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TOXICOLOGICAL INTERACTION OF CHROMIUM AND GENTAMICINE SULPHATE ON KIDNEY FUNCTIONS.

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ABSTRACT
The present study was done to explore relation between kidney failure problem in Egypt and chromium toxicity with or without aminoglycoside. Chromium levels in tanning hides dyes and output of some factories were determined. Area of tainting hides in SOR MAGRY EL AIONE in Egypt used for collection of samples. Samples from certain solutions used in tanning hides, water discharge in these factories, water discharge of Kaha factories and Vetrac Company was used. Nephrotoxic effect of chromium on kidney functions of rabbits treated with and without gentamicin sulfate was studied. Four groups of rabbits each of four rabbits were used in this study. First group was given 1.8 PPM (mean of chromium level in analytic samples) potassium dichromate (VI) for 21 successive days in drinking water. Second group injected intramuscular by gentamicin sulfate (5ml/kg b.w) for seven days. Third group given pot. Dichromate for 21 successive days and gentamicin sulfate for seven days by doses and route as that given to first and second groups. Fourth group kept as control. Chromium and gentamicin sulfate levels were determined in serum and kidney of treated animals at the end of experiments (21 days). Serum chromium and gentamicin sulfate levels showed marked increase in group taken pot. Dichromate and gentamicin sulfate in comparison to first and second group. Chromium in the kidney tissues was increased in third group in comparison to first group taken pot. Dichromate (VI) Serum urea and Creatinine levels were also monitored. Serum urea and creatinine levels were increased in all groups in comparison to control group. Histopathological alteration of kidney was detected. Kidney in the group taken 1.8 PPM pot. Dichromate showed cellular infiltration and necrosis in renal tubule. Kidney in group taken gentamicin sulfate showed fibrosis in glomerular basement membrane and necrosis. Lesions in kidney of third group (pot. Dichromate & gentamicin sulfate) showed greater marked lesions in the kidney in comparison to first and second group.
INTRODUCTION
Industrial uses of chromium centered on the production of dyes and tanning of hides (Terry, 1995). Chromium is an essential trace element. Chromium has many oxidation states, of which the trivalent and hexavalent states are the most stable. Exposure to chromium and its salts takes place via cement, wood ash, plating baths, green baize of gaming tables, matches leather, tanning agent, leather gloves, welding fumes, coated zinc and galvanized iron sheets (Bang-Pedersen, 1982). Industrial and agricultural discharge is considering the primary source of metal poisoning (El Nabawi, et al, 1987). Several metals are toxic to the kidney either from occupational or environmental exposures (WHO, 1991). Chromium is essential for glucose metabolism, but the margin between required concentration and toxic concentration is often small (Bowen, 1966). Toxic effect of chromium are mediated via reactive oxygen intermediates during reduction of chromate (VI) to chromate (III) in tissue cells (Sugiyama, 1992). Chromium and its salts induce cytotoxicity, these observations suggest that chromium produces reactive oxygen species which may mediate many of the untoward effects of chromium (Bagchi, et al 1995). Chromium increased urinary excretion of low molecular weight proteins (B2-Microglobline and retinol-binding protein (RBP)) (Bernard and Lauwerys, 1991). Such increase may reflect tubular cell dysfunction, or damage or competition for absorption. B2-M is synthesized by all nucleated cells and is present on their membranes as a component of histocompatibility antigens. Healthy subjects excrete little B2-M in urine (L 100 μg/24 hr) but excretion is increased with renal tubular dysfunction (Goyer and Cherion, 1995). Chromium enhanced excretion of urinary lipid metabolites (Bagchi, et al 1995 and Bagchi, et al 1997). After oral or dermal absorption of chromium (VI) the kidney is the main target organ for chromium accumulation, which might result in acute tubular necrosis in human (Dartsch, et al., 1998). Same author adds that kidney epithelial cells are 10 times more sensitive towards chromium than liver epithelial cells and this might explain the known nephrotoxicity in vivo. Chronic renal failure seems to be responsible for marked elevation of serum chromium (Brodner, et al., 1998)
Renal, tubular necrosis after ingestion of chromate or dichromate salts has been demonstrated in animals and in humans following acute intoxication (Lonnard and Norseth, 1986). B-Glucuronidase and renal cell antigen may be increased in workers with chronic exposure (Mutti, 1989). Tubular proteinuria was reported after acute exposure to chromium (Franchini and Mutti, 1988). Chronic nephrotoxicity of solvents has been investigated in a group of workers in the foot wear industry (Caudarella, et al 1981)
Gentamicin is an aminoglycoside antibiotic derived from micromonaspora purpurea with bactericidal effect for many gram-negative pathogens (Black, et al., 1983). Gentamicin is widely used in veterinary and human medicine Aminoglycosides. Aminoglycoside nephrotoxicity usually develops over 7-10 days, polyuria and renal concentration defect may proceed a fall in glomerular filtration rate (Bennett, 1986). Amino-

