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Acute toxic effects of benzamide derivative on renal histomorphology of BALB-C mice

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ABSTRACT

Introduction: For developing a new drug, it must be tested successively on animals and humans for its efficacy and side effects. This includes a series of studies to establish the safety of drug. In these studies, the effects are observed in certain organs like brain, heart, lungs, kidneys, gastrointestinal tract, liver, and reproductive organs. The present study was performed on Balb-C mice to see the toxic effects of benzamide derivative on the histomorphology of kidneys.

Materials & Methods: This experimental study was carried out at Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar, from February to July 2018. Thirty two (32) male BALB/c mice were used in this study. The benzamide derivative was administered orally in incremental doses.

Results: By increasing the dose of benzamide derivative up to 100mg/kg, toxicity induced histopathological changes were observed in the histological sections of kidneys of Balb-C mice in the form of lymphocyte infiltration, increase in urinary space, glomerulosclerosis, peri glomerulosclerosis, tubular swelling, tubular necrosis, and vascular congestion.

Conclusion: Before a drug is declared safe for human consumption, safety studies are carried out in animals. This compound (benzamide derivative) causes toxicity in kidneys with increasing doses.

Keywords: drug testing, renal toxicity, animal studies for drugs, kidney histomorphological changes

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

The development of a new drug for human use is a meticulous process involving a multidisciplinary integrated approach. It usually begins with the discovery or synthesis of a potential new compound or revelation of a new drug target. Subsequently an attempt is made to understand the interaction of the drug with its biologic targets.[1] In addition to the efficacy of a prospective molecule, there are definite concerns about its safety. The studies about the safety of a new drug are carried out in experimental animals. These studies form the basic component of preclinical phase of new drug development.[2,3]

Sensitive methods for detecting nephrotoxicity of drugs in preclinical studies are extremely important in all stages of drug development process to prevent nephrotoxic drugs from entering the market as the cost of management of nephrotoxicity and its associated morbidity are high. The susceptibility of kidney to drug toxicity is largely due to its morphology and function. After filtration process, the filtrate is subjected to a modification process wherein its components are concentrated to many folds (as much as 100 fold) in the complex structure of tubules and collecting ducts.[4]

Nephrotoxicity resulting from drug exposure has been estimated to contribute 19-25% of all cases of Acute Kidney Injury (AKI) in critically ill patients.[5]

The kidneys are significantly exposed to potential nephrotoxins due to high rate of drug and toxin delivery to it, as it has a high blood flow amounting to approximately 25% of cardiac output. The renal tubular cells, especially of the proximal tubules are vulnerable to toxic effects of drugs and metabolites due to their role in concentrating and reabsorbing glomerular filtrate by apical and basolateral transport mechanisms resulting in increased nephrotoxicity.[6-9] This leads to high levels of circulating toxins in proximal tubular cells.[10]

Metabolism and excretion of exogenously administered drugs and toxins are also performed by the kidneys. The drug-induced renal injury primarily occurs in patients with underlying risk factors like diabetes mellitus and hypertension which increase the vulnerability of kidneys to drugs. The nephrotoxic effects of drugs and other agents (herbal products and toxins) are enhanced by certain factors, which may be patient-specific, kidney related, and drug related. These factors may play their role as a single entity or in combination. The nephrotoxicity may express itself as acute kidney injury, tubulopathy, proteinuric renal disease and chronic kidney disease.[11]

The gross anatomy of mouse kidney has been described as similar in many respects to the kidneys of other mammals.[12,13] The mouse kidney is unilobar. A median longitudinal section shows a cortex which follows the convex border and a pyramidal-shaped medulla with the broad base against the cortex and the apex ending in a single papilla surrounded by the pelvis.

Various areas and regions of kidney where a drug may exert its effects include glomeruli, tubules, interstitium and blood vessels.

