glucose over 250 mg/dL under fasting conditions were used for the study. Plasma glucose was estimated by GOD-POD method.

Estimation of Triglycerides and Cholesterol

Blood samples were collected from the retro-orbital plexus of the rat and serum was separated by centrifugation at 3000 rpm for 3 minutes. Triglycerides level was estimated in the unhemolysed serum by (GPO-PAP Method) using Triglycerides Test Kit (Coral Clinical System.) Total cholesterol levels were estimated in the unhemolysed serum by (CHOD-PAP Method) using *Cholesterol Kit (Span Diagnostics Pvt. Ltd. India)*.

Estimation of free radicals and anti-oxidants

Test and standard drugs were given orally daily for 21 days and on the day of the experiment the animals were then sacrificed and various parameters was calculated in tissue and serum as described earlier. Liver, pancreas, kidney and muscle tissue were homogenized (5%) in ice cold 0.9 % saline with a Potter - Elvehjem glass homogenizer for 30 sec. The homogenate was then centrifuged at 800xg for 10 min followed by centrifugation of the supernatant at 12,000-x g for 15 min and the obtained supernatant was used for the following estimations[17].

Antioxidants viz. superoxide dismutase SOD (nmol/g wet tissue)[18], and reduced glutathione GSH (nmol/g wet tissue)[19] and free radicals viz. extent of lipid peroxidation LPO by estimating MDA(nmol/g wet tissue)[18]and nitric oxide NO (mmol/g wet tissue)[20]were estimated in homogenate.

Estimation of Leptin

Leptin was estimated in serum by commercially available ELISA kit. The kit was obtained from Kinesis Dx catalog no. K11-0562.

Statistical Analysis

The statistical analysis was carried out using paired and unpaired t test. The values are represented as Mean ± SD. $P < 0.05$ was considered significant.

RESULTS

The study was conducted in department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University. The results are as under:

Table 1. Effect on Blood Glucose Level (mg/dl)

Day	Group A	Group B	Group C	Group D
0	96.5±18.97	104.66±12.83	93.33±16.23	89.16±13.76
3rd	99.66±17.66	274±11.93	277.83±9.08	276.33±113.62
7th	97.66±6.74	279.83±12.62	202.5±3.78	268.5±113.21
21th	97.16±6.73	285.66±11.20	138.16±6.31	139±10.37

Table 2. Effect on Plasma Triglycerides and Cholesterols Level and Serum Leptin on Day 21

Parameters	Group A	Group B	Group C	Group D
Leptin (ng/ml)4.5±0.14	4.6±0.11	4.4 ±0.15	4.5±0.15	
Triglycerides(mg/dl)	74.33±1.87	97.5±4.92	81.83 ± 5.7	81.66±8.26
Cholesterols (mg/dl)	108± 6.72	142.6±10.63	117.5 ± 8.96	147.3±8.73

Table 3. Effect on Lipid Peroxidation (nmol/g wet tissue) and Nitric Oxide (mmol/g wet tissue) on Day 21

Groups & Tissue Homogenate Lipid Peroxidation	Group A	Group B	Group C	Group D	*P* – value
Liver	217.5± 27.02	196 ±20.89	218.66 ±28.73	211.5±20.44	0.285
Kidney	196 ± 11.11	158.66 ± 26.25	187.5 ± 12.38	192±13.99	0.026*
Pancreas	216.5 ± 16.63	179.16 ± 16.63	220.16 ± 19.27	195.5±12.83	0.017*
Muscle	187.33 ± 9.77	132.33 ± 21.33	190 ± 12.21	173.33±10.50	0.046*
Groups & Tissue HomogenateNitric Oxide					
Liver	232±6.75	197.5±7.23	216.5±4.08	204.5± 5.61	0.092
Kidney	202.83±5.84	167.5±7.44	184.66±9.09	183.16 ± 7.08	0.004*
Pancreas	199.33±7.04	157.33±14.06	190.16±9.19	184.16 ± 11.7	0.017*
Muscle	198.33±8.21	164±8.69	187.5±9.37	184± 8.24	0.002*

Table 4. Effect on Superoxide Dismutase (units/ g wet tissue) and Glutathione (mmol/g wet tissue) level on Day 21