MATERIAL AND METHODS

Sampling: Total of twenty samples was collected. Ten samples represent certain dyes used in tanning hides in factories of Sor Magra El Aieon in Egypt. Ten samples from some industrial factory discharge in Kalubia governorate.

Analysis of samples: Dyes and water samples were filtered and 0.1 ml of nitric acid was add to each 100 ml and kept in refrigerator till analysis. All the samples were analyzed for determination of chromium using flame type air-acetylene atomic absorption spectrophotometer Tahan et al (1994). Gentamicin sulfate levels was determined in serum and urine according to (Kirshbaum and Arret, 1959). Gentamicin was extracted from kidney homogenate according to (Haddad et al, 1987) then determined as mentioned before. Serum urea levels were determined according to (Tabacco, 1979) and serum creatinine according to (Husdan and Rapoport, 1968). Kidney sections submitted for histopathological examination according to (Dray and Walling, 1973).

The data obtained in this study were calculated as mean + standard error, and they were statistically analyzed by the student’s (t) test. All statistical analysis were carried out according to (Johnston, 1972).
RESULTS
The results in Table (1&2) indicated that the concentration of chromium in some dyes used in tanning of hides ranged from 0.747 to 8.487 ppm with a mean value of 1.8 PPM. Chromium level in water out put of some industrial locality ranged from 0.774 to 2.750 PPM with mean value of 1.127 PPM. Such data exceed the permissible limits (0.05 PPM).

Table (1): Concentrations of chromium (PPM) in some tanning hides dyes and some water out put of some industrial locality in comparison to permissible limit.

<table>
<thead>
<tr>
<th>Hides dye samples</th>
<th>Cr (PPM)</th>
<th>Water discharge of some locality</th>
<th>Cr (PPM)</th>
<th>Permissible limits (p,l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.747</td>
<td>Near out put of Kaha factory</td>
<td>0.774</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>8.486</td>
<td>Out put of Kaha factory</td>
<td>0.841</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>0.84</td>
<td>Near out put of Vetcac factory</td>
<td>0.88</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>1.13</td>
<td>Near out put of Vetcac factory</td>
<td>0.938</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>Out put of Vetcac factory</td>
<td>0.949</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>1.09</td>
<td>Out put of carpet factory in Moshtohor</td>
<td>1.056</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>1.15</td>
<td>Moshtohor out put(EL-Namol canal)</td>
<td>1.0867</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>0.78</td>
<td>Moshtohor canal</td>
<td>0.783</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>1.3</td>
<td>Out put of tanning hid factory in area of Sore Magra El Aion</td>
<td>1.210</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>1.28</td>
<td>Out put of another tanning hid factory in area of Sore Magra El Aion</td>
<td>2.752</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Over permissible limits
- Less than the permissible limits

Table (2): Concentration of chromium (PPM) in some dyes and water out put of some industrial locality.

