

Protective Effect of Selenium Against Oxidative Stress Induced Potassium Dichromate Toxicity in Male Rats

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Abstract:

The purpose of this study was to investigate the potentially protective effect of selenium against potassium dichromate toxicity and to evaluate the toxicity of potassium dichromate in male rats. One indicator of oxidative stress is the presence of statistically significant levels of malondialdehyde in liver and kidney tissue as a result of the ability of potassium dichromate to produce stress-inducing electrolytes. Increased oxidized fat, decreased high-density lipids, and increased low-density lipids have been observed in male rats. Injury to the liver cell, kidneys, decreased activity of catalase and glutathione enzymes were observed in the liver and kidneys, and increased (TBARS) levels were observed in the liver and kidneys, noting histological changes in the kidneys and liver that confirm enzymatic changes. On the other hand, histological sections of the kidneys of the treated group showed potassium dichromate-induced contraction of the renal glomeruli and dilation of Bowman's sac, congestion, and hemorrhage of the vessels near the Malpighian body, and increased thickness of its walls. The presence of protein casts in the lumen of the renal tubules, haemorrhage between the tubules, dilation, and leukocyte infiltration and I. This study was performed in adult male white rats (24 rats), divided into 4 equal groups (6 rats) each. At the end of the experiment, blood was drawn from the rat ear vein in all groups for evaluation. Biochemical parameters in blood were analyzed, and samples from liver, kidney were collected from rats for histological examination.

Keywords: selenium. Oxidative stress. tanning. Chromium. Electrolytes.

Introduction:

Leather tanning is considered a major environmental pollutant due to the quantity and quality of waste generated during its production. The raw materials such as chromium salts and other chemical compounds that can directly affect workers in the field. Continuous exposure to these mineral toxins, which accumulate in the tissues of workers' bodies, can lead to diseases, including some that are considered carcinogenic. Additionally, the tanning process can produce free radicals,

which are derived from molecular oxygen and nitrogen oxides such as nitric oxide. These reactive oxygen species can damage biological compounds such as nucleic acids, proteins, and lipids. Reactive intermediates produced during the process usually remain tightly bound to the active site of the enzyme until the reaction is completed. However, they can escape from the active site of the enzyme, leading to a disturbing effect on cellular processes.

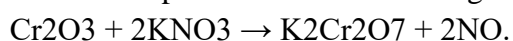
The main biological compounds that are damaged by free radicals (FR) include:

- Nucleic acids, which can lead to mutations.
- Proteins, which can cause cellular malfunction due to structural changes.
- Polyunsaturated fatty acids (PUFA).
- When a PUFA molecule reacts with a free radical, it produces a lipid free radical.

The lipid free radical (L•) rapidly reacts with molecular oxygen, forming a peroxy radical (LOO•) through Reaction 2. The LOO• radical can then attack another polyunsaturated lipid molecule to produce a lipid hydroperoxide and another lipid free radical through Reaction 3. This new lipid radical can be converted into another lipid peroxy radical, continuing the lipid peroxidation process as a chain reaction. Reaction 1 shows the reaction of a lipid hydroperoxide with a hydroxyl radical (OH•) to form a lipid free radical and water.(1)

***Chromium** is one of the most abundant elements in the earth's crust and exists in many oxidized forms, including zero-valent, trivalent, and hexavalent metals. The toxicity and carcinogenicity of chromium are mainly due to the oxidation state of chromium. Chromium is commonly used in the manufacture of metal alloys, paints, and inks, and in leather tanneries. Trivalent chromium salts are also used in cosmetics, glass manufacturing, and photography, where chromium is an essential element accounting for about 40% of hexavalent chromium depletion. The production of potassium dichromate occurs during the reaction of potassium nitrate with Cr₂O₃ according to the following equation.

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The compound hexavalent chromium is toxic even at concentrations below 14 mg/kg and is classified as a carcinogen due to its ability to produce oxidative stress that affects kidney and liver function in mammals (4). High exposure to hexavalent chromium can cause cancer, anemia, or gastrointestinal damage, and inhalation of this compound by workers has been shown to cause lung and stomach cancer (5).

