Original Research Paper

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Zoology

TOXICITY OF ANILINE ON KIDNEY OF MALE ALBINO RAT.

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ABSTRACT Aniline is a widely used chemical in a variety of industrial products and in almost all hair dye formulation. It is documented as a cause of clinical nephrotoxicity. The purpose of following study to investigate the histological alterations in kidney by aniline. For this study 24 male albino rats (*Rattus norvegicus*) weighing between 200-300gm were used. They were randomly divided into three groups. Group I as a control provided normal saline water and the group II, group III were exposed to aniline at 20mg/kg bw/day orally for 15 and 30 days duration respectively. The result revealed that the kidney structure showed fragmentation of brush border in the epithelial lining of proximal convoluted tubule, tubular dilation and hemorrhage in glomerular cells. As well as in 30 days duration the more effect also showed nuclear pyknosis, fragmentation of glomerular tuft and thickening of glomerular basement membrane. Therefore, it is concluded that the aniline causes histological changes as increase the exposure of duration may resulting in improper ultrafiltration of blood by kidney.

KEYWORDS : Aniline, albino rat, kidney histology.

INTRODUCTION

Aniline is a clear to slightly yellow liquid with a pungent odor. It does not readily disappear at room temperature. Aniline is a little soluble in water and mixes readily with most organic solvents. Aniline is used to create a number of yields such as agricultural chemicals, synthetic dyes, antioxidants, polyurethane foam stabilizers for the rubber industry, herbicides, varnishes and explosive etc. (ATSDR, 2002). The general population may be displaying to aniline by eating food or drinking water containing aniline, but these amounts are usually very small. If you work in a place that makes products like dyes, varnishes, herbicides, and explosives, you may be exposed to aniline at high amount (Anuradha *et al* 2004). Aniline detected also in tobacco smoke, so people who smoke or breath in second-hand smoke may also be exposed to aniline. Aniline produced commercially by catalytic vapor phase hydrogenation of nitrobenzene (Hummaddi, 2012). Paraphenyl-diamine (PPD) is analog of aniline provokes one of the most prominent edema, and it appears to be grossly specific and selectively localized in the head and neck. which is responsible for intense local irritation. The PPD toxicity altered vascular permeability and involvement of the parasympathetic nervous system (sentilkumaran *et al*, 2015).

The highest body loads were found for production of aniline and further processing in the large-scale chemical industry. High exposures were also determined for release of aniline as a decomposition product in iron, steel and aluminum foundries and use of liquid dyeing formulations with residual aniline (scientific commity, 2003). Oral absorption of aniline amounts in rats is more than 80 % and 90 %. In the body, aniline is widely distributed, the highest concentrations being found in red blood cells, plasma, kidney, liver, bladder and the gastrointestinal tract in rat. Due to their basicity, aniline and N-acetylaniline undergo enterogastric cycling (Kalpal *et al*, 2007). Aniline is able to cross the placenta. The concentration of aniline was slightly higher in fetal than in maternal blood of rats, while the half-life was 1.5 h in both fetal and maternal blood plasma (Benya and Cornish,1994). The textile printing and dyeing industry use aniline is a water-intensive industry requiring a large volume of freshwater at various steps of printing and therefore, the volume of wastewater produced is equally large (chung *et al*, 1982). N-methylaniline reacts aggressively with strong acids and oxidants. N-methylaniline is injurious if swallowed or inhaled or absorbed through the skin can cause methaemoglobinaemia, central nervous system effects, eye and skin irritation, liver and kidney damage, gastrointestinal irritation with nausea, vomiting and diarrhea (Abebe *et al*, 2013).

Para-phenylene Diamine (PPD) is a common element in most of the hair dye preparations used for accelerates the process of dyeing and can caused local toxic effects or systemic when applied topically or ingested. PPD is derivative of aniline, aniline is aromatic amine, a colorless solid when pure. It is used mainly as a fur and hair dye and as a chemical intermediate in the production of numerous substances, including dyes and polymers (HSDB, 2009). The PPD frequently used by women for beauty in Africa and the Middle East and Indian sub-continent (Hashimet al. 1992). PPD is mixed with henna leaves of *Lawsonia alba*, as color enhancement to decorate the hands and feet in special Sudanese social events, such as wedding ceremonies. Whereas, a serious PPD intoxication problems were recorded in Morocco and India due to the most popular hair dyes contain PPD, among other ingredients (Singhet al, 2008). Severe exposure to high level of PPD may cause severe dermatitis, eye irritation and tearing, Asthma, renal failure, vertigo tremors, convulsions and coma, while ingestion of PPD produces rapid developments of edema of face, neck, pharynx tongue and larynx with respiratory distress which often needs tracheostomy (Kumar 1988).

