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NEPHROLITHIATIC POTENTIAL OF TOPIRAMATE IN MALE ALBINO RATS

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ABSTRACT

The present study is to investigate the nephrolithiasis produced by topiramate in male albino rats. For this study the male albino rats were divided into three groups containing six animals in each and received distilled water, Topramate 4.5mg/kg, Topiramate 18mg/kg orally for 28 days. On 14th and 28th day, the following parameters like serum urea, BUN, creatinine, urine calcium, phosphorus, creatinine, uric acid, antioxidant enzymes MDA, GSH, SOD, catalase and renal histopathology were monitored after the treatment period. Statistical analysis was done by one way ANOVA with Dunnet multiple comparison test. There was a significant change in urine volume, kidney weight and elevated serum urea, creatinine, calcium and phosphorus as compared to control rats. Lipid peroxidation products MDA were significantly increased. Antioxidant enzymes SOD, GSH and catalase levels were significantly lowered than the control group. Histopathological study of kidney also supported the above results. These observations enable us to conclude that topiramate produces nephrolithiasis as dose dependent manner.

KEYWORDS

Nephrolithiasis, Topiramate, Oxidative stress and Metabolic acidosis.

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INTRODUCTION

Topiramate is a sulphamate substituted monosaccharide licenced for the monotherapy and adjunctive treatment of generalized tonic - clonic seizures and prophylaxis of migraine headaches[1,2]. Topiramate is also being investigated or prescribed in an off label fashion for an increasing number of conditions including bipolar disorder, alcoholism, smoking cessation, obesity and binge eating, type2 diabetes, bulimia nervosa, post-traumatic stress disorder, infantile spasms, neuropathic pain, cluster

headache and cocaine dependence. In 2008 alone, over 7 million prescriptions for TPM were filled in the united states[3].

Several reports of post marketing surveillance of topiramate indicate that there is an increased risk of kidney stone formation while using topiramate as monotherapy and adjunct therapy. Among its multiple Pharmacological actions, TPM inhibits renal carbonic anhydrase, leading to metabolic acidosis[4]. The resulting hypocitraturia, elevated urinary PH and hypercalciuria have been shown to increase urinary saturation with respect to brushite and predispose Topiramate users to kidney stone formation. Brad *et al* suggested that induction of progressive profound hypocitraturia with increasing doses of topiramate. The escalating FDA approved and almost unquantifiable off label use of Topiramate underscores the need to estimate the true stone prevalence[5]. There is no systematic scientific experimental preclinal evidence for the formation of renal stone by Topiramate treatment. The present study is to evaluate if the topiramate at two clinically equivalent doses will produce any biochemical and histopathological changes associated with nephrolithiasis.

MATERIALS AND METHODS

Animals

Male Albino rats (*Wistar strain)*, weighing 250-300g were used as the experimental animals. They were obtained from animal house (Reg No: 752/02/ a/CPCSEA) Medical college, Thiruvananthapuram. The animals were fed on standard rodent pellet diet (MFD at: Baramati Agro Ltd, Baramath) and water ad libitum. The animals were maintained under standard conditions of relative humidity, 12 hrs light-dark cycle, adequate ventilation and ambient room temperature (22 ± 2^0 C) and there were acclimatized to laboratory conditions 10 days prior to the commencement of the experiment. All the experiments were carried out between 9.0am to 4.pm.

Ethical clearance

The study protocol was approved by the institutional animal ethics committee, Medical College, Thiruvananthapuram.(IAEC Approval No:07/04/2012/ MCT).

Drugs and chemicals

Topiramate

Topiramate reference sample was supplied by BAL Pharma Limited, Bangalore, Karnataka. Dose level of topiramate administered for rat was obtained by multiplying the clinical dose with the factor of 0.018, thus obtaining the animal dose for 200g body weight. Topiramate 4.5mg/kg and 18mg/kg was dissolved in distilled water and administered orally to rats.

Experimental design

The male albino rats were designed into three groups containing six animals in each and kept in cages. All animals had free access to regular diet and drinking water *ad libitum* for 28 days.

Group IA (6rats): control group, received distilled water orally daily for 28 days.

Group IB (6 rats): Received Topiramate 4.5mg/kg orally daily for 28 days.

Group IC (6 rats): Received Topiramate 18 mg/kg orally daily for 28 days.

Change in body weight and water intake were monitored at initial and final day of treatment period.