* **Selenium** plays an important role in cellular function when present in low concentrations and is necessary for the synthesis of many antioxidant enzymes. It is also involved in the synthesis of three iodine dehydrogenases that facilitate the conversion between thyroid hormones. (3)

Materials and method:

24 male Westar rats were used, and their weights ranged from 150 ± 10 grams. The rats were left to acclimatize for a week before the experiments began. The rats were exposed to a temperature of 25.C and to 12 hours of light darkness. They were fed standard food and had free access to water. The animals were randomly divided into four groups (6 mice).

The protocol has been described for animal housing and care are described in the Laboratory Animal Care and Use Manual.

The duration of the experiment lasted (10) days and the treatment was as follows:

1. The first group (control) took saline solution by peritoneal injection at a dose of (1 ml / kg) daily for 10 days
2. The second group (selenium group) was treated by peritoneal injection with sodium selenite compound in saline solution at a dose of (1 ml / kg) in two doses, one on the first day and another on the sixth day of the experiment.
3. The third group (chromium group) was treated with potassium dichromate at a dose of (0.4%) for 10 days.
4. The fourth group (selenium group and potassium dichromate) was injected by peritoneal injection with potassium dichromate at a dose of (0.4%) for 10 days and was treated with two doses of soda selenate compound Its amount (1 ml / kg) on the first and second day on the sixth day of the experiment.

At the end of the experiment, the rats were weighed, and blood samples were taken to conduct various blood measurements and to define the effects on liver and kidney function. The rats were slaughtered to evaluate the percentage of oxidative signs in the kidneys and to collect and analyze the results.

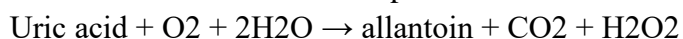
*Blood Measurement:

One milliliter of blood collected in heparinized sample tubes can be analyzed to measure various blood parameters, including hemoglobin (Hb), total red blood cell count (RBC), mean cell size (MCV), mean intramuscular hemoglobin (MCH), mean body hemoglobin concentration (MCHC), total white blood cell count (WBC), hematocrit (HCT), and total platelet count. These measurements can be obtained using an automated blood test analyzer, such as the Advia 60 Hematology System.

Biochemical Measurements

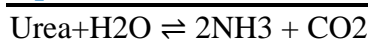
Uric acid reacts with oxygen and water to produce hydrogen peroxide. The generation of H₂O₂ can be determined by measuring the catalytic activity of uric acid peroxidase. This reaction also generates 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHBS) and aminofenarone (PAP-4), react to form the pigment quinolone magenta (17).

The overall reaction can be represented as follows:



Renal function measurement*

The percentage of urea was determined from the following reaction using a commercially available panel



In an alkaline environment, ammonium ions react with salicylate and hypochlorite to form the green dicarboxylic acid indophenol (2,2).

Measurement of creatinine*

Creatinine was measured using a commercial kit based on the principle of the colored complex that creatinine forms with picric acid in an alkaline medium, and the rate of formation of this compound was measured. One volume of sodium hydroxide was diluted with four volumes of redistilled water and mixed with one volume of the dilute sodium hydroxide to make a reagent mixture.

Results and Discussion:

Humans are in constant contact with toxic agents. Toxic substances in the food we eat, the water we drink, and the air we breathe. Toxic agents can be absorbed through the gastrointestinal tract, skin and lungs (7). The kidney is an important organ that is greatly affected to the Exposure to chromium compounds. administration of $\text{K}_2\text{Cr}_2\text{O}_7$ is nephrotoxic and results in acute Renal failure (ARF). 5 an administration of $\text{K}_2\text{Cr}_2\text{O}_7$ leads to an increase in urea and creatinine levels Blood plasma, urinary pH decreased, increased Urinary excretion of glucose and proteins Decreased excretion of sodium in the urine Doses of $\text{K}_2\text{Cr}_2\text{O}_7$ lead to the development of the acute condition Tubular necrosis is largely located to the proximal small loop of henly (8). It was discovered in this experiment that the rats treated with potassium dichromate decreased their weight after 10 days, due to the lack of food intake and lack of activity, in contrast to the treatment with sodium selenite which led to an increase in the weights of those groups, indicating recovery from the effects.