The potential drug used in this trial is a sample compound molecule with the chemical formula of '(E)-N-(1-methyl-4-oxoimidazolidin-2-yliedene) N-benzamide' being studied at the Department of Pharmacology IBMS, KMU Peshawar.

This compound is in the form of orange colored solid needle shaped crystals, with a melting point of 182^{o} C. This is soluble in acetone and methanol while insoluble in water.

By using thin layer chromatography (TLC) with ethyl-acetate and *n*-hexane 50% (1:1) and Rf value calculated at 0.5, this compound is visible under ultraviolet light.

The study was conducted to determine the acute histomorphological changes in kidneys of BALB/c mice treated with different doses of the molecule '(E)-N-(1-methyl-4-oxoimidazolidin-2-yliedene) N-benzamide'

MATERIALS & METHODS

This experimental study was carried out at Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar from February to July 2018.

Thirty two male BALB/c mice were used in this study. The mice were purchased from National Institute of Health (NIH), Islamabad. The mice were kept in cages in animal house of IBMS under normal environmental conditions with a 12:12 hours period of light and dark. Commercial diet was prepared for them. A period of one week was given for acclimatization. Food and water were provided *ad libitum*.

The histomorphological examination of the kidney specimens taken after sacrificing the animals with a special reference to acute toxicity on kidney of BALB/c mice was carried out to determine the safety of the said molecule. In addition, levels of blood urea nitrogen (BUN) and serum creatinine were estimated by taking blood samples at the time of animal scarification to determine the toxic effects of the said molecule on the function of the kidneys.

For the acute study of the said molecule, the mice were divided into four groups each consisting of eight mice. Simple randomized technique was used for sampling mice. Group 1 was control whereas group 2, 3 and 4 were made for doses of 1mg/kg, 10mg/kg, and 100 mg/kg respectively and kept in separate cages. Color codes were defined for various groups and the tails of mice were marked with permanent markers for identification of group as well as dose. Weight of each mouse in each group was recorded by electronic scale and entered in a chart for calculating dose of the drug.

The dose of each mouse was calculated according to the weight of mouse. The drug was dissolved in carboxy methyl cellulose (CMC) and made homogenous by a stirrer.

The drug was administered orally by using a syringe with a rounded metallic tip. Every mouse from experimental groups was taken out from the cage, held in one hand, placed horizontally and with the other hand its head was stabilized by the animal attendant. The other person filled the desired dose in syringe, put it gently in the mouth of mouse which engulfed it till a feeling of give way was reached. Then the drug was administered into the stomach of mouse. The animals were observed constantly during initial 24 hours regarding vomiting, activity, and other behavioral changes. The observations were recorded in a Performa.

The animals of control as well as experimental groups were observed for seven days after administration of the drug and then sacrificed by cervical dislocation. Blood samples were drawn in 3 ml disposable syringes by cardiac puncture for estimation of BUN and serum creatinine. The dissection was carried out and both the kidneys were taken out. The morphological features of kidneys like appearance, color and texture were noted. The weight of both the kidneys was recorded. Both the kidneys were preserved in 10% formalin solution.

The formalin preserved kidneys were fixed in the same fixative for 12-24 hours. The specimens were dehydrated by alcohol 70% for 2 hours and cleared in xylene. Then the specimens were embedded in paraffin at 58^{0}C and subjected to microtome sectioning at five-micron slices for preparation of slides. The slide specimens were stained with eosin and hematoxylin.

The histological slides were examined under the microscope at different powers to see any changes in the glomeruli, tubules, vessels and interstitium. Photographs were taken by a digital camera on the light microscope connected to the computer.

The carcasses and remains of the dissected experimental animals were disposed of as per procedures mentioned in "The Khyber Medical University Rules 2011 for animals' Scientific Procedures (Issue I)"

Data were entered and analyzed by using SPSS 20.. Mean and standard deviation were calculated for continuous data while frequency and percentages were calculated for categorical data. Independent T test was applied to compare variables among two groups while ANOVA was applied for comparison among multiple groups. Chi-square test was applied for comparison of categorical data where p value ≤ 0.05 was considered as statistically significant.

