SUBLETHAL EFFECTS OF FOOD FLAVOUR FURFURAL ON HAEMATOLOGICAL INDICES AND VARIOUS SERUM BIOMARKERS IN MALE ALBINO RATS

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ABSTRACT

This study aims to assess the effect of furfural, an aldehyde commonly used as food flavor in many foods, on the hematological and biochemical aspects in male albino rat. The experimental animals used in this study were divided into four groups. The control group was given a daily volume of the vehicle dimethyl sulfoxide (DMSO) whereas the other three groups were orally administered furfural for 8 weeks in sublethal doses of 3.18, 6.36 and 12.72 mg/kg b.w. respectively. The obtained results revealed a decrease in red blood cells (RBCs) count, packed cell volume (PCV) and hemoglobin content and an increase in mean cell volume (MCV) and mean cell haemoglobin content (MCH) in rats received furfural in a dose dependent manner. The white blood cells (WBC) count was decreased especially eosinophil percentage which was depleted in the low and medium doses groups. Furfural elevated serum liver enzymes activities and total bilirubin concentration. The serum total protein and albumin levels were increased in medium and high doses groups, while globulin level, was elevated only in low dose group. Furfural elevated serum urea level but did not affect serum AFP and CA 19.9 levels in all groups. In conclusion, furfural administration to rats is highly able to induce a sort of macrocytic anemia, leucopenia, esinopenia as well as marked disturbances in liver and renal functions, but there is no evidence of carcinogenic effect detected by tumor markers.

KEY WORDS: Furfural; Haematological Indices; Liver; Kidney; Tumour Markers.

1. INTRODUCTION

Furfural (furan-2-carboxy-aldehyde, OC₄H₃CHO) was first extracted in 1832-1840 through the bran distillation (Latin: furfur) by diluted sulfuric acid (Adams et al., 1997). Furfural is a colorless liquid with pleasant aroma and it is partially soluble in water. It has a
significant use as a selective solvent of mineral oil products, in chemistry, in the rubber industry, plastic surgery and polymer industry (Begic-Janeva, 1991). It is also present in orange juice, brandy and Japanese sake and it is also derived from a variety of agricultural by products (Blitzer and Boyer, 1982; International Programme on Chemical Safety, IPCS, 1999). In the organism, furfural occurs by pentose dehydration (Petkovic, 1990). In Europe, furfural is used as a flavor in foods such as baked goods, frozen dairies, meat products, candy, puddings, beverages, and gravies (Burdock, 2010). Furfural is a carcinogen classified in EU as a Category 3 carcinogen. In mice, it increased the incidence of hepatocellular adenomas and carcinomas in males and of hepatocellular adenomas and forestomach papillomas in females as indicated by National Toxicology Program (NTP, 1990). After oral administration, furfural is rapidly absorbed from the gastrointestinal tract into blood and distributed to the tissues, principally the liver and kidney. Furfural is eliminated slowly and in an unchanged form through the kidneys and lungs. The liver oxidizes it into pyromucic acid (C₄H₃OCOOH) which is toxic to hepatocytes, but it is conjugated with glycine and mostly excreted in urine (Maruyama et al., 1992; Cekić et al., 2003). Furfural is unsaturated cyclic aldehyde, it is a known hepatotoxic substance (Mishra, 1992; Malikand and Pandey, 2013), and it changes the activity of some enzymes in the liver in acute (Jonek et al., 1975) and chronic (Kaminska, and Gruszecka, 1977) experiments. Furfural may cause morphologic changes in the liver (Kiso et al., 1994) that in the acute experiment are manifested in diffuse necrosis associated with regeneration of hepatocytes and terminate within a few days with liver recovery. It is presumed that liver damage is induced by oxidation of furfural. In the chronic experiment, furfural induces cirrhotic changes (Kiso et al., 1994) associated with pseudolobule formation, enlargement of the portal area, and destruction of the border plaque. In the liver parenchyma, a marked bridging necrosis and hydropic degeneration of hepatocytes develop. Simultaneously, various degrees of liver insufficiency may become evident (Koura et al., 1999; Ballmer et al., 1993). Liver transaminases, AST and ALT are important markers of liver damage and are routinely done in modern laboratories. Also, γ-GT and alkaline phosphatase (AP) are increased in cases where there is damage to liver parenchyma under the influence of many harmful substances which are detoxified in the liver every day (Divald et al., 1990; Malikand and Pandey, 2013). Renal cortical adenomas or carcinomas in furfural exposed male and female mice and a renal in NTP (1990).
The aim of this study is to clarify the hazard effects of oral furfural use in different sublethal doses on some haematological and biochemical parameters including liver and kidney functions tests in male albino rats.