Toxic Potassium Dichromate. (9)

Results of rat blood, biochemical markers, plasma, and tissue markers in the group injected with potassium dichromate had lower red blood cell counts and increased levels of hemoglobin and hematocrit (MCH and MCV) compared to the control group and MCHC levels were higher than those???. In the control group, there was no change in the control group given doses of sodium selenite solution (10). High-density lipids were also reduced, low-density lipids increased, and their levels adjusted with sodium selenite. Cholesterol and triglyceride levels can also be elevated due to toxicosis. In the context of oxidative stress, we showed that treatment with potassium dichromate leads to increased activity of the liver function enzymes lactate dehydrogenase and glutamate transpeptidase. MDA increases. In kidney tissues, catalase activity and renal glutathione concentrations decreased and thiobarbituric acid (11). (TBARS) concentrations increased, which are indicators of renal oxidative stress. Due to the toxicity of potassium dichromate, exposure to potassium chromate has been shown to generate electrolytes that induce oxidative stress in many tissues. Its production leads to the oxidation of lipids in cell membranes and selenium exposed to the environment has been shown to act as an antioxidant against free radicals (18).

Histological sections are shown. Kidneys were detected in the potassium dichromate-treated group Renal glomeruli contraction, Bowman's capsule dilatation, congestion and hemorrhage Blood vessels near the Malpighian globules which increase their wall thickness was noted.

Presence of cast protein in the lumen of the renal tubules, bleeding and expansion, infiltration of white blood cells between the urinary tubules, as well as the presence of necrosis and secretions in the cytoplasm of the cells lining the urinary tubules, as well as the presence of edema in some places in the renal cortex was observed. It was also found that the group treated with sodium selenite + potassium dichromate was shown to improve liver and kidney tissue(19).

Table 1 Erythrocyte analysis

Parameters	Control	Se	K2Cr2O7	K2Cr2O7 + Se
BODY WEIGHT				
INITIALT	159.20 ± 9.29	159.00 ± 8.95	160.00 ± 7.91	162.20 ± 8.56
FINAL	170.0 ± 8.40	172.80 ± 10.21 b	165.45 ± 9.91 a	171.40 ± 9.42
CHANGES	8.10 ± 1.55	8.67 ± 1.26 b	3.34 ± 1.76 a	5.67 ± 1.13
LIVER WEIHT	6.69 ± 0.50	6.88 ± 1.26 b	6.16 ± 0.59	7.10 ± 0.84
KIDNEY WEIHT	1.15 ± 0.10	1.16 ± 0.09	1.11 ± 0.12	1.18 ± 0.09

Values represent the mean ± SE of 6 samples

a means significantly different from the control group

b means significantly different from the k2cr2o7 group

p < 0.05

Table2. Kidney glutathione (U/g tissue) of rat treated control k2cr2o7 & Se & k2cr2o7 + Se

Group	Control	Se	k2cr2o7	k2cr2o7 + se
Mean	0.348	0.326	0.436	0.350
St Div	0.013	0.023	0.037	0.021
St Error	0.0116	0.020	0.034	0.019
	C	C	abd	c

Table3 (Experimental groups)

Changes in erythrocyte values	Control	se	K2cr2o7	Se + k2cr2o7c
RBC (10 ⁶ /mm ³)	4.60 ± 0.24	4.65 ± 0.33 ^b	3.93 ± 0.34 ^a	4.41 ± 0.32 ^a
Hb(g/d L)	13.11 ± 0.88	13.31 ± 0.91 ^b	10.20 ± 0.87 ^a	12.32 ± 0.81 ^b
Ht%	42.01 ± 5.77	45.41 ± 5.57 ^b	33.65 ± 4.70 ^a	37.21 ± 4.79 ^{ab}
MCV(F L)	90.88 ± 8.44	98.66 ± 9.88 ^{ab}	85.22 ± 9.31 ^a	88.31 ± 12.13 ^a
MCH(Pg)	27.41 ± 0.54	28.60 ± 0.43	28.34 ± 0.65	26.98 ± 0.44
MCHC(g/d L)	31.51 ± 3.37	31.41 ± 3.79	31.45 ± 4.90	31.25 ± 4.90

Body, liver, and kidney weights (g) in the control k2cr2o7 & Se & k2cr2o7 + Se treated rats.