Assessment of Nephrolithiasis

Collection and analysis of urine

All rats were kept in individual metabolic cages and urine sample of 24 hr were collected on 14^{th} and 28^{th} day. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4^0C. Urine was analysed for creatinine, calcium, phosphorus, protein, and uric acid (Table No.3).

Serum Analysis[6]

After the 14^{th} and 28^{th} day experimental period blood samples were collected from individual rats of all groups by retro orbital bleeding under ether anaesthesia. Serum was separated by centrifugation at 10,000rpm for 10 min and analysed for uric acid[7], urea[8], BUN, calcium, phosphate[9], creatinine, sodium and potassium (Table No.1 and 2).

Kidney homogenate analysis[10]

After the experimental period, all the animals from each group were sacrificed by cervical dislocation followed by ether anaesthesia. The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and one of them was preserved in 10% formalin. The

other one was divided into two pieces. One for kidney homogenate analysis and other for the assessment of oxidative parameters.

For kidney homogenate analysis a sample of 100mg of the dried kidney was boiled in 10ml of 1N HCL for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10min and the supernatant was separated and analysed for phosphate and calcium (OCPC).

Estimation of oxidative stress[11,12]

Preparation of Tissue homogenate

500gm of kidney was sliced, two volumes of a solution containing 140mM potassium phosphate buffer (pH7) was added. Mixture was taken in a clean sterile centrifuge bucket and homogenized to get a 25% tissue homogenate. The homogenate was centrifuged at 15,000 rpm at 4^0c for 15 minutes and the supernatant stored at 4^0c and used for the antioxidant enzymes.

Homogenate protein determination

The amount of soluble protein present in the homogenate was determined by the standard method proposed by Biuret *et al* using bovine serum albumin as the standard.

Superoxide dismutase assay[13]

Superoxide dismutase assay was done by Marklund and Marklund method. The assay was based on the conversion of Nitro Blue Tetrazolium (NBT) to NBT-diformazan (which absorbs light at 560 nm) by superoxide anions. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in an experimental sample.

Catalase assay[14]

Catalase assay was done by Aebi *et al* method. The assay was based on the principle that catalase catalyses the decomposition of hydrogen peroxides to water and oxygen; in which the decomposition of peroxide is followed spectrophotometrically at 240 nm.

Lipid peroxidation assay[15]

MDA produced in renal tissue was measured by *Ohkawa et al method* was based on the principle that the end product of lipid peroxidation MDA forms a 1:2 adduct with thiobarbituric acid and the red pigment produced was extracted with n-butanol-pyridine mixture and estimated by the absorbance at 532nm.

$$\text{MDA} = \frac{Absorbance}{Molar\ absorptivity} D.F \times 10$$

D.F=dilution factor

Glutathione Assay[16]

Renal GSH was measured by *Modified beutler et al method* and was based on the principle that reduced glutathione (GSH) interacts with 5-5'dithiobis 2-nitrobenzoic acid (DTNB) to form the coloured product 2-nitro-5-thiobenzoic acid, which is measured at 412nm. The antioxidant enzyme levels and MDA was expressed in units per milligram of protein.

HISTOPATHOLOGICAL STUDIES[17]

Left kidney was fixed in 10% dehydrated with ascending grades of ethyl alcohol, embedded in paraffin wax, sliced on a rotary microtome, stained with haemotoxylin and eosin and histomorphological features were examined under high power.

STATISTICAL ANALYSIS

The data were expressed as mean ±standard error means (SEM). Difference among experimental groups was determined by one way ANOVA followed by Dunnet multiple comparison test. In all experiments p value less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Urinary supersaturation with respect to stone forming constituents is one of the causative factors in calculogenesis (Daddola *et al*., 2008). It was accepted that hypercalciurea is one of the risk factors in the pathogenesis of renal stone (Moe *et al*., 2005)[18].

The present study was undertaken to investigate the effect of nephrolithiatic potential of Topiramate at two clinically equivalent doses[19]. Up to date, to our knowledge there is no published data showing the effect of short term injection of topiramate on various parameters of Nephrolithiasis in male albino rats. In the present study male rats were selected as an experimental animal because the urinary system of male rats resembles that of humans and also earlier studies have shown that amount of stone deposition in female rats was significantly less. The dose level 4.5mg/kg and 18mg/kg were selected on the basis of clinical doses (50mg/kg and 200mg/kg orally). Rats treated with topiramate 4.5 mg /kg and 18 mg/ kg for

28 days shown significant increase (three fold) in Nitrogenous waste products as compared to the control group (Figure No.1).

