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Evaluation of Some Kidney Functions of Rates Treated with Sodium Benzoate

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ABSTRACT

The present study was conducted in the Department of biology at the College of Science toinvestigate some of the effects that can be caused by benzoate sodium treatment on the functional efficiency of the rats' kidneys by evaluating some functional indicators in addition to studying the histological changes of the treated rat kidneys. (36) Female rats *Rattus norvegicus* were used with average age 55 days and weights ranging from 175-200 g. They were divided into three equal groups. Each group included 12 female rats, the control group (C) injected daily with normal saline, the first treatment group (T1) injected daily with sodium benzoate at a100 mg/kgbody weight. The second treatment group (T2) injected daily with sodium benzoate at a concentration of 300 mg/kg, and the experimental period was two weeks. Animals were sacrificed after the end of the experimental period, and blood samples were taken from the abdominal vein to study some blood parameters that included (RBCs,WBCs,Hb, and Lymphocytes) and some serological tests to study levels (urea, creatinine, sodium, and potassium). The kidney was also removed to study histological changes. The results of the study of blood parameters showed a significant decrease in red blood cells (RBCs) and hemoglobin (Hb) due to sodium benzoate treatment compared with the control group.On the other hand, sodium benzoate caused a significant increase in white blood cells (WBCs) and lymphocytes compared to the control group. A significant increase in serum sodium, potassium, urea, and creatinine levels was observed in the first and second treatment groups compared with the control group. The microscopic examination of the kidneys showed the effect of sodium benzoate in the structure of urinary tubules. It caused degeneration in some urinary glomeruli and the expansion of urinary tubules with cells infiltration , congestion and calcification in some urinary tubules. We conclude from this study that sodium benzoate negatively affects the functional efficiency of the urinary system.

Keywords: *sodium benzoate, Rats, kidney, blood parameters, serological tests*

1. INTRODUCTION:

Sodium benzoate is a chemical compound. The chemical formula ($C_7H_5NaO_2$) has a high solubility in the water, reaches 660 g\L, disintegrates with heating, is odorless, and is used as a preservative (Kuboto k, Ishizaki T 1991**).** Sodium benzoate has an antimicrobial impact on the food that continues for several years. It is also used as a preservative, especially for keeping foods with high acidities such as Gaseous drinks and fruit juices (FDA,2011). Also used with milk and meat vegetable products (Zengin *et al.*, 2011).In addition to using for conservation in cosmetics, mouthwashes, and Pharmaceutical products (Nishna *et al.*, 2012). As a result of the wide use of sodium benzoate, many studies have been conducted to determine their toxicity and effect in various physiological systems for different types. It has been found that sodium benzoate can inhibitcell division in aquatic organisms (Straatton and Corke, 1982). It also owns toxic developmental effects that have led to several deformities in frog embryos (Dawson *et al.*,1996)**.**

The toxic effects of benzoate were observed in various body organs of mammals. The symptoms of acute toxicity of high doses of sodium benzoate in humans result in gastrointestinal irritation and effects on the central nervous system (SCF, 1994). The high doses of sodium benzoate caused the release of histamine and prostaglandin (Kreindler *et al.*,1980), in addition to ulcers and change of gastric secretion (Schaubschlager *et al.*,1991). Also, Sodium benzoate has been shown to impact growth indicator and body weight, thus leading to a reduction in body weight. Reduction of body weights was observed in mice treated with sodium benzoate at levels (200 mg/kg of body weight). In another study by the researcher Taheri (2002), When pregnant rats were treated with sodium benzoate with concentrations (9.3 and 18.6mmol/kg of body weight), Led to a decline in the weights of embryos, in addition to the lower of weights and diameters of placenta compared to control group. Hu *et al.* (2008) showed that the effect of different sodium benzoate concentrations on isolated lymphatic node cells from mice, which altered the structure and destroyedthe plasma membrane of lymphocytes. On the other hand, Yilmaz *et al.* (2009) noted the effect of sodium benzoate on human's lymphocytes in *vitro* at levels dose

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(200 and 500 µg/mL). Also caused in the decrease of white blood cells (WBCs)) and hemoglobin concentration of rats treated with sodium benzoate at dose levels 60 and 120 mg/kg (Eberechukwu *et al*..,2007), in addition to the increment of creatinine,urea, and uric acid (Lu and Shen, 2006).