RESULTS

Data regarding the grouping, numbering, bodyweight before and after experiment, and kidney weight are provided in Figure 1(a-d).

Reduced motor activity, mild sedation and reduction in food intake were observed in experimental groups. Water intake was normal in all groups.

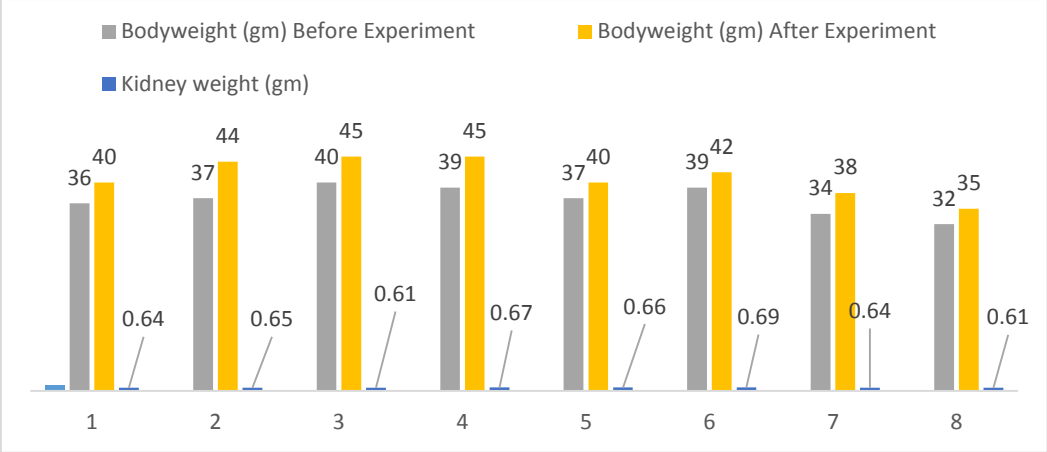


Figure 1a: Morphological data of acute toxicity study (Group 1; Control).

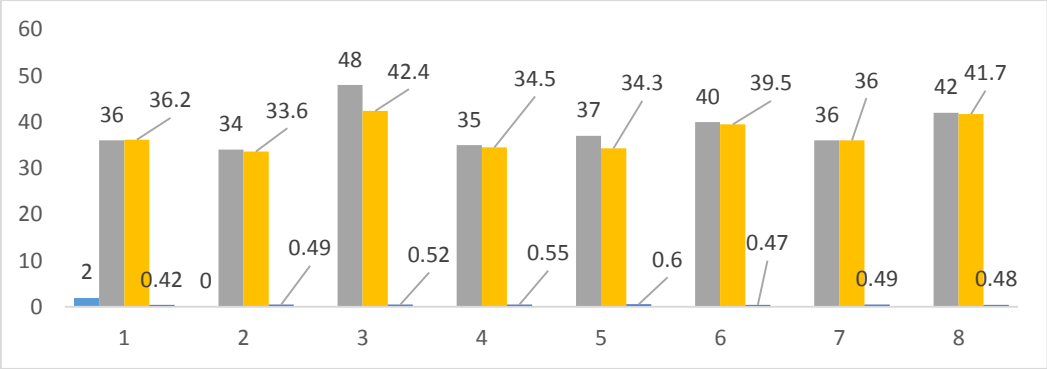


Figure 1b: Morphological data of acute toxicity study. (Group 2; Experimental 1mg/kg)