There is very diminutive information is available on the toxicity of aniline on kidney of rat particularly on histopathology. Thus the present study was performed to determine the effect of aniline on kidney of rat.

MATERIAL AND METHOD

Animal model

The present study was conducted on healthy male albino rat (Rattus noervegicus). Weighing between 150-250g. All animal procedures were reviewed and approved by the Animal Ethics Committee. Rats were maintained in clean polypropylene cages with saw dust bed this cage covered by stainless still wire lid. The animal kept in highly hygienic, ventilated room under constant conditions of temperature, relative humidity (50–60%), and lighting 12 h light and 12h dark cycles. Animals were provided with a standard profitable rat diet and distilled water.

CHEMICAL

Aniline is an oily liquid, which is colorless when freshly distilled, but darkens in exposure to air and light it becomes brown, oily liquid. The Molecular formula of Aniline is C6-H7-N. The Molecular Weight is 93.127. Aniline has pungent odor. It has 8.1ph and 184.1 Boiling Point. The Solubility of aniline is partial in normal water and highly in boil water. Synonyms of aniline is amino benzene; benzenamine, phenylamine.

Structural formula

Experimental design

In our study the total 24 male albino (Rattus norvegicus) rat were used in the experimental protocol. The animal divided in three groups. The group I served as a control received normal saline water. Group II and III were experimental received aniline 20mg/kg/day for fifteen and thirty days respectively. The experimental animals were preserved in the animal house on daily observations.

Body and Kidney weight

Weight of each animal was recorded before and after treatment. After

treatment rat were sacrificed by using chloroform kidney was dissected out and weighed.

Histopathological study of kidney

After termination of the experiment all rat were sacrificed kidney of rats of all rats were immediately collected and fixed in bovine fixative for a period of at least 24 h before histopathological study. The tissues were dry in an ascending grade of alcohol (ethanol), cleared in xylene. Samples were then embedded in paraffin wax and 5 micron sections were prepared with a rotary microtome. These thin sections were stained with hematoxylin and eosin (H&E), mount, observed for pathological changes under a binocular microscope. The photomicrographs were taken with the help of digital camera Nikon Coolpix 8400 attached to the light microscope (Nikon Eclipse E200) and magnified to required size.

Statistical analysis

Data were conveyed as the mean ± standard deviation (SD). Differences between groups were determined by Student T- test and a *P* value <0.05 was considered significant.

RESULT

Body and kidney weights

Body weights and the mean absolute and body-weight-related kidney weights were affected by the treatment in relation to their respective controls. The body weight of rats as compared with control was decreased and in the treated rat the kidney weight was increased significantly with higher doses of aniline.

No of Animal	Treatment	Initial body weight(gm)	Final body weight(gm)	Kidney weight after experiment
6	15days saline	261.17 ± 1.92	264.0 ± 1.75	934.165±21.58
6	15days Aniline	262.83 ± 2.30	236.83 ± 1.96	1034.50±28.13
6	30days saline	263.17 ± 2.06	269.83 ± 2.48	1123.005±15.465
6	30days Aniline	259.67 ± 2.99	210.50 ± 1.82	1142.01±12.35

Data are expressed in Mean ± S.D. of six animals *P value is 0.05 i.e. significant.

Histology

No histopathology was observed in the control groups I rats kidneys. Cortical zone of rats kidneys displayed urinary corpuscles and glomeruli. Glomeruli of kidney corpuscles were made up of parietal cells and podocytes with foot processes, the Glomerular Basement Membrane (GBM), foot processes and filtration slits were observed in normal structure.

In group II and III of kidney observed with pyknosis of nuclei in proximal convoluted tubule, fragmentation in glomerular tuft, fragmentation in brush border of epithelial lining of proximal convoluted tubule, tubular dilation thickening of glomerular basement membrane.