<table>
<thead>
<tr>
<th>Value</th>
<th>Hides dye</th>
<th>Water out put in some industrial locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>0.747</td>
<td>0.774</td>
</tr>
<tr>
<td>Max.</td>
<td>8.487</td>
<td>2.750</td>
</tr>
<tr>
<td>Median</td>
<td>1.140</td>
<td>0.943</td>
</tr>
<tr>
<td>Mean.</td>
<td>1.8</td>
<td>1.127</td>
</tr>
<tr>
<td>SE</td>
<td>0.746</td>
<td>0.186</td>
</tr>
</tbody>
</table>
Serum chromium levels in group taken pot. Dichromate or group taken gentamicin sulfate and pot. Dichromate is presented in table (3). Increase in serum chromium levels was detected in group taken pot. Dichromate and gentamicin sulfate. Serum gentamicin level in group taken both gentamicin and Pot. Dichromate showed high significant increase in comparison to group taken gentamicin sulfate only. Serum gentamicin sulfate was 15.36± 1.3 and 8.53 ± 1.1 respectively as clear in table (3). There is no significant difference in serum gentamicin sulfate in group administered gentamicin sulfate and pot. Dichromate and that administered gentamicine sulfate only. Gentamicin sulfate levels were 22.41± 2.17 and 20.29± 1.33 in third and second group respectively. Effect of pot. Dichromate or gentamicin sulfate or both on serum urea and creatinine levels were detected in table(3). Increase in serum urea levels was showed in group taken pot. Dichromate and group injected by gentamicin sulfate as compared to control group. Urea levels were 40.56± 1.8, 38.56 ± 1.22 and 25.8 ± 2.14 mg/dl respectively. Third group that taken pot. Dichromate and gentamicin sulfate showed high significant increase in serum urea level as compared to first and second group (taken pot. Dichromate and gentamicin sulfate only respectively). Serum creatinine levels showed high significant increase in first and second group in comparison to control group. Serum creatinine levels were 3.96 ± 0.5, 4.92 ± 0.54 (mg/dl) for first and second group respectively in comparison to 1.72 ± 0.21(mg/dl)of control group. Administration of Pot. Dichromate and gentamicin sulfate caused increase in creatinine levels as compared to first and second group. Serum creatinine levels were 6.16± 0.69 mg/dl in third group in comparison to 3.96± 0.5 mg/dl and 4.92± 0.54 mg/dl in first and second group respectively.

**Table (3):** Serum chromium levels (PPM), gentamicin sulfate, urea and creatinine of Newzeland rabbit administered (1.8ppm) pot. Dichromate or gentamicin sulfate (IM 5ml/kg b.w.) or pot. Dichromate and gentamicin sulfate in comparison to control group at the end of 21 days of experimental. (Mean ± S.E).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Cr (ppm)</th>
<th>Gentamicin sulfate(ug/ml)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Daily administration of 1.8 ppm pot. Dichromate in drinking water.</td>
<td>0.282± 0.02</td>
<td>——</td>
<td>40.56± 1.8</td>
<td>3.96± 0.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>Administration of (5ml/kg b.w.) gentamicin sulfate for 7 days</td>
<td>——</td>
<td>8.53 ± 1.1</td>
<td>38.56± 2.22</td>
<td>4.92± 0.54</td>
</tr>
<tr>
<td>Group 3</td>
<td>Daily administration of 1.8 ppm pot. Dichromate in drinking water with IM injection of (5ml/kg) gentamicin sulfate for 7 days.</td>
<td>0.68**± 0.10</td>
<td>15.36**± 1.3</td>
<td>57.4**± 3.17</td>
<td>6.16± 0.69</td>
</tr>
<tr>
<td>Group 4</td>
<td>Control group</td>
<td>——</td>
<td>——</td>
<td>25.8± 2.14</td>
<td>1.72± 0.21</td>
</tr>
</tbody>
</table>

*Significant at p ≤ 0.05

** High significant p≤ 0.01
• Chromium levels in kidney tissues were showed in table (5). The data indicated an increase in chromium level of kidney in third group (pot. dichromate and gentamicine sulfate) in comparison to first group (pot. Dichromate). Chromium in kidney tissue was 0.346 ± 0.06 and 0.618 ± 0.08 PPM in the first and third group respectively.