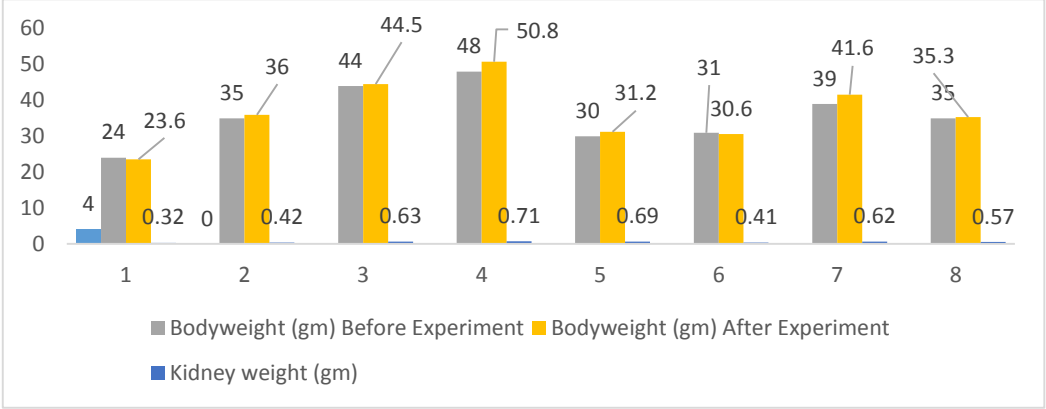


Figure 1c: Morphological data of acute toxicity study. (Group 3; Experimental 10mg/kg)

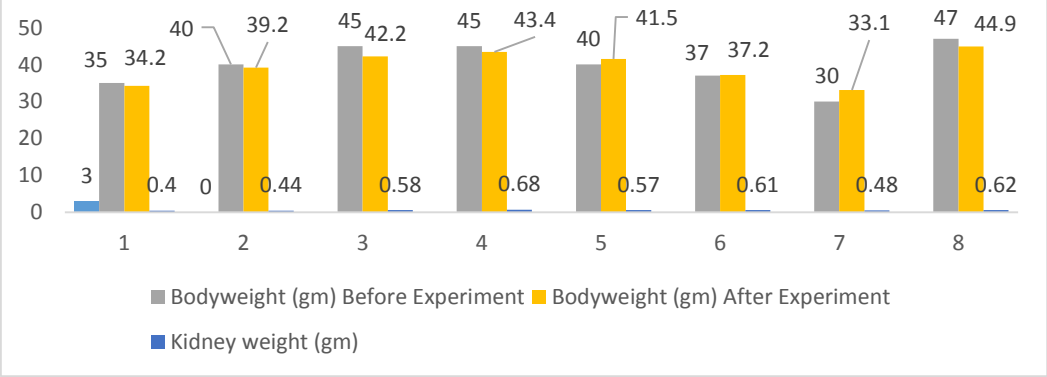


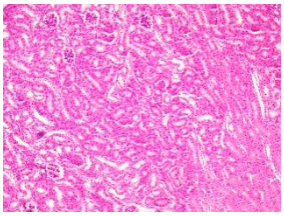
Figure 1d: Morphological data of acute toxicity study. (Group 4; Experimental 100mg/kg)

Levels of BUN (Blood Urea Nitrogen) and serum creatinine in acute toxicity study

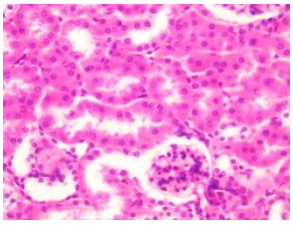
The blood samples taken at the end of 7 days period were sent to laboratory for estimation of BUN and serum creatinine. The results showed that the levels of BUN and serum creatinine were within normal range in all the groups.

Histological results of acute toxicity study

The appearance of the microscopic slides of kidneys from control group of acute toxicity study are depicted in Figure 2. It shows the normal structure of glomeruli, tubules, interstitium and vasculature.