* (Kidney functional parameters)

Table 4. Serum urea and creatinine levels in the control

Parameters	Control	Se	K2Cr2O7	K2Cr2O7 + Se
Urea (mg/dL)	33.24 ± 7.07	33.60 ± 7.6 ^b	70.03 ± 17.71 ^a	31.41 ± 6.7 ^b
Creatinine (mg/dL)	0.11 ± 0.05	0.27 ± 0.048 ^b	1.10 ± 0.33 ^a	0.33 ± 0.19 ^b

(Malondialdehyde (MDA) levels in kidney homogenate of the control)

Table5. k2cr2o7 & Se & k2cr2o7 + Se treated rats

Parameters	Control	Se	K2Cr2O7	K2Cr2O7 + Se
MDA (nmol /mg kidney tissue)	84.41 ± 13.95	83.01 ± 26.75 ^b	156.21 ± 17.03 ^a	95.01 ± 12.00 ^b

Values represent the mean ± SE of 6 samples

a means significantly different from the control group

b means significantly different from the k2cr2o7 group

p<0.05

Table6. Total protein in kidney tissue

Parameters	Control	Se	K2Cr2O7	K2Cr2O7 + Se
protein (nmol /mg kidney tissue)	85.41 ± 45.95	83.80 ± 43.75 ^b	.21 ± 33.03 ^a 85	88.01 ± 29.81 ^b

Values represent the mean ± SE of 6 samples

a means significantly different from the control group

b means significantly different from the k2cr2o7 group

p< 0.05

Table7. Kidney an oxidant enzymes activities of the control)

Parameters	Control	Se	K2Cr2O7	K2Cr2O7 + Se
Superoxide dismutase (units/ng protein) (SOD)	58.21 ± 8.77	56.65 ± 9.28 ^b	30.21 ± 7.90 ^a	52.66 ± 9.33 ^b
Glutathione peroxiase (units/ng protein) (GPx)	33.40 ± 7.01	35.80 ± 8.25 ^b	15.55 ± 3.61 ^a	34.95 ± 7.65 ^b

Values represent the mean ± SE of 6 samples
a means significantly different from the control group
b means significantly different from the k2cr2o7 group
p< 0.05

*Kidney TBARS end catalase of rat treated with

Table8. k2cr2o7 & Se & k2cr2o7 + Se

Parameters	Control	se	K2cr2o7	Se + k2cr2o7
TBARS (mmol/mg protein)	23.65 ± 0.70	21.65 ± 0.74	31.1 ± 1.53	26.71 ± 1.01
Catalase (U/g tissue)	489.27 ± 41.42	499.43 ± 38.10	353.73 ± 25.31	416.00 ± 11.613

Values represent the mean ± SE of 6 samples
a means significantly different from the control group
b means significantly different from the k2cr2o7 group
p≤ 0.05