Gentamicin sulfate levels in kidney tissues were cleared in table (4).

**Table (4): Residue of chromium levels (PPM) and gentamicine sulfate in kidney of Newzeland rabbit administered (1.8 PPM) pot. Dichromate or IM injection of (5 ml/kg) gentamicin sulfate or pot. Dichromate and gentamicin sulfate. (Mean ± S.E).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Cr (ppm) after 21 days of treatment</th>
<th>Gentamicin sulfate (μg/ml) after 21 days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Daily administration of 1.8 PPM pot. Dichromate in drinking water.</td>
<td>0.346 ± 0.06</td>
<td>---</td>
</tr>
<tr>
<td>Group 2</td>
<td>Administration of (5ml/kg) gentamicin sulfate for 7 days.</td>
<td>---</td>
<td>20.29 ± 1.33</td>
</tr>
<tr>
<td>Group 3</td>
<td>Daily administration of 1.8 PPM pot. Dichromate in drinking water with IM injection of (5ml/kg) gentamicin sulfate for 7 days.</td>
<td>0.618 ± 0.08</td>
<td>22.41 ± 2.17</td>
</tr>
<tr>
<td>Group 4</td>
<td>Control group</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

*Significant at p ≤ 0.05  ** High significant p ≤ 0.01

Histopathological study revealed that kidney of group taken pot. Dichromate showed renal tubular necrosis cellular and infiltration Fig (1,2&3). Kidney tissue of second group showed necrosis and fibrosis in glomerular basement membrane Fig (4,5&6). Kidneys of third group (taken pot. Dichromate and gentamicin sulfate) showed marked necrosis in proximal tubule and thickened in glomerular basement membrane Fig (7,8&9). Normal kidney of control group showed in Fig.(10&11).
Fig. (1&2): Kidney of rabbit (taken 1.8 PPM pot. Dichromate) showing renal tubular necrosis. (H&E)(X40)

Fig. (3): Kidney of rabbit (taken 1.8PPM pot. Dichromate) showing cellular infiltration sulfate showed necrosis. (H&E) (X 40)

Fig. (4): Kidney of rabbit injected IM 5ml/kg, b.w gentamicine (H&E)(X 40).
Fig. (5): Kidney of rabbit injected IM 5ml/kg b.w gentamicine sulfate showed fibrosis in glomerular pasment membrane. (H&E) (X40)

Fig. (6): Kidney of rabbit injected IM (5ml/kg b.w) gentamicine sulfate showed marked fibrosis. (H&E) (X40)

Fig. (7): Kidney of rabbit (pot. Dichromate & gentamicine sulfate) showed necrosis in proximal tubule. (H&E) (X40)
Fig. (8&9): Kidney of rabbit (pot. Dichromate & gentamicine sulfate) showed thickened in glomerular basement membrane. (H&E) (X 40)