2. MATERIALS AND METHODS

2.1. Experimental animals
Male albino rats (Rattus norvegicus) weighing 140-160 g were obtained from the animal house of Institute of Ophthalmology, Giza, Egypt. They were housed in plastic cages with good aerated covers at normal temperature (25±5 °C) and daily 12-hours light-dark cycle. The animals were fed standard diet pellets and tap water ad libitum. They were kept under observation for 2 weeks to exclude any inter current infection and for proper acclimatization. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals of Canadian Council on Animal Care (CCAC, 1993).

2.2. Furfural
2-Furancaboxaldehyde, known commercially as furfural, was obtained from Aldrich Company, Gillingham, England. The different doses of furfural-dissolved in dimethyl sulfoxide (DMSO)-were given daily to rats by gastric intubation between 8-10 AM. Three doses were chosen on the basis of the oral LD50 value which was previously determined by Jenner et al. (1964) and equals 127 mg / kg. b. w.

2.3. Experimental design
The experimental animals used in this study were divided into four groups, six animals for each. One group was kept as control and was given a daily respective volume of the vehicle (DMSO) whereas the other three groups were orally administered 1/40, 1/20 and 1/10 of LD50 that approximately correspond to 3.18, 6.36 and 12.72 mg/kg b.w. respectively.

All groups were treated for 8 weeks. At the end of this period, rats were sacrificed and blood samples were obtained from jugular vein in two tubes, one containing ethylenediaminetetraacetic acid solution 15% (50 μl EDTA/5 ml blood) for determination of blood indices and the other without EDTA. After blood coagulation in the 2nd tube, blood was centrifuged at 3000 rpm for 15 minutes. Serum was obtained from each tube and kept at -30 °C till used for biochemical determination.
2.4. Haematological Investigations

Counting of the red blood cells (RBCs) and white blood cells (WBCs) was carried out by Neubauer slide, using saline (0.9 % NaCl) (Wintrobe et al., 1981) and Turk's fluid (Miale, 1972) respectively. The hematocrit value was determined according to Dacie and Lewis (Dacie and Lewis, 1991) and the hemoglobin content was measured as described by Lucky (1977). Also, mean cell volume (MCV), mean cell haemoglobin content, and mean cell haemoglobin concentration percent (MCHC) were calculated according to Dacie and Lewis (1991). Blood film was prepared, fixed in methanol and then stained with Giemsa stain according to the methods of Houwen (2000).

2.5. Biochemical assays

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined according to the method of Bergmeyer et al. (1978), using reagent kits purchased from Spinreact Company (Spain). Serum gamma-glutamyltransferase (γ-GT) activity was determined according to Beleta and Gella (1990). Serum alkaline phosphatase (ALP) activity was assayed according to the method of Belfield and Goldberg (1971) using reagent kit obtained from BioMerieux Chemical Company (France). Serum lactate dehydrogenase activity was estimated by kits obtained from Stanbio Laboratories (USA) according to the method of Bühl and Jackson (1978). Total bilirubin concentration was determined according to the method of Jendrassik and Grap (1938) using reagent kits purchased from Diamond Diagnostics Chemical Company, Egypt.

Serum total protein and serum albumin levels were estimated according to Henry (1964) and Doumas et al. (1971) using reagent kits purchased from Diamond Diagnostics Chemical Company, Egypt. Serum globulin level was calculated by subtracting albumin level form total protein concentration. In addition, the ratio of albumin to globulin was calculated. Serum α-fetoprotein (AFP) concentration was determined by a radioimmunoassay kit [Double antibody kit, Diagnostic Products Corporation (DPC), USA] according to the methods of Waldmann and McIntire (1974) and Wepsic (1981). Serum carbohydrate antigen (CA)-19.9 was estimated by an immunoradiometric assay kit (Coat-A-Count GI-MA IRMA, DPC, USA) according to the method of Ferbourg et al. (1988). Both AFP and CA-19.9 concentrations were measured in the Radioactive Isotope Unit, National Research Center, Dokki, Cairo, Egypt.
Serum urea and creatinine concentrations were measured based respectively on the methods of Patton and Crouch (1977) and Henry (1974) using reagent kits produced by Diamond Diagnostics (Egypt). Serum uric acid level was determined according to the method of Barham and Trinder (1972) using reagent kits obtained from Spinreact Company (Spain).