Many studies have shown the effect of sodium benzoate on the kidney functions, which is responsible for the purification of blood from the toxins produced by the metabolism (Walter and Boron, 2004). Dewangan (2009) noted a gradually significant increase in each of urea and creatinine levels in serum with increased sodium benzoate doses, also histopathological change in kidney tissue. The study of Abd-ALgadir *et al*. (2009) showed significant increases in creatinine, uric acid, and urea values when the rats were orally administered with sodium benzoate at concentrations (100, 500, 1250 mg/kg body weight).

2. MATERIALS AND METHODS:

2.1 Experimental Animals

Twenty-seven female rats***Ratuss norvegicus*** obtained from the College of Veterinary Medicine / Al-Qadisiyah University were used with average age (55) days and weights (175-200 g). Experimental animals were placed in special plastic cages with metal lattices. Cages were cleaned and sterilized with Antiseptics and the cleaning of the oral perfusion bottles and ventilation room. Experimental animals subjected to laboratory conditions suitable temperature 20 - 25 °C.The animals were provided during the duration of the experiment with water (*ad libitum*) and a standard diet(9% protein and 3000 calories).

2.2 Sodium Benzoate

Sodium benzoate was used in this study obtained from the chemistry department /Science College/ Al-Qadisiyah University. Two concentrations of benzoate were used (100 and 300mg/kg of body weight). The animals were injected Intraperitoneal *(ip)* with (1 ml per animal).

2.3 Experimental Design

The study included an effect of *ip* injection of sodium benzoate at two different concentrations on renal functional efficiency. (36) Female rats were divided into three groups, and each group included (12) animals (Figure 1). Control group: included 12 animals injected with normal saline for two weeks. The first treatment group (T1): included 12 animals injected with sodium benzoate at a concentration of (100 mg/kg) for two weeks. The second treatment group (T2): included 12 animals injected with sodium benzoate at a concentration of (300 mg/kg) for two weeks.

2.4 Samples Collection

At the end of the treatment period, the rats were anesthetized using chloroform, the animals were dissected, and blood samples per (2ml) of each animal were withdrawn directly from the abdominal vein (Method name here)**.**A fraction of the blood samples were thenplaced intubes with Potassium EDTA anticoagulant for estimation of some of the physiological parameters,which included (RBC,Hb,WBC,Lymphocytes).The other part of the blood sample has been putting in a gel tube without anticoagulant to estimatesome of the biochemical parameters, which included (K^+,Na^+,creatinine, and urea).The kidneys were removed and placed in sterile tubes with(10%) formalin for studying pathophysiological change in the renal section.

Comment [LADB1]: Format the subsections as in the template

2.5 Hematological Examination

For determination of total RBCs and WBCs count was used hemocytometer. Hayem's solution as a blood sample dilator was used for total RBCs count in the slid chamber. For total WBCs count, Turk's solution withconcentration 1-2 % acetic acid to diluted blood sample (Stibbe *et al.*, 1985). Hemoglobin levels in blood were estimated by using the Sahli method. The differential count of leucocytes was used to determine lymphocytes numbers, and the blood smears were stained with Leishman's stain. The counts of lymphocytes are based on the characterization of the cell nucleus and its size, which is small in size, and its nuclei occupy most of the size of the cell.

Comment [LADB2]: Proper paragraph

2.6 Biochemical Examination

The spectrophotometer technique was used for the estimation of sodium and potassium levels in the serum. (Na kit and K kit) were supplied by (Human Company. German). According to the manufacturer's instructions, sodium and potassium levels were measured at length waves, 410 nm and 578nm, respectively.

To estimate of serum urea and creatinine concentration used colorimetric method, an examination based on the manufacturer Reagents by Randox Company (Bartels and Bohmer, 1972).

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2.7 Histopathological Examination

Histological sections were prepared by method (Luna, 1968). Diagnosis of the pathistological changes that are resulting from the injection of the sodium benzoate. Photomicrographs of sections have been taken by a camera lens mounted on the optical microscope at ×100 magnification.

2.8 Statistical Analysis

The experiment results were analyzed by using a t-test to determine the significant differences among groups at a level than 0.05 (Shiefler, 1980).