Fig. (10&11): Normal kidney of the control group. H&E (X 40)
DISCUSSION
Chromium is a widely used industrial chemical extensively used in paints, metal finishes, steel including stainless steel manufacturing, alloy cast iron and wood treatment. On the contrary, chromium (III) salts such as chromium polynicotinate, chromium chloride and chromium picolinate are used as micronutrients and nutritional supplements (Bagchi, et al 2001).
Industrial and agricultural discharges are considered the primary source of metal poisoning in Egypt (EL-Nabawi et al, 1987). The present study indicated that the chromium levels in certain dyes (in leather industry) were reached 0.747 PPM as a minimum level and 8.487 PPM as maximum value with mean value (1.8 PPM) which used as an experimental dose in this study. Chromium in the water discharge of certain factories is higher than the permissible limit as detected by WHO (1984). The present study indicated that leather dye samples contain higher level of chromium. Where industrial uses of chromium centered on the production of dyes and tanning of hides (Terry, 1995).
The strong epidemiological occurrence of chromium helps us to study the interaction of chromium and one of common used aminoglycoside (gentamicin sulfate) on the kidney and serum levels of these compounds. Serum chromium level was increased in group taken 1.8 PPM pot. Dichromate in drinking water and 5 ml IM injection of gentamicin sulfate than that group taken pot. Dichromate only. This results may be due to nephrotoxic effect of both pot. Dichromate (VI) and gentamicin sulfate (Lonnard and Norseth, 1986, Mutti, 1989, Franchini and Mutti,1988, Bennett,1986, Michael and Richard,1989, Whiting and Simpson,1983). Exposure to chromium induce an alteration of structure and function of the kidney plasma membrane (Dey,2001) Renal failure seems to be responsible for marked elevation of serum chromium (Brodner, et al 1998). Serum gentamicin levels increased in group taken pot. Dichromate and gentamicin sulfate in comparison to group taken gentamicin sulfate only. This result may be due to nephrotoxic effects of chromium and gentamicin sulfate (Langard and Norseth, 1986, Mutti, 1989, Franchini and Mutti, 1988, Bennett, 1986, Michael and Richard, 1989, Whiting and Simpson, 1983). Such nephrotoxic effects lead to greater marked impairment of renal function as a result of the combination of pot. Dichromate and gentamicin sulfate compared with either test material given alone as first and second group. This explanation is supported by the histopathological finding of kidney in third group (greater marked lesions than other group) as in Fig. (7,8&9).
Chromium and gentamicin sulfate residues levels in kidney tissues were detected. Chromium showed increase level in group taken pot. Dichromate and gentamicin sulfate. This results were attributed to that kidney is the main target organ for chromium accumulation (Dartsch et al 1998). Same author added that uptake of Cr. (VI) through the general anion transport system of the cell membrane might be the only facet of cellular uptake and toxification. Concerns residues of gentamicin sulfate (ug/gm) in kidney of rabbits showed no difference between group administered gentamicin sulfate or group administered pot. Dichromate and gentamicin sulfate.

Increase in serum urea and creatinine were detected either in group taken pot. Dichromate or group taken gentamicin sulfate in comparison to control group. Third group taken pot. Dichromate & gentamicin sulfate showed marked increase in serum urea and creatinine level. This result agreed with Beech et al (1977) and Brodner et al, (1998). This result may be attributed to chromium and aminoglycoside nephrotoxicity (Langard and Norseth, 1986, Mutti, 1989, Franchini and Mutti, 1988, Bennett, 1986, Michael and Richard, 1989, Whiting and Simpson, 1983). Chromium caused cytotoxicity by induces an oxidatives stress through enhanced production of reactive oxygen species leading to genomic DNA damage and oxidative deterioration of lipids and proteins. A cascade of cellular events occur including enhanced production of superoxide anion and hydroxyl radicals, increased lipid peroxidation DNA fragmentation, activation of protein kinase c, apoptotic cell death (Bagchi et al 2001). Histopathological finding supports this explanation Fig (7,8 & 9).

Results concerning histopathological lesion of kidney rabbits treated with pot. Dichromate was represented by cellular infiltration and renal tubular necrosis. These findings were in agreement with (Langard and Norseth, 1986 and Dartsch et al, 1998). Renal lesions in rabbits injected by gentamicin sulfate were represented by fibrosis in glomerular basement membrane and necrosis. These finding are agreed with Bennett, (1986) and Ngeleka et al (1990). These result may be explained as chromium (VI) induce an oxidative stress resulting in tissue damaging effects that may contribute to the toxicity of this cations (Bagchi et al,1997). Chromium induce an alteration on structure of the kidney (Dey, 2001) Kidney in rabbits administered pot. dichromate and gentamicin sulfate showed greater marked lesion than first and second group. These results may be due to the augmentation of nephrotoxicity as a result of combination of pot. Dichromate and gentamicin sulfate.