Groups& Tissue Homogenate Superoxide Dismutase (units/ g wet tissue)	Group A	Group B	Group C	Group D	*P* – value
Liver	35.61±4.3	43.08±10.63	39.66±3.2	44.16±3.3	0.332
Kidney	54.7±1.31	58.08±1.31	56.78±2.79	55.38±1.4	0.012*
Pancreas	49.41±4.5	54.91±3.05	50.33±3.1	50.41±3.1	0.032*
Muscle	45.33±2.9	49.66±3.5	46.91±2.9	52.16±3.9	0.295
Groups& Tissue HomogenateGlutathione (mmol/g wet tissue)					
Liver	5.7±0.91	7.5±0.53	6.4±0.73	6.28±0.49	0.020*
Kidney	7±0.50	10.4±0.72	8.33±0.56	9.23±0.82	0.024*
Pancreas	7.15±0.70	8.71±.94	7.5±0.89	7.4±0.70	0.010*
Muscle	7.61±.66	9.65±1.02	8.41±1.22	9.33±1.17	.631

In Group A all animals completed study. In Group B, 3 animals died during the study and rest completed the study. In Group C, 2 animals didn't develop required blood glucose level and hence excluded from study. In Group D, one animal died and one didn't develop required blood glucose so excluded from study.

After Streptozotocin administration, on day 3 the blood glucose level was markedly increased (274±11.9 mg/dl) as compare to control(99.7±17.7 mg/dl). Group D i.e. treatment group showed a decrease in blood glucose level by 2.9% at day 7and 49.7% at day 21. Group C i.e. Standard showed a decrease in blood glucose level by 50.27% at day 21. (Table 1). The extract of *Phyllanthus niruri* showed statically significant decrease in blood glucose level at day 21 (*p* - 0.041). At day 7 plant was showing slight decrease in blood glucose level which was not statistically significant.

On day 21, blood Cholesterol and Triglycerides level were assessed. In Group B blood cholesterol and triglyceride level were higher than Group A which was control group indicating deranged lipid profile There was significant difference in blood triglyceride level between Group B and Group D. In Group D, the level was significantly less as compared to Group B (p value 0.05). The result shows Group D was having better Triglyceride Profile than Group B.Group B and

Group D were having similar cholesterol level (p value 0.36). It seems that extract of *P. niruri* didn't offer any change in Cholesterol level (Table 2).

On day 21, blood Leptin level was assessed. In Group B blood leptin level was higher than Group A (Table 2). Group D showed decrease in leptin level, as compared to Group B which was not statistically significant (p value 0.18).

On day 21, markers of oxidative stress i.e. extent of Lipid Peroxidation (LPO) and Nitric Oxide (NO) level were assessed in four different tissues homogenate i.e Liver, Kidney, Pancreas, Muscle. Both are indicators of oxidative stress. In Group B, the level of both were increased on day 21 compared to Group A in all tissue samples showing increase in oxidative stress.

The extent of LPO level significantly decreased in Group D as compared to Group B in tissue sample of Kidney, Pancreas and Muscle having p value 0.026, 0.017 and 0.046 respectively. (Table 3)

The NO level significantly decreased in Group D as compared to Group B in tissue sample of Kidney, Pancreas and muscle having p value 0.004, 0.017 and 0.002 respectively. (Table 3)

On day 21, anti-oxidants i.e. Glutathione (GSH) and Super-oxide Dismutase (SOD) level were assessed in four different tissues homogenate i.e Liver, Kidney, Pancreas, Muscle. Both are part of inherent defense mechanism against oxidative stress. In Group B, the level of both these anti-oxidants were reduced on day 21 compared to Group A in all tissue samples showing decreased defense against oxidative stress.

The SOD level significantly increased in Group D as compared to Group B in tissue sample of Kidney and Pancreas having p value 0.012 and 0.032respectively. (Table 4)

The GSH level significantly increased in Group D as compared to Group B in tissue sample of Liver, Kidney and Pancreas having p value 0.02, 0.024 and 0.01 respectively. (Table 4)

DISCUSSION

In the present study, diabetes in rats was induced by Streptozotocin and we selected the *Phyllanthus niruri* commonly known as stone breaker. The study includes the status of various diabetic parameters in streptozotocin-induced diabetic rats. The present study was therefore intended to examine the effect of methanolic dried aerial part of *P. niruri* streptozocin-induced diabetic rats, using metformin as a reference drug.