A significant decrease in urine volume was evident in topiramate treated group. This was more significant in topiramate 18 mg /kg than 4.5 mg /kg (Figure No.2 and 3). An increased urinary calcium concentration is a factor favouring nucleation and precipitation of CaOx on apatite (Calcium Phosphate) from urine and subsequent crystal growth (Atef *et al.,* 2010; Biren *et al*., 2011. Increased serum urinary phosphorous was observed in topiramate treated rats. (4.5 mg/kg and 18 mg/kg). Topiramate at a dose level (4.5 mg/kg and 18 mg/kg) significantly decreased the body weight of rats during the treatment course. Parmar *et al*., reported that renal stone deposition in kidneys resulted severe weight loss. Kidney weight of topiramate treated rats have significantly increase than the control group $P<0.0001$. This is due to the deposition of promoters in kidney, leads to the formation and aggregation of stone in kidney. Previous reports on Nephrolithiasis supported this result (Anitha *et al*., 2008) (Table No.4).

From different experimental reports, it was observed that the development of the renal stones would leads to the production of Reactive Oxygen Species (ROS), development of oxidative stress followed by injury and inflammation. Renal injury and inflammation appears to play a significant role in stone formation. Oxidative stress is an imbalance between fine radical production and antioxidant activity. So measurement of lipid peroxidation products eg. MDA and free radical scavenging antioxidant enzymes like superoxide dismutase and catalase, antioxidant proteins. GSH are good markers for studying the effect of oxidative stress. Topiramate treatment with 4.5 mg/kg and 18 mg/kg for 28 days showed significant decrease in catalase, SOD and GSH activity.

Catalase is considered as a first line defensive antioxidant enzyme since it regulates H2O2 levels. The experimental evidence indicated that carbonic anhydrase inhibition would lead to cell death and oxidative stress. Parmar *et al* reported that ethylene glycol administered for 28 days resulted significant reduction in antioxidant enzyme activity and increased MDA activity. He suggested that this was due to the stone formation in kidney that leads to oxidative stress. There was a significant increase of MDA concentration $P<0.0001$ in renal tissue of rats treated with topiramate 4.5 mg/kg and 18 mg/kg. Topiramate at a dose level 18 mg/kg showed significant increase in MDA level as compared to the topiramate 4.5 mg/kg; suggesting the involvement of oxidative stress in nephrolithiasis.

Microscopic examination of kidney sections derived from topiramate treated rats showed polymorphic irregular crystals deposits inside the tubules which causes dilation of the proximal tubules along with intestinal inflammation and necrosis. Topiramate18 mg/kg for 28 days treatment resulted the formation of comparatively larger crystals than topiramate 4.5 mg/kg treated groups.

Kidney weight of topiramate treated rats have significantly increase than the control group $P<0.0001$. This is due to the deposition of promoters in kidney, leads to the formation and aggregation of stone in kidney (Table No.5).

Topiramate challenge in high dose (18mg/kg) showed highly significant change in biochemical parameters as compared to low dose (4.5mg/kg). But antioxidant enzyme levels on topiramate challenge 18 mg/kg resulted mild change as compared to topiramate 4.5mg/kg but decreased urine volume and increased kidney on topiramate treatment 18mg/kg also supports the nephrolithiatic potency of topiramate as compared to topiramate 4.5mg/kg and control group. Renal histopathology and urine microscopy gave a concurrent result. From the above findings we interpreted that topiramate produces a dose dependent effect on nephrolithiasis.

Table No.1: Effect of topiramate on serum nitrogenous substance

(n=6, animal=male albino rat, values are expressed as mean±SEM)

S.No	Groups (days)	Treatment Drug/Dose/route	Urea(mg/dl) (mean±SEM)	BUN(mg/dl) (mean±SEM)	Creatinine(mg/dl) (mean±SEM)	Uric acid(mg/dl) (mean±SEM)
1	IA: 14 28	Distilled Water (p.o) 0.5ml/100g (28 days)	 37.87±0.4346 39.34±0.8933	 17.69±0.2029 18.13±0.2266	 0.2842±0.03379 0.3514±0.0236	 1.2310±0.06849 1.460±0.0464
2	IB: 14 28	Topiramate4.5mg/kg (p.o) for 28 days	 69.45±0.6730* 106.50±2.516*	 32.43±0.3143* 49.74±1.175*	 2.645±0.2185* 5.114±0.3718*	 7.631±0.7090* 20.640±1.003*
3	IC: 14 28	Topiramate18mg/kg (p.o) for 28 days	 78.09±0.6598* 135.3±1.476*$^{\#\#\#}$	 36.47±0.3081* 63.17±0.6891*$^{\#\#\#}$	 3.068±.1116* 8.452±0.2220*$^{\#\#}$	 9.923±.1085* 26.88±0.4258*$^{\#}$