3. RESULTS AND DISCUSSION

3.1 Hematological Study

Results of the effect of *ip* injection of sodium benzoate on some hematological parameters,which included (RBCs,WBCs,lymphocytes,and Hb(hemoglobin)) illustrated in Table (1) and figure (3),which showed to significant different ($p<0.05$) in all parameter in each of treated groups compared with control.The total number of RBCs and hemoglobin results recorded a significant decrease in treated groups with sodium benzoate at two concentrations (100 and 300 mg/kg), and the reduction was more significant in T2 in comparison with T1. Simultaneously, the results (total WBCs, and lymphocytes) revealed a significant increase in each of the two treated groups compared tothe control group and each of the other (table 1 and figure 3)**.**

The current study reports a significant decrease in the total number of RBCs and hemoglobin, while there was a significant increase in the total number of WBCs and lymphocytes. Results of the present study were in agreement with Abdel Aziz and Zabut (2010), whose found a decrement in the total count of RBCs and the level of Hb concentration, in addition to an increment of the total count of WBCs and lymphocytes in male rats that orally administration with sodium benzoate at levels dose (500 mg/kg) for 12 and 26 days, respectively. While contrasts with Eberechukwu et al. (2007), who found that sodium benzoate is caused in reducing total count WBCs at level (60 and 120 mg/kg). The significant difference in the hematological parameter is treated groups may belong to the effect of sodium benzoate. It was noticed through previous studies that the effect of sodium benzoate on the hematopoietic centers resulting in increased or decreased effectiveness, which negatively affects the blood standards, which is reflected on the physiological functions of the natural body of the animal, Dewangan (2009) showed that the use of the sodium benzoate at three levels of doses (25,100, and 400 mg/kg of body weight) for 28 days significantly reduced RBCs and hemoglobin levels. This may be due to kidney dysfunction, which was evident through the study of histological changes in the current study. It is known that the kidneys are the main organ of regulating the erythropoiesis through the secretion of the erythropoietin hormone, which plays an important role in erythropoiesis. Therefore any damage or functional defect in the kidneys can be directly reflected on the erythropoiesis, as the low secretion of the erythropoietin that leads to anemia (Brugnara and Eckard, 2011). This is suggested functional dysfunction of kidneys is associated with reduced erythropoietin. On the other hand, the increase of WBCs and lymphocytes may be immune response due to inflammatory action resulting from treatment with sodium benzoate. Yilmaz et al. (2009) demonstrated increased indices of sister chromatid of human cells lymphocytes treated with benzoate sodium at levels 200 and 500 µg/mL. This may be lead to an increase in the number of lymphocytes. All these can indicate that sodium benzoate may have a critical role in inflammation action.

3.2 Biochemical Study

The result of concentration each of (Na^+,K^+,Urea, and creatinine) in serum illustrated in (table 2 and figure 4) Shows the presence of significant differences at level 0.05 in all studied biochemical parameters in rats treated with sodium benzoate compared with the control group. The levels of positive ion (Na^+, K^+) registered significant increment ($p<0.05$) in T1 and T2 compared with the control group, and this higher in T2 was more than T1.On the other hand, the concentration of urea and creatinine also revealed a significant increase ($p<0.05$) in treated groups in comparison with the control, and the rise was more pronounced in the T2 group compare with the T1 group (Table 1 and Figure 4).

The current study results revealed a gradual significant increment of serum levels of urea, creatinine, sodium, and potassium in treated rats that injected with sodium benzoate at doses levels (100 and 300 mg/kg for two weeks) compared with the control group. The results of an accurate study agreed with Abdel Aziz and Zabut (2012). They showed that the addition of sodium benzoate with feed could affect the function and metabolism

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of the body and lead to an increase in urea and creatinine values. As Cortan (2005) confirmed, the difference of urea and creatinine values due to treatment with sodium benzoate is a clear indication of renal dysfunction, especially glomerular filtration, that possibly leads to the accumulation of these metabolic products in the blood.

The decline in urea and creatinine can be attributed to the effect of sodium benzoate on the levels of glycinin serum, which in turn affects the level of metabolites such as urea and creatinine. Sodium benzoate is removed from the body by coupling with glycine, and high sodium levels mean more glycine loss through coupling. Thus lower blood glycine is reflected in the secretion rate of both urea and creatinine. As known, lower glycine levels can reduce the secretion of creatinine and urea in urine, resulting in the accumulation of these substances and increases of its values in the blood (Oyewole et al..,2012).