2.6. Statistical Analysis
The data were analyzed using the one way analysis of variance (ANOVA) (Rao et al., 1985) followed by LSD analysis to compare various groups with each other. Results were expressed as mean ± standard error (SE). Values of $P>0.05$ were considered statistically non-significantly different, while values of $P<0.05$ and $P<0.01$ were significantly and highly significantly different respectively. F-probability expresses the general effect between groups.

3. RESULTS
3.1. Haematological effects
The administration of furfural to albino rats for 8 weeks produced significant decrease of red blood cell count, haematocrit (PCV) and hemoglobin content at the medium and high doses (6.36 and 12.72 mg/kg b.w.) in a dose dependent manner. However, these parameters were not significantly affected at low dose (3.18 mg/kg b.w.). In contrast, Mean cell volume (MCV) of red blood cells as well as mean cell haemoglobin (MCH) were highly significantly increased as a result of administration of medium and high concentration of furfural in a dose dependent manner. Mean cell haemoglobin concentration (MCHC) was not significantly (P>0.05; LSD) affected as a result of all tested doses of furfural. ANOVA results revealed that the effect between groups on RBC count, MCH and MCV was very highly significant (P<0.001; F-prob.) while the effect on hematocrit, Hb and MCHC was only significant (P<0.05; F-prob.) (Table 1).

The white blood cells (WBC) or leucocytes count was significantly decreased due to furfural administration for 8 weeks; the percentage decreases were 26.804, 41.842 and 40.689 % respectively as a result of low, medium and high doses of furfural. Both lymphocytes and monocytes percentages were detectably decreased in furfural-administered animals although these changes are not significant. Eosinophils percentage was highly significantly depleted as a result of low and medium doses administration. Basophils percentage was also not significantly affect. ANOVA results revealed that while the effect between groups on WBC
count and neutrophil percent was highly significant (P<0.01; F-prob.), the effect on eosinophil percent was very highly significant (P<0.001; F-prob.) (Table 2).

3.2. **Biochemical Effects**

Serum ALT, AST, γGT, ALP and LDH activities as well as total bilirubin concentration were remarkably increased as a result of furfural administration. The LDH activity and total bilirubin concentration were increased in a dose dependent manner. The low dose produced the most potent effect on AST activity, but the high dose seemed to be the most effective on ALT, γGT, ALP and LDH activities and total bilirubin concentration in serum. One-way ANOVA indicated that the general effect was significant (P<0.05; F-prob.) on LDH activity, highly significant (P<0.01) on γGT activity and total bilirubin concentration and very highly significant (P<0.001) on ALT, AST and ALP activities (Table 3).

The serum total protein and albumin levels were significantly (P<0.05; LSD) and highly significantly (P<0.01; LSD) increased as a result of medium and high doses of furfural, respectively (Table 4). Serum globulin level, on the other hand, was significantly elevated due only to medium dose while it was remarkably decreased as a result of low and high doses. A/G ratio was significantly (P<0.01; LSD) increased as a result of low and high dose of furfural (Table 4). Serum AFP and CA 19.9 levels was non-significantly affected as a result of all tested doses of furfural (Table 4) as indicated by one-way ANOVA and LSD test (P>0.05).