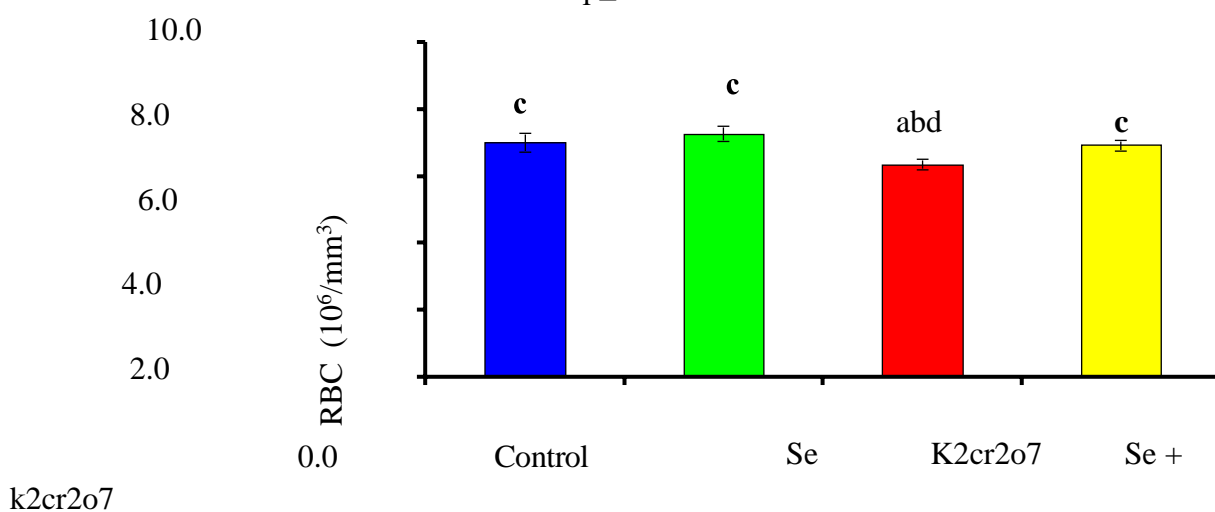


Figure 1: Red blood cell counts (RBC) (10⁶/mm³) of rat treated with. sodium selenite and

potassium dichromate Significance at $P < 0.05$.

^a Comparison of control and other groups

^b Comparison of (se) and other groups

^c Comparison of k2cr2o7 and other groups; ^d Comparison of se + k2cr2o7 and

other groups

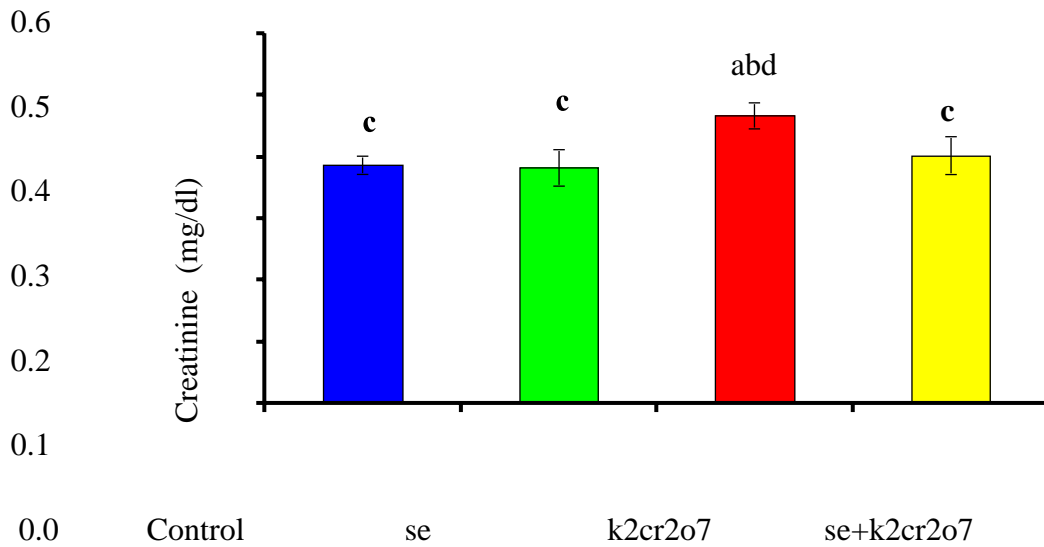


Figure2: Serum creatinine (mg/dl) of rat treated with (se) and (k2cr2o7).

Significance at $P < 0.05$.