*p<0.0001,**p<0.001***,p<0.01 compared to group IA(control)

#p<0.01,##p<0.001,###p<0.0001 group IC compared to group IB

Data were analysed by one way ANOVA followed by Dunnet's multiple comparison test

Table No.2: Effect of Topiramate on serum electrolytes

(N=6 animal=male albino rat, values are expressed as mean±SEM)

S.No	Groups	Treatment Drug/dose/route/duration	Calcium mg/dl (mean±SEM)	Phosphorus mg/dl (mean±SEM)
1	IA 14 28	Distilled water/0.5ml/100g/(p.o) for 28 days	 5.057±0.329 4.021±0.70	 3.744±0.43 4.1970±0.50
2	IB 14 28	Topiramate4.5mg/kg(p.o) (28 days)	 9.53±0.79* 27.59±0.78*	 10.71±0.48* 16.05±1.43*
3	IC 14 28	Topiramate 18mg/kg(p.o) (28 days)	 14.92±0.24#* 34.39±0.46#*	 13.16±0.40*# 20.96±0.35*#

*p<0.0001,**p<0.001***,p<0.01 compared to group IA(control)

$^{\#}$p<0.01,$^{\#\#}$p<0.001,$^{\#\#\#}$p<0.05, group IC compared to group IB.

Data were analysed by one way ANOVA followed by Dunnet's multiple comparison and student's t test

Table No.3: Effect of topiramate on urine nitrogenous substances

(n=6, animal=male albino rats, values are expressed as mean ±SEM)

S.No	Groups	Treatment Drug/dose/route/duration	Creatinine g/24hr (mean±SEM	Uric acid mg/dl (mean±SEM)	Protein mg/dl (mean±SEM)
1	IA 14 28	Distilled water/0.5ml/100g(p.o) for 28 days	 0.9126±0.09 1.066±0.07	 6.8810±0.28 7.4600±0.45	 1.4480±0.12 2.0960±0.32
2	IB 14 28	Topiramate4.5mg/kg(p.o) for 28 days	 1.793±0.13** 3.605±0.18*	 12.23±0.56* 22.35±0.86*	 3.133±0.13*** 5.018±0.27*
3	IC 14 28	Topiramate 18mg/kg(p.o) for 28 days	 2.918±0.18*# 5.641±0.26*#	 14.33±0.51*### 27.17±0.66*###	 3.540±0.26**¥ 5.841±0.14*¥

*p<0.0001,**p<0.001***,p<0.01 compared to group IA(control)

$^{\#}$p<0.01,$^{\#\#}$p<0.001,$^{\#\#\#}$p<0.05,¥ no significance group IC compared to group IB

Data were analysed by one way ANOVA followed by Dunnet's multiple comparison test

Table No.4: Effect of Topiramate on Nephrolithiasis- Renal antioxidant enzyme levels
(N=6, Animal =male albino rat, Values are expressed as mean±SEM)

S.No	Groups	Treatment Drug/dose/route/duration	Catalase(µm of H2O2 decomposed/min (mean±SEM)	SODnm/mg of protein (mean±SEM)	GSHnm/mg of proteinmean± SEM	MDA nm/mg of protein mean±SEM
1	IA	Distilled water 0.5ml/100g (p.o) for 28days	4.028±0.6277	9.192±0.5813	2.698±0.3110	4.716±0.3169
2	IB	Topiramate .5mg/kg p.o for 28 days	1.079±0.0782*	4.591±0.2020*	0.8808±0.0383*	9.183±0.2784*
3	IC	Topiramate18 mg/kg p.o for 28 days	0.8486±0.0565*###	2.892±0.2509*##	0.4917±0.0389*##	14.52±0.3816*##

***p<0.01,**p<0.001,*p<0.0001,compared to group IA
###p<0.05, ##p<0.01, # no significant change as group IC compared to group IB
Data were analysed by one way ANOVA followed by Dunnet's multiple comparison test

Table No.5: Effect of Topiramate on Kidney weight (28th day)(n=6, animal=male albinorat, values are expressed as mean±SEM)