Present results were in agreement with Ibekwe et al. (2007), who is a confirmed the increase of sodium and potassium levels in the serum of treated rats with sodium benzoate at levels doses (60 and 120 mg/kg of body weight) for two weeks. The increasing in the levels of electrolytes (Na+ and K+) in the serum due to injection with sodium benzoate observed in the current study may be belong to the effect of sodium benzoate on controlling mechanisms of regulation and balance of electrolytes in the body fluid, as it was noted in previous studies that sodium benzoate effect on glucocorticoid that produced by the adrenal cortex glands, this leading to increased levels these hormones in serum in response to the effect of oxidative stress caused by sodium benzoate treatment (Sabour, 2015) glucocorticoids have Mineralocorticoids activity in reabsorption of Na+ and K+ ions and increase their concentration in blood as a result of their affinity to binding with Mineralocorticoids Receptors MR, this affinity is increase when increasing levels of glucocorticoids in blood, Stimulation of Mineralocorticoids Receptors MR leads to the retention of Na+ where epithelial Na Channels that located in apical membrane of in distal and collecting urinary tubules are more effective (Khurana,2006). On the other hand, a high concentration of Na+ and K+ ions may be the result of the direct effect of benzoate on the effectiveness of the enzyme Na + / K + ATPase (Choudhary and Rathinasamy, 2013), Which negatively affects the regulation and balance of the electrolytes inside and outside the cell, which leads to disruption of cellular function, thus reflected on the homeostasis of body fluids

3.3 Histopathological study

Histopathological sections obtained from rat's kidney (figure 5) showed the effect of sodium benzoate in the structure of urinary tubules. Compared with the control group, which normally appeared (a),while the section obtained from rats treated with sodium benzoaterevealed histological changes include damage in the structure of the renal glomeruli with varying degrees and detachment of epithelial cells from the basement membrane.Also, infiltration of inflammatory cells,especiallylymphocytes, and dilation of urinary tubules(b and d),in addition to necrosis and severe congestion, especially in T2 with clear vascular calcification (c and d).

Our results of the kidney section obtained from rats treated with sodium benzoate showed some Histopathological changes compared to the control group. Severe histopathological alterations in the renal tissue section of rats treated with sodium benzoate, especially in T2 groups as seen in our study, and all these alterations show to the potential of sodium benzoate to cause renal impairment at the structural and functional levels, sodium benzoate exposure in present study proved renal glomeruli damage, necrosis, and severe congestion, in addition to calcification. The Current study is Consistent with the findings of previous research by (Abd-AlGadir *et al.*, 2009), whose found the effect of sodium benzoate at two concentrations 100 and 500 mg/kg on renal structure and function. These may be due to metabolic products of sodium benzoate. The sodium benzoate is removed from the body after its association with glycine on the form of Hippuric acid, which is also called Benzoyl glycine, it is expelled in urine (Elsa and Miljøstyrelsen, 2000). this compound is associated with urinary tubules injury (Edamatsu *et al.*, 2014). Also, the causes of pathohistological changes may belong to cellular damage due to oxidative stress, which can result from sodium benzoate injection. Studies have shown that exposure to sodium benzoate significantly affects the incidence of oxidative damage by interfering with biological activities in the body and, therefore, interferes with the body's homeostasis (Kale, 2015). Oxidative stress is caused in the generation of free radicals (ROS), leading to the dysfunction of mitochondria, which reflects on cellular activity. It is also known that mitochondria play an important role in maintaining cell viability and growth by providing energy to activate intracellular enzymes (Kakkar *et al.*,2000). On the other hand, oxidative stress and inflammation are factors that possibly cause vascular calcification, especially in renal tissue; Therefore, Vascular calcification is the most common complication associated with chronic kidney disease (Masahide *et al.*, 2009). in the current study, we showed clear vascular calcification in renal tubules of treated rats with 300mg/kg of sodium benzoate, which may indicate that the sodium benzoate exposure contributes to the development of chronic kidney disease And causes complications such as Cardiovascular diseases, As calcification causes the loss of artery elasticity (Janzen and Vuong, 2001).

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Conflict of Interest:

"The authors declare that they have no conflict of interest."

5. CONCLUSIONS:

In thisstudy, In this study, it was noticed that sodium benzoate has a negative effect on renal efficiency by reducing erythropoiesis in addition to inflammatory action on renal tissue. It also caused disruption of function in regulation and balance of (urea, creatinine, $Na^{+,}$ and K^{+}) and disorganization of renal tubules, all of these effects threaten the occurrence of chronic kidney disease and its complications, including cardiovascular diseases.