^a Comparison of control and other groups

^b Comparison of (se) and other groups

^c Comparison of k2cr2o7 and other groups; ^d Comparison of se + k2cr2o7 and other groups

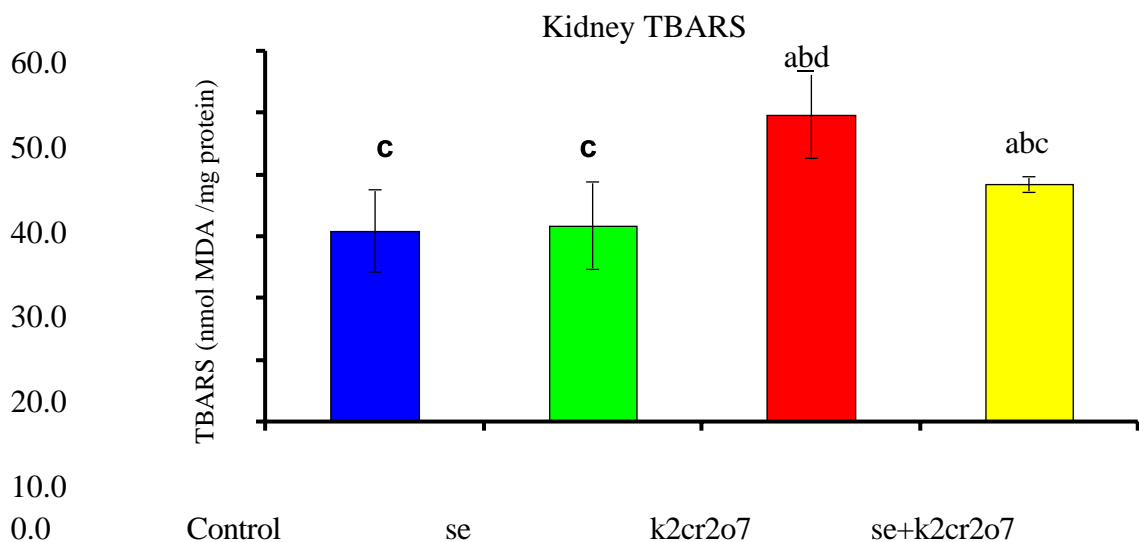


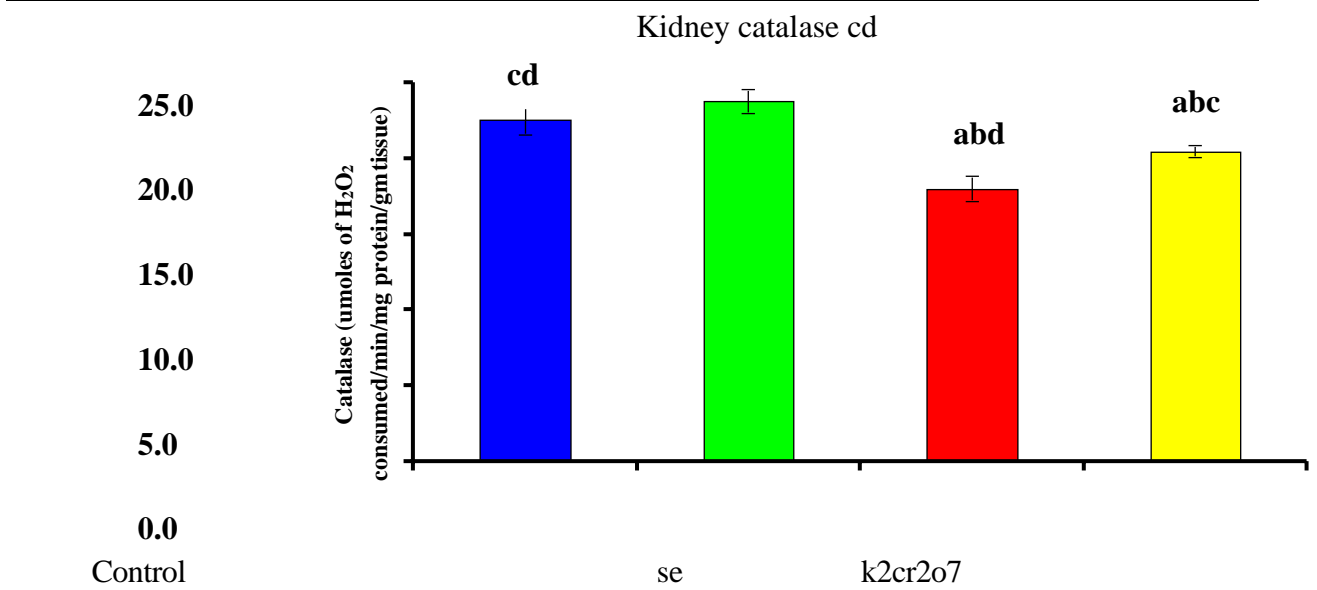
Figure3: Kidney (TBARS) (nmol MDA /mg protein) of rat

treated with (se) and k2cr2o7 Significance at $P < 0.05$.