Serum creatinine and uric acid levels were not significantly changed in furfural administered rats. Serum urea level, on the other hand, was highly significantly increased as a result of all tested doses of furfural; the percentage increases were 32.665, 56.903 and 42.820 respectively due to low, medium and high doses respectively (Table 5). One-way ANOVA revealed that the effect between groups on urea level and A/G ratio was highly significant and significant respectively.
<table>
<thead>
<tr>
<th>Group</th>
<th>Total RBCs count x 10^6/µl</th>
<th>%</th>
<th>PCV %</th>
<th>%</th>
<th>Hb (g/dl) %</th>
<th>%</th>
<th>MCH Pg %</th>
<th>%</th>
<th>MCV (Fl) %</th>
<th>%</th>
<th>MCHC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.732 ± 0.122 a</td>
<td>46.481 ± 0.229 a</td>
<td>13.549 ± 0.292 a</td>
<td>23.549 ± 0.845 c</td>
<td>81.430 ± 1.842 c</td>
<td>29.287 ± 1.031 c</td>
<td></td>
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<tr>
<td>Low dose</td>
<td>6.036 ± 0.497 a + 5.303</td>
<td>45.130 ± 0.594 ab</td>
<td>- 2.906</td>
<td>12.169 ± 0.384 b</td>
<td>- 2.804</td>
<td>20.902 ± 1.898 c</td>
<td>- 11.847</td>
<td>82.311 ± 7.252 bc</td>
<td>+ 1.082</td>
<td>27.224 ± 1.074 a</td>
<td></td>
</tr>
<tr>
<td>Moderate dose</td>
<td>4.641 ± 0.0282 b - 21.528</td>
<td>43.604 ± 1.806 bc</td>
<td>- 6.189</td>
<td>12.587 ± 0.289 ab</td>
<td>- 7.100</td>
<td>28.310 ± 1.149 b</td>
<td>+ 19.396</td>
<td>98.635 ± 6.727 b</td>
<td>+ 21.128</td>
<td>28.958 ± 1.368 a</td>
<td></td>
</tr>
<tr>
<td>high dose</td>
<td>2.702 ± 0.141 c - 52.861</td>
<td>41.574 ± 0.123 c</td>
<td>- 10.557</td>
<td>11.692 ± 0.694 b</td>
<td>- 13.705</td>
<td>43.837 ± 1.695 a</td>
<td>+ 84.880</td>
<td>156.611 ± 4.887 a</td>
<td>+ 92.320</td>
<td>28.176 ± 1.435 a</td>
<td></td>
</tr>
</tbody>
</table>

F-probability     | P < 0.01 | P < 0.05 | P < 0.05 | P < 0.001 | P < 0.001 | P > 0.05 |
LSD at 5%         | 0.887    | 2.831    | 1.318    | 4.303      | 16.500    | -        |
LSD at 1%         | 1.210    | 3.861    | 1.798    | 5.869      | 22.503    | -        |

- Data are expressed as Mean ± SE. Number of animals in each group is six.
- Means, which share the same superscript symbol(s), are not significantly different.
Table 2: The differential WBCs count (% of total) of control male albino rats and those daily administered a low, moderate and high dose of furfural/kg, after 8 weeks of administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total WBCs count x 10^3/µl</th>
<th>%</th>
<th>Lymphocytes</th>
<th>%</th>
<th>Neutrophil (segmented)</th>
<th>%</th>
<th>Monocytes</th>
<th>%</th>
<th>Eosinophil</th>
<th>%</th>
<th>Basophil</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.980 ± 1.009 a</td>
<td></td>
<td>75.5 ± 2.473 a</td>
<td></td>
<td>17.333 ± 1.202 b</td>
<td></td>
<td>1.666 ± 0.333 a</td>
<td></td>
<td>7.000 ± 0.683 a</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Low dose</td>
<td>5.841 ± 0.578 b</td>
<td>-24.804</td>
<td>70.00 ± 5.360 a</td>
<td>-7.285</td>
<td>26.000 ± 1.341 a</td>
<td>+50.003</td>
<td>0.833 ± 0.477 a</td>
<td>-50.00</td>
<td>3.000 ± 0.730 b</td>
<td>-57.143</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Moderate dose</td>
<td>4.641 ± 0.393 b</td>
<td>-41.842</td>
<td>74.833 ± 2.856 a</td>
<td>-0.883</td>
<td>21.166 ± 1.351 b</td>
<td>+22.113</td>
<td>1.000 ± 0.258 a</td>
<td>-39.975</td>
<td>3.666 ± 0.333 a</td>
<td>-47.628</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>High dose</td>
<td>4.733 ± 0.553 b</td>
<td>-40.689</td>
<td>69.333 ± 4.402 a</td>
<td>-8.168</td>
<td>22.000 ± 5.190 ab</td>
<td>+26.925</td>
<td>0.500 ± 0.498 a</td>
<td>-69.988</td>
<td>8.000 ± 0.806 a</td>
<td>+14.286</td>
<td>0</td>
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<tr>
<td>F-probability</td>
<td></td>
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</tr>
<tr>
<td>LSD at 5%</td>
<td>1.986</td>
<td></td>
<td>4.634</td>
<td></td>
<td>1.957</td>
<td></td>
<td>2.669</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LSD at 1%</td>
<td>2.708</td>
<td></td>
<td>6.321</td>
<td></td>
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</tr>
</tbody>
</table>