Conclusion: Population that might be at higher risk to a toxic metal that may cause nephrotoxicity such as people or other life stock exposed in the work place to chromium (industrial area of tanning hides or foot wear industry) should be closely monitored for renal effect. Certain drugs (aminoglycoside) augmented the nephrotoxic effect of the toxic metal (chromium).
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المختصر العربي

دراسة التداخل السمعي للكروم وسلفات الجناحيين وآثاره على كفاءة الكلى.

نبيلة محمود عبد العليم - قسم الطب الشرعي والسموم

كلية الطب البيطري - جامعة الزقازيق - نفرع بها

هدف البحث إلى دراسة التأثير المسمار لكل من الكروم وسلفات الجناحيين على كفاءة الكلى. مفهوم نماسية الكروم في بعض الشخصيات المبتخلطة في دبابة وضعف الجالود وكتلخت في مياه صرف بعض هذه المستخدمين. كما تم قياس نسبة الكروم في صرف بعض المصانع في محافظاطه القاهرة وفي مصرف مشتر (محافظة القليوبية). وقد أوضحت الدراسة تواجد الكروم نسبًا عالية جداً خاصة في بعض الشخصيات المستخدمة في مياه الجالود حيث أن بعض هذه الشخصيات تم استخدام تحت نماسية (عند ارتفاع الماء في الكروم). أظهرت صحة مياه الكروم نسبة عالية جداً من الكروم وصلت إلى 8.69 جزء في المليون. كما تم دراسة التأثير السمعي للكروم سداسي التكافؤ (داي كرومبيضوم) مفردًا وعند تعاطية مع مضادات الحيوية سلفات الجناحيين على الكلى.

تتستخدم علاج أربعة مجموعات كل مجموعة 4 أرانب. ووضع داي كرومبيضوم بنسبة 0.8 جزء في المليون في مياه الصرف لمجموعة الأولى لمدة 21 يوم وقعت المجموعة الثانية سلقات الجناحيين مع داي كرومبيضوم لمدة 7 أيام. أما المجموعة الثالثة فقد تناولت 0.8 جزء في المليون من داي كرومبيضوم في مياه الصرف لمدة 21 يوم وقعت سلقات الجناحيين بجرعة 5 جم لكل كيلوغرام من وزن الجسم لمدة 7 أيام. استخدمت المجموعة الرابعة كمجموعة ضابطة. تم فحص عينات بعد 21 يومًا في كل المجاميع.

أوضحت النتائج الدراسة زيادة نسبة الكروم في مصل حيوانات المجموعة الثالثة عن المجموعة الأولى. كما أظهرت نسبة سلالات الجناحيين في مصل حيوانات المجموعة الثالثة عن المجموعة الثانية.

أوضح الدراسة زيادة تركز الكروم في نسيج الكلى في مصل حيوانات المجموعة الثالثة (داي كرومبيضوم مع سلقات الجناحيين). بينما لم يظهر سلقات الجناحيين في نسيج الكلى أي تغيرات معنوية. سجلت الدراسة زيادة في كمية البيولينا والكروماتين في مصل حيوانات المجموعات الأولى والثانية والثالثة عند مقارنتها بالمجموعة الضابطة. وأوضح القياس البيولوجي لعينات الكلى تأثير المجموعة الثالثة (داي كرومبيضوم) وسلقات الجناحيين (أكثر من المجموعة الأولى والثانية). حيث حدثت موت تدهور في خلايا أنيبتيات الكلى. ونتيجة النتائج السابقة أن الكروم سداسي التكافؤ له تأثير سمي على الكلى ويزيده هذا التأثير عند تعاطي البيض الحيوي سلقات الجناحيين. حيث أن الماداتين لمها تؤثر ضار على الكلى.