P. niruri, belongs to family Euphorbiaceae, an indigenous antidiabetic plant popularly used in South India for diabetes mellitus. Hypoglycemic effect *P. niruri* is documented[21]. Our present result with *P. niruri* also showed significant antihyperglycemic effect in diabetic rats after 21 days of treatment, after seven days of treatment the blood glucose was reduced but the effect was not statistically significant. Significant decrease in fasting plasma glucose after 21 days may indicate that *P.niruri* can be beneficial on prolonged use, however the decrease in fasting plasma glucose started from after 7days itself. This may have insulinomimetic activity or may affect insulin resistance also[22].

Group B rats exhibited increases in plasma Cholesterol and Triglycerides level. Diabetes is known to associated with increased plasma and cholesterol and triglyceride levels[23]. Currently, *P.niruri* extract administration led to significant decline in triglyceride level. The significant decreases in triglycerides is similar to the result reported earlier in hyper lipemic rats treated with *Phyllanthus niruri* extract[24]. We did not find significant reduction in plasma cholesterol level which is similar to previous study[22]. This finding suggests that the plant extract may have activity like fibrates class of drug which act via PPAR-g and indicated for treatment of hypertriglycridemia[25].

Sustained hyperglycaemia leads to increased lipid peroxidation, production of superoxide, and resulting in oxidative stress[26,27]. GSH and SOD are important endogenous anti-oxidative defence against oxidative stress[8]. SOD is a free radical scavenger which protects cells from oxidative damage[28].Group B animals were found to be have lower level of GSH and SOD on day 21 in all the tissue homogenate i.e Liver, Kidney, Pancreas, Muscle compared to Group A. The treatment group i.e. Group D was found to have statistically significant higher level of GSH and SOD in all tissue homogenate of kidney, pancreas and showing anti-oxidant effects. These finding are similar to results reported by authors[29,21].All tissue homogenate of Group B was having higher concentration than Group A. Kidney, Pancreas and

muscle homogenate showed significant reduction in level indicating less oxidative damage. These finding are similar to results reported by authors[29,21]. Anti-oxidant activity in Kidney and liver supports its activity as stone-breaker and prevention of hepatotoxicity[30,31].Group D showed decrease in Serum Leptin level, although the result was not statistically significant but it was following similar trend reported earlier in study[22]. Decrease in Leptin level can be corelated with decrease in insulin resistance as both are positively corelated. On long term use the extract can be effective in reversing insulin resistance.

CONCLUSION

In light of the present results, our work indicates that methanolic extracts of *P.niruri* have good anti-diabetic activity. Our study showed significant improvement in anti-oxidant parameters i.e. increase in GSH & SOD while decrease in NO, LPO in various tissue homogenate (Muscle, kidney, Liver and Pancreas) after 21 days of administration of extract of *P.niruri* indicating its anti-oxidant potential. Downward trend in Leptin level may be correlated with decrease in insulin resistance.