- Data are expressed as Mean ± SE. Number of animals in each group is six.
- Means, which share the same superscript symbol(s), are not significantly different.
- Stuff cells are zero in all groups.
Table 3: The liver function enzymes and total bilirubin in serum of control male albino rats and those daily administered a low, moderate and high dose of furfural/kg, after 8 weeks of administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>ALT (U/L)</th>
<th>%</th>
<th>AST (U/L)</th>
<th>%</th>
<th>γ GT (U/L)</th>
<th>%</th>
<th>ALP (U/L)</th>
<th>%</th>
<th>LDH (U/dl)</th>
<th>%</th>
<th>Total bilirubin (mg/dl)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>41.300± 3.309 b</td>
<td></td>
<td>154.00 ± 3.443 c</td>
<td></td>
<td>15.333 ± 0.882 c</td>
<td></td>
<td>203.100 ± 1.050 b</td>
<td></td>
<td>90.383 ± 8.490 b</td>
<td></td>
<td>0.566 ± 0.042 ba</td>
<td></td>
</tr>
<tr>
<td>Low dose</td>
<td></td>
<td>64.681 ± 4.246 a</td>
<td>+ 5.303</td>
<td>248.600 ± 20.907 a</td>
<td>+56.612</td>
<td>19.600 ± 0.219 b</td>
<td>+27.829</td>
<td>332.000 ± 7.399 a</td>
<td>+63.466</td>
<td>93.906 ± 6.389 b</td>
<td>+3.898</td>
<td>0.675 ± 0.054 b</td>
<td>+19.257</td>
</tr>
<tr>
<td>Moderate dose</td>
<td></td>
<td>63.375 ± 2.897 a</td>
<td>+53.450</td>
<td>207.400 ± 7.779 b</td>
<td>+34.675</td>
<td>19.012 ± 1.258 bc</td>
<td>+23.994</td>
<td>329.066 ± 11.078 a</td>
<td>+62.071</td>
<td>100.150 ± 0.980 b</td>
<td>+10.806</td>
<td>0.883 ± 0.079 a</td>
<td>+56.007</td>
</tr>
<tr>
<td>high dose</td>
<td></td>
<td>66.425 ± 5.571 a</td>
<td>+60.835</td>
<td>201.400 ± 3.443 b</td>
<td>+30.773</td>
<td>24.050 ± 2.393 a</td>
<td>+56.851</td>
<td>388.366 ± 37.933 a</td>
<td>+88.757</td>
<td>117.566 ± 3.692 a</td>
<td>+30.075</td>
<td>0.921 ± 0.069 a</td>
<td>+62.721</td>
</tr>
</tbody>
</table>

F-probability: P < 0.001
LSD at 5%: 12.200 33.679 4.206 59.323 16.655 0.185
LSD at 1%: 16.639 45.933 5.737 80.908 22.715 0.252

- Data are expressed as Mean ± SE. Number of animals in each group is six.
- Means, which share the same superscript symbol(s), are not significantly different.
Table 4: The total protein, albumin, globulin, A/G ratio, fetoprotein and CA 19.9 in serum of control male albino rats and those daily administered a low, moderate and high dose of furfural/kg, after 8 weeks of administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Total protein (g/L)</th>
<th>%</th>
<th>Albumin (g/L)</th>
<th>%</th>
<th>Globulin (g/L)</th>
<th>%</th>
<th>A/G ratio</th>
<th>%</th>
<th>α-fetoprotein (U/ml)</th>
<th>%</th>
<th>CA 19.9 (U/ml)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (control)</td>
<td></td>
<td>84.579 ± 3.912 c</td>
<td></td>
<td>46.375 ± 4.754 c</td>
<td></td>
<td>40.113 ± 3.355 ab</td>
<td></td>
<td>1.225 ± 0.186 c</td>
<td></td>
<td>1.096 ± 0.075 a</td>
<td></td>
<td>3.750 ± 0.201 a</td>
<td></td>
</tr>
<tr>
<td>Furfural treated</td>
<td>Low dose</td>
<td>85.498±4.621 bc</td>
<td>+1.080</td>
<td>55.025±3.139 bc</td>
<td>+18.652</td>
<td>30.475±2.813 c</td>
<td>-24.027</td>
<td>1.874±0.192 ab</td>
<td>+52.979</td>
<td>1.133±0.142 a</td>
<td>+3.376</td>
<td>4.00±0.223 a</td>
<td>+6.661</td>
</tr>
<tr>
<td></td>
<td>Moderate dose</td>
<td>103.778±5.617 a</td>
<td>+22.699</td>
<td>60.975±3.017 ab</td>
<td>+31.482</td>
<td>42.803±2.573 a</td>
<td>+6.706</td>
<td>1.442±0.068 bc</td>
<td>+17.714</td>
<td>1.300±0.069 a</td>
<td>+18.613</td>
<td>3.65±0.134 a</td>
<td>-2.661</td>
</tr>
<tr>
<td></td>
<td>High dose</td>
<td>101.916±7.549 ab</td>
<td>+20.498</td>
<td>65.675±3.008 a</td>
<td>+41.617</td>
<td>34.566±0.761 bc</td>
<td>-13.828</td>
<td>1.908±0.113 ab</td>
<td>+55.755</td>
<td>1.316±0.148 a</td>
<td>+20.073</td>
<td>3.850±0.268 a</td>
<td>+2.661</td>
</tr>
<tr>
<td>F-probability</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.01</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>16.504</td>
<td>10.494</td>
<td>7.576</td>
<td>0.439</td>
<td>-</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>LSD at 1%</td>
<td>22.509</td>
<td>14.313</td>
<td>10.332</td>
<td>0.60</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