S.No	Groups	Treatment Drug/dose/route/duration	Kidney wt(g) (mean±S.E.M)
1	IA	Distilled water 0.5ml/100g(p.o) for 28 days	0.825±0.03
2	IB	Topiramate4.5mg/kg (p.o.) (28 days)	1.32±0.17**
3	IC	Topiramate 18mg/kg (p.o.) (28 days)	1.51±0.08**

P**<0.001 compared to group IA
Data were analysed by one way ANOVA followed by Dunnet's multiple comparison test

HISTOPATHOLOGY

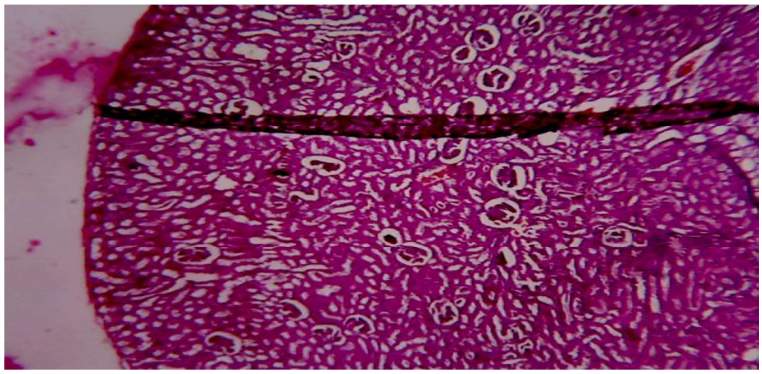

Figure No.1: Control group (IA): Normal organization of tubular epithelial cells and glomeruli

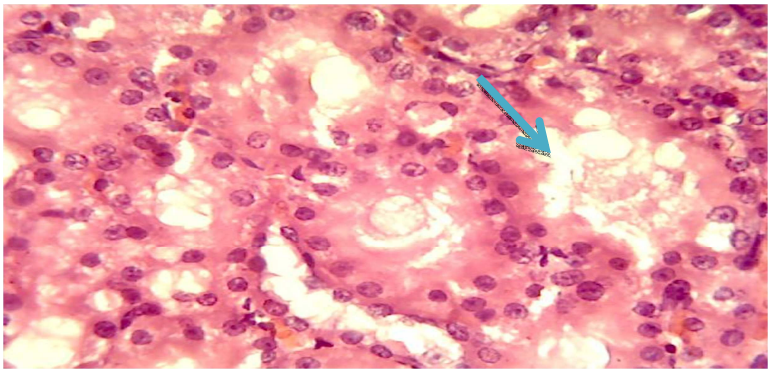

Figure No.2: Topiramate 4.5mg/kg (group IB): Glomerularatropy, tubulardialation, deposistion of crystals

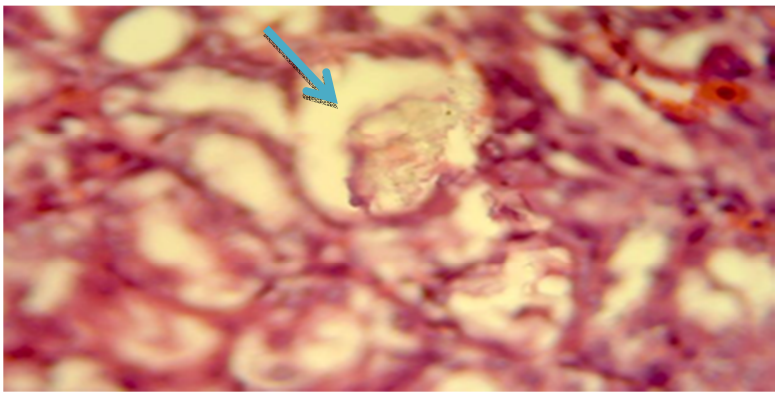

Figure No.3: Topiramate 18 mg/kg(groupIC): Tubular necrosis, inflammation, tubulardialation, deposistion of crystals

CONCLUSION

Correlating the results obtained from biochemical parameters and antioxidant enzyme assays we concluded that, Topiramate at two clinically equivalent doses caused nephrolithiasis. The exact mechanism behind the nephrolithiasis remains unknown. One mechanism could be due to metabolic acidosis and carbonic anhydrase inhibition. This metabolic acidosis remains as one factor for oxidative stress in kidney. Studies are also awaited to confirm the mechanism behind the renal stone formation caused by topiramate.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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