REFERENCES

1. Forouhi NG, Wareham NJ. Epidemiology of diabetes. Medicine (Abingdon) **42**(12): 698–702 (2014).
2. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. *AMJ,* **7**(1): 45-48 (2014).
3. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk Factors Contributing to Type 2 Diabetes and Recent Advances in the Treatment and Prevention. *Int. J. Med. Sci.*; **11**(11): 1185-1200 (2014).
4. Yang Y, Chan L. Monogenic Diabetes: What It Teaches Us on the Common Forms of Type 1 and Type 2 Diabetes. *Endocr Rev.;* **37**(3): 190–222 (2016).
5. Venkataraman K, Kannan AT, Mohan V. Challenges in diabetes management with particular reference to India. *Int J Diabetes Dev Ctries.;* **29**(3): 103–109 (2009).
6. Matough FA, Budin SB,Zariyantey A Hamid ZA, Alwahaibi N,Mohamed J. The Role of Oxidative Stress and Antioxidants in Diabetic Complications. *Squ Med J.*; **12**(1):5-18 (2012).
7. Ullah A, Khan A, Khan I. Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharmaceutical Journal;* **24**: 547–553 (2016).
8. Birben E,Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative Stress and Antioxidant Defense. *WAO Journal;* **5**: 9–19 (2012).
9. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*; **2**(4): 320-330 (2012).
10. Narendra K *et al*. Phyllanthus niruri: A Review on its Ethno Botanical, Phytochemical and Pharmacological Profile. *Journal of Pharmacy Research* **5**(9):4681
11. Arulmozhi DK, Veeranjaneyulu A,Bodhanka SL. Neonatal streptozotocin-induced rat model of Type 2 diabetes mellitus: A glance. *Indian J Pharmacol*; **36**:217-21 (2004).
12. Eleazu *et al*. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk(to humans. *Journal of Diabetes & Metabolic Disorders*, **12**:60 (2013).
13. Nahdi AMT, John A, Raza H. Elucidation of Molecular Mechanisms of Streptozotocin-Induced Oxidative Stress, Apoptosis, and Mitochondrial Dysfunction in Rin-5F Pancreatic *â*-Cells. Oxidative Medicine and Cellular Longevity Volume 2017, Article ID 7054272, 15 pages.
14. Park HK, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism*.; **64**(1): 24–34 (2015).
15. Filho GP, Mastronardi C, Wong ML, Licinio J. Leptin therapy, insulin sensitivity, and glucose homeostasis. *Indian J Endocrinol Metab.;* **16**(Suppl 3): S549–S555 (2012).
16. Deeds MC *et al*. Single Dose Streptozotocin Induced Diabetes: Considerations for Study Design in Islet Transplantation Models. *Lab Anim*.; **45**(3): 131–140 (2011).
17. Das D, Banerjee RK. Effect of stress on the antioxidant enzymes and gastric ulceration. *Mol Cell Biochem*; **125**(2):115-25 (1993).
18. Sairam K, Priyambada S, Aryya NC, Goel RK, Gastroduodenal ulcer protective activity of *Asparagus racemosus*: An experimental, biochemical and histological study, *J Ethnopharmacol*, **86**: 1 (2003).
19. Sedlak J, Lindsay RH. Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent, *Anal Biochem*, **25**: 192 (1968).
20. Miranda KM, Espey MG, Wink DA. A rapid, simple spectophotometric method for simultaneous detection of nitrate and nitrite, *Nitric oxide: Biol Chem*, **5**(1): 62 (2001).
21. Mazunder UK, Gupta M, Rajeshwar Y.

Antihyperglycemic effect and antioxidant potential of *phyllanthus niruri* (euphorbiaceae)(in streptozotocin induced diabetic rats. *European Bulletin of Drug Research.* **13**(1): (2005).

22. Jagtap S *et al*. Protective effects of phyllanthin, a lignan from *Phyllanthus amarus*, against progression of high fat diet induced metabolic disturbances in mice. *RSC Adv.*, **6**: 58343-58353 (2016).
23. Verges B. Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia,* **58**: 886–899 (2015).
24. Khanna AK, Rizvi F, Chander R. Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats. *J Ethnopharmacol.*; **82**(1):19-22 (2002).
25. Gurgle HE, Blumenthal DK. Drug therapy for dyslipidemias. Goodman&Gillmans Pharmacological basis of therapeutics. 13th/e p.605.
26. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care.;* **19**(3): 257-67 (1996).
27. Maxwell SR *et al*. Antioxidant status in patients with uncomplicated insulin- dependent and non-insulin-dependent diabetes mellitus. *Eur J Clin Invest.;* **27**(6):484-90 (1997).
28. Hatwalne MS. Free radical scavengers in anaesthesiology and critical care. *Indian J Anaesth.*; **56**(3): 227–233 (2012).
29. Bavarva JH, Narasimhacharya AVRL (2007) Comparative Antidiabetic, Hypolipidemic, and Antioxidant Properties of Phyllanthus niruri. in Normal and Diabetic Rats, *Pharmaceutical Biology,* **45**(7): 569-574.
30. Pucci ND. Effect of *phyllanthus niruri* on metabolic parameters of patients with kidney stone: a perspective for disease prevention. *Int Braz J Urol;* **44**(4): 758–764 (2018).
31. Manjrekar AP *et al*. Effect of Phyllanthus niruri Linn. treatment on liver, kidney and testes in CCl4 induced hepatotoxic rats. *Indian J Exp Biol.;* **46**(7):514-20 (2008).