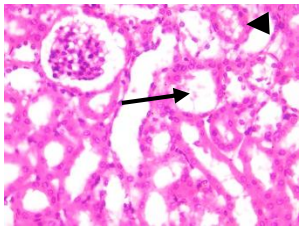
Control Group (10x)



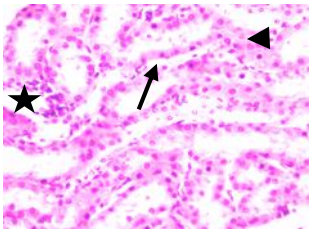
Control Group (40x)

Fig. 2: Microscopic appearance of kidney from control group (10x & 40x). Shows normal appearance of the renal parenchyma & vasculature.

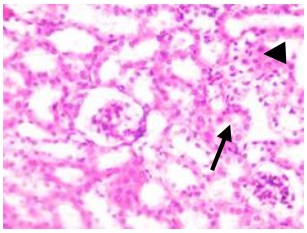
The appearance of kidneys under the light microscope in groups 2, 3 and 4 of acute toxicity study are shown in Figure 3.



Group 2 (40x)



Group 3 (40x)



Group 4 (40x)

Fig. 2: Microscopic appearance of kidney tissue from acute group 2 (40x) showing evidence of tubular swelling and necrosis (arrow), & tubulorrhexis (arrowhead). Group 3 (40x) shows evidence of tubular swelling & necrosis (arrow), vascular congestion (arrowhead) and interstitial inflammation (asterisk). Group 4 (40x) shows tubular swelling (arrow) and interstitial inflammation (arrowhead)

DISCUSSION

A total of 32 BALB-C mice were used in this experimental study. These mice were further subdivided into four groups of 8 each. For each group, there was a defined dose of the drug; 1mg/kg, 10mg/kg, and 100mg/kg of body weight for groups 2, 3 and 4 respectively while group 1 was the control group.

The comparison of body weight of mice within the acute toxicity study indicates that there was no statistically significant change in body weight before drug administration and at the time of sacrifice ($p=0.141$). Though there was decrease in food intake after administration of drug, it did not significantly affect the body weight in acute toxicity study.

The blood samples were taken at the time of sacrifice of animals to determine BUN and serum creatinine. Due to drug toxicity of any compound, the effect on the kidney is depicted by a rise in the levels of BUN and serum creatinine. No significant difference was observed among subgroups of this study ($p>0.05$).

BUN and creatinine are the traditional biomarkers for assessment of renal function. But these markers do not possess the sensitivity and selectivity required to detect renal damage caused by any nephrotoxic agent before the marked progression of the disease. There is a need for developing second generation biomarkers for acute kidney injury which will help in drug development and planning procedures for product testing. Based on such biomarkers clinical trials can be designed for assessment of efficacy and safety of potential candidate drugs.

The results of present study indicate the estimated biomarkers (BUN and serum creatinine) were not useful for detection of renal injury caused by the molecule which was used in our study. This is supported by the findings in the study of novel biomarkers of acute kidney injury in children by Sandokji & Greenberg 2020,[14] and Pavkovic et al., 2016.[15]

Role and significance of renal biomarkers in the early detection of acute renal injury has been emphasized by Al-Naimi et al. 2019.[16] Some of these markers are kidney injury molecule-1 (KIM-1) as described by Al-Kuraishy 2019,[17] and Vijayasimha 2019,[18] cytokines described by Al-Harbi NO et al., 2019,[19] and Osteopontin described by Zhao H et al., 2019.[20]

On histological examination of the kidneys, the tubular swelling and interstitial inflammation were significantly associated with the drug ($p>0.05$) while other morphological features like color and texture did not change. Moreover, tubular necrosis, periglomerular fibrosis, vascular congestion and thrombosis were not caused by the new molecule used in this study at low doses ($p>0.05$).

The findings of this study are consistent with the literature. The mechanisms by which drugs produce nephrotoxicity are multiple and early detection of renal injury is critical to prevent renal dysfunction. Timely intervention helps to prevent further renal damage.[21]

CONCLUSION

This compound of benzamide derivative has shown nephrotoxicity in increasing doses in BALB/c mice. Further studies are required to explore its safety for human use.

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