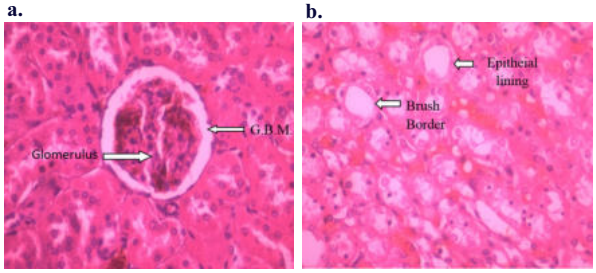
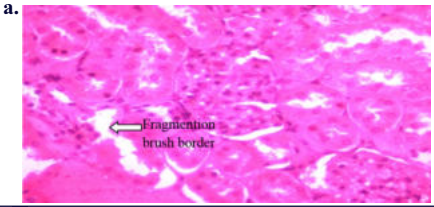


Figure-1 Transverse section of kidney 100x -showing normal a) glomerulus, glomerular basement membrane.(GBM) b) epithelial lining and brush border of pct and dct c) nucleus glomerular tuft.



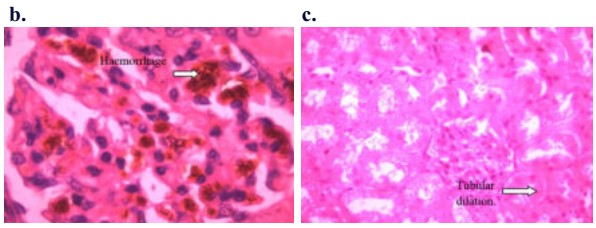


Figure 2 transverse section of kidney a)100x-showing framentation of brush border in pct b)40x-showing haemorrhage c)100x showing tubular dilation.3.

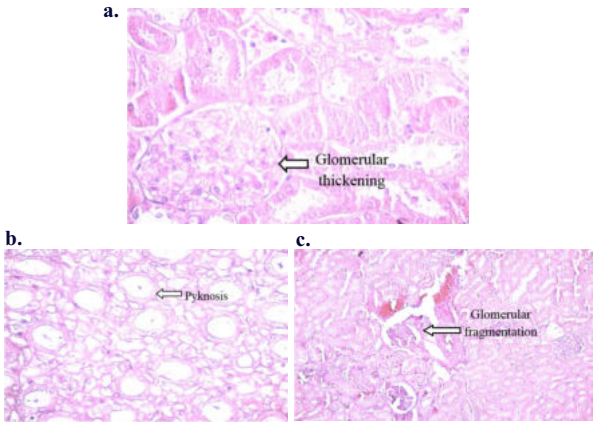


Figure 3- transverce section of kidney showing a)100x- thikning of glomerular basement membrane b) 100x-pyknosis c) glomerular fragmentation.

DISCUSSION

In the present study, aniline was found to cause of nephrotoxicity, depending on the concentration of the dose and time after administration. In rat kidney, high doses of aniline elicited increasing weight of kidney, which are associated with degrees of kidney damage. Results presented here suggest that aniline lead to moderate and severe microscopic and ultra-structural glomerular degenerative changes in both treated groups II and III. These observations were agreement with (Prasadet al. 2011). Who confirmed that the degree of the tissue damage is related to the dose of the ppd.

PPD is the synonyms of aniline (Sulimanet al 1995). Indicated that high application of the PPD to the skin lead over many years to glomerular injury and glomerulosclerosis in human and rat. The previous study state that PPD cause of renal injury is probably direct nephrotoxicity of PPD compound (Kumar, 2010). The metabolic products of PPD have a high urinary excretion rate and their oxidation produces quinone-diamine is a potentially nephrotoxic substance (Ashrafet al., 1994). Hence, the results of this study suggest that aniline causes glomerular hypertrophy, capillaries congestion associated by diffused hyaline and thickening f GBM. Similarly, the PPD mediated hepatotoxicity is evident from histological observation (hypertrophy, hyperplasia of portal tract, hepatocytes necrosis, hemorrhages, fibrin deposition within central vein and around the hepatic cords, fibrinous exudates as well as inflammation of the portal tract (Bharali and Dutta, 2009).

CONCLUSION

These studies concluded that aniline causes various glomerular histological and subcellular structure changes, resulting in impairment of renal function and metabolism. These results therefore provide valuable insight on the effects of higher dose of aniline exposure in rats. Aniline cause kidney damage depending on the dose concentration and time after administration. High doses of aniline demonstrated kidney function impairment.

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