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Appendix

Table 1. Hematological parameter (RBCs,WBCs,lymph,and Hb) of rats treated with sodium benzoate

Parameter	**Control**	**T1**	**T2**
Total RBCs (×10^{12}/L)	7.39±0.119^{a}	6.35±0.16^{b}	5.26±0.15^{b}
Total WBCs (×10^{9}/L)	5.23±0.21^{a}	7.30±0.15^{b}	8.94±0.24^{b}
Lymph %	3.92±0.13^{a}	4.81±0.10^{b}	5.76±0.15^{c}
Hb (gm/L)	14.34±0.65^{a}	11.92±0.10^{b}	10.15±0.31^{c}

Value .represent mean ± standard error, a different letter (a, b, and c) represent significant different ($p<0.05$)

Table 2:Biochemical parameter (Na^{+},K^{+}, urea, and creatinine) of rats treated with sodium benzoate

Parameter	Control	T1	T2
Na+(mmol /L)	85.45±0.58^{a}	112.21±0.21^{b}	135.9±0.80^{c}
K+ (mmol /L)	1.65±0.07^{a}	2.50±0.01^{b}	4.06±0.11^{c}
Urea(mg/dl)	44.4±1.61^{a}	105.03+2.10^{b}	136.27+4.32^{c}
Creatinine	0.4±0.02^{a}	0.85±0.01^{b}	1.35±0.03^{c}

Value .represent mean ± standard error, a different letter (a, b, and c) represent significant different ($p<0.05$)

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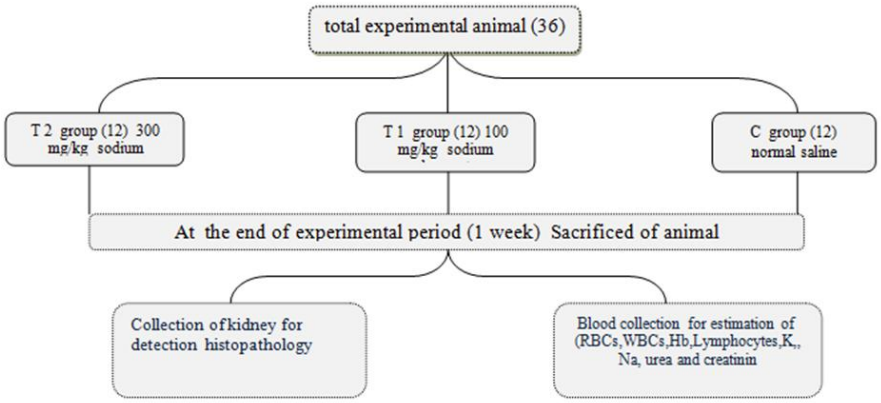

Figure (1) : Schematic diagram showing experimental design

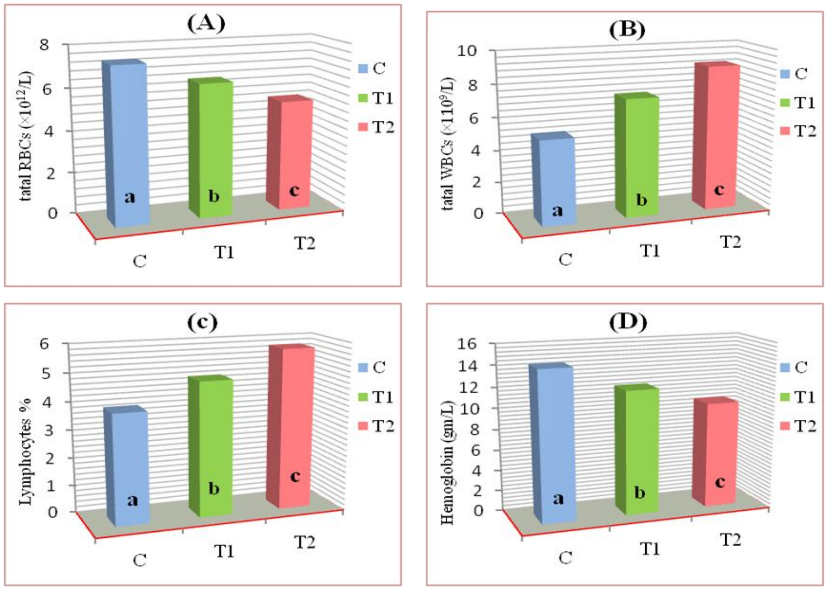

Figure 3: show to the impact of *ip* injection with sodium benzoate on hematological parameter of rats as follow total RBCs ($\times 10^{12}$/L) in (A),total WBCs($\times 10^{9}$/L) in (B) ,Lymphocytes % in (C) and Hemoglobin (gm/L) in (D) the columns labeled with Different letter show to significant difference at level o.o5 between experimental groups ,c (control),T1(first treatment),and T2(second treatment)