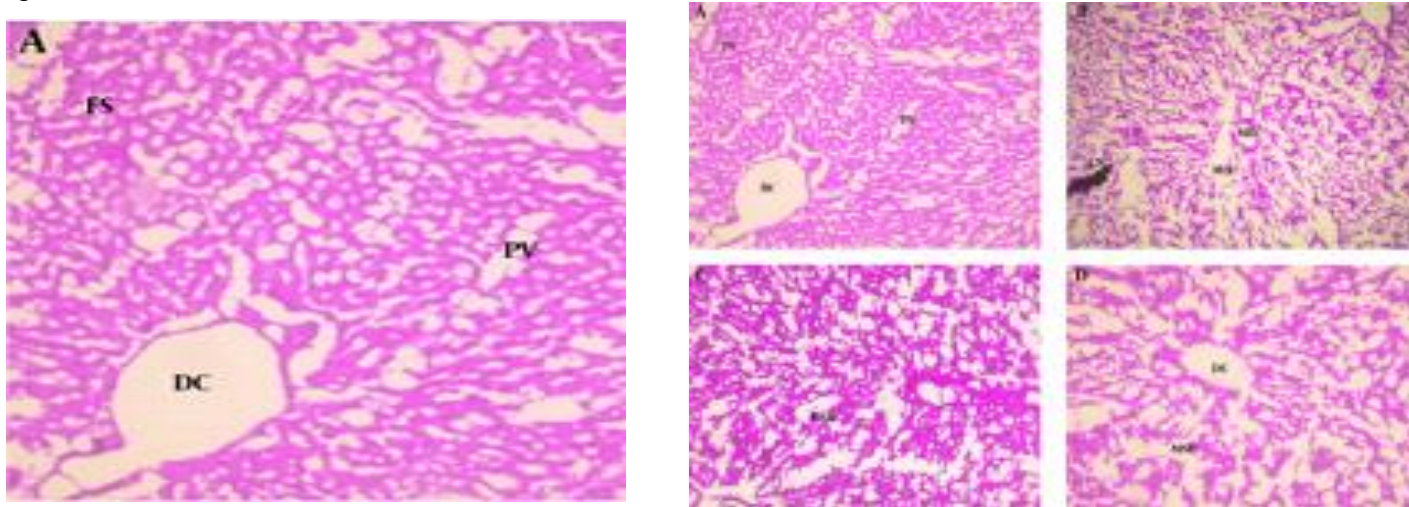
^a Comparison of control and other groups

^b Comparison of (se) and another group

^c Comparison of k2cr2o7 and other groups; ^d Comparison of se + k2cr2o7 and other groups



Figuer4: Kidney catalase (umoles of H₂O₂ consumed/min/mg protein) of rat treated with (se) and (k2cr2o7) Significance at $P < 0.05$.



^a Comparison of control and other groups
^b Comparison of (se) and other groups
^c Comparison of k2cr2o7 and other groups; ^d Comparison of se + k2cr2o7 and other groups

LIST OF ABBREVIATION

- CAT – catalase
- LDH: lactate dehydrogenase
- MDA:malondialdehyde
- MCH: mean corpuscular hemoglobin
- MCHC: mean corpuscular hemoglobin concentration
- MCV: mean cell volume
- GPx: glutathione peroxidase
- GR: glutathione reductase
- GSH: reduced glutathione

GST: glutathione-S-transferase

Hb: hemoglobin

Ht: haematocrit

HDL: high density lipoproteins

SOD: superoxide dismutase

TBARS: thiobarbituric acid reactive substances

WRC: red blood cell

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Fig4. Kidney catalase (μ moles of H_2O_2 consumed/min/mg protein) of rat treated with (se) and (k_2cr_{2o7}).

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