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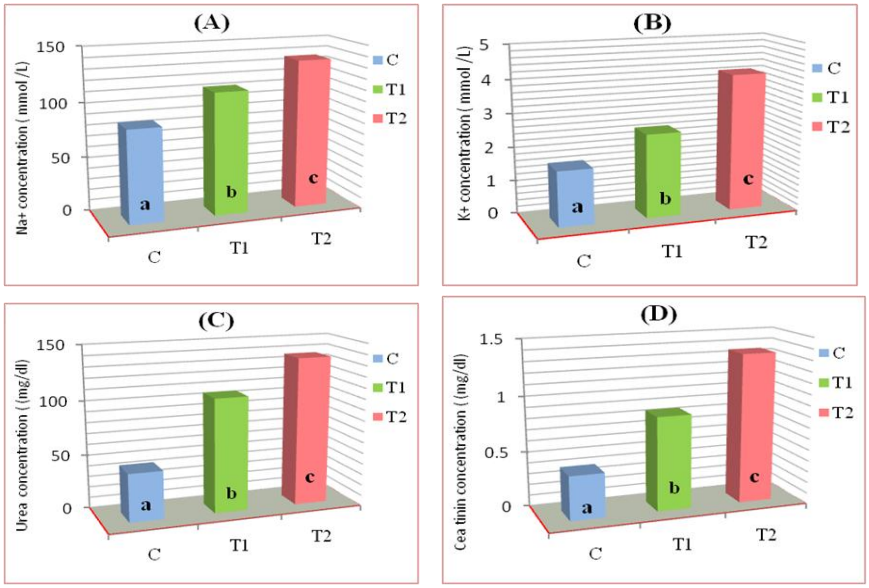

Figure 4: show to the impact of *ip* injection with sodium benzoate on biochemical parameter of rats as follow Na (mmol/L) in (A),K (mmol/L) in (B) ,Urea(mg/dl) in (C) and Creatinine (mg/dl) in (D) the columns labeled with Different letter show to significant difference at level o.o5 between experimental groups ,c (control),T1(first treatment),and T2(second treatment)

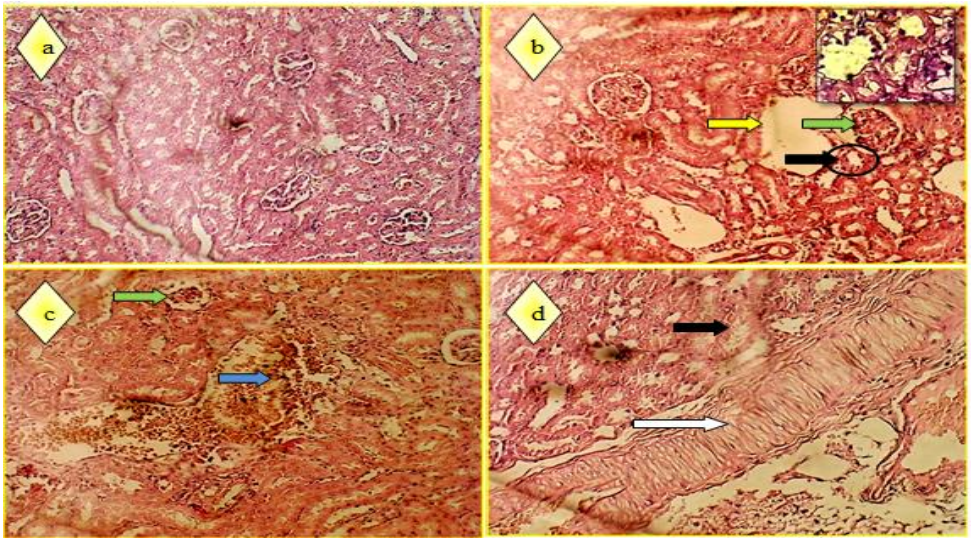

Figure 5 : histological section in kidney of rats treated with sodium benzoate (E *and* H stain with 100X) , control (a) , T1(b) ,and T2 (c and d) ,show to infiltration of lymphocytes (black arrows),damage of urinary glomerular tissue (green arrows) ,vaculation between urinary tubules(yellow arrows),cell infiltration with bleeding(blue arrow),calcification in urinary tubule(white arrow) , while normal structure in section of control (a).