- Data are expressed as Mean ± SE. Number of animals in each group is six.
- Means, which share the same superscript symbol(s), are not significantly different
Table 5: The kidney function parameters in serum of control male albino rats and those daily administered a low, moderate and high dose of furfural/kg, after 8 weeks of administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Uric acid (mg/dl)</th>
<th>%</th>
<th>Creatinine (mg/dl)</th>
<th>%</th>
<th>Urea (mg/dl)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.100 ± 0.150 a</td>
<td></td>
<td>1.091 ± 0.059 a</td>
<td></td>
<td>36.583 ± 3.429 b</td>
<td></td>
</tr>
<tr>
<td>low dose</td>
<td></td>
<td>3.550 ± 0.117 a +</td>
<td>14.516</td>
<td>1.100 ± 0.031 a +</td>
<td>0.917</td>
<td>48.533 ± 2.890 a</td>
<td>+ 32.665</td>
</tr>
<tr>
<td>Moderate dose</td>
<td></td>
<td>2.966 ± 0.088 a -</td>
<td>4.322</td>
<td>1.183 ± 0.134 a +</td>
<td>8.432</td>
<td>57.400 ± 2.818 a</td>
<td>+ 56.903</td>
</tr>
<tr>
<td>high dose</td>
<td></td>
<td>3.000 ± 0.223 a -</td>
<td>3.225</td>
<td>1.125 ± 0.104 a +</td>
<td>3.116</td>
<td>52.250 ± 2.219 a</td>
<td>+ 42.820</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F-probability</th>
<th>P &gt; 0.05</th>
<th>P &gt; 0.05</th>
<th>P &lt; 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSD at 5%</td>
<td>-</td>
<td>-</td>
<td>8.472</td>
</tr>
<tr>
<td>LSD at 1%</td>
<td>-</td>
<td>-</td>
<td>11.554</td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± SE. Number of animals in each group is six.
- Means, which share the same superscript symbol(s), are not significantly different.
4. DISCUSSION

Furfural has been classified as GRAS (Generally Recognized as Safe) by the Flavor Extract Manufacturers Association (FEMA) (Adams et al., 1997). The substance 2-furancarboxaldehyde was identified as a high priority for assessment of human health risk because it was considered to present greatest potential for exposure (GPE) and had been classified by other agencies on the basis of carcinogenicity (Canadian Environmental Protection Act, 1999a and b). The present study shows that the administration of furfural to albino rats for 8 weeks produced significant decrease of red blood cell count, white blood cells (WBC), haematocrit (PCV) and hemoglobin content at the medium and high doses in a dose dependent manner. However, mean cell volume (MCV) of red blood cells as well as mean cell haemoglobin (MCH) were highly significantly increased as a result of administration of medium and high concentration of furfural in a dose dependent manner. These results were consistent with the findings of Jonker (2000b) study on Fischer 344 rats were administered microencapsulated furfural via the diet for 13 weeks, there were some haematological changes as decrease in erythrocytes count in males in the highest dose group with increased cell volume and mean corpuscular haemoglobin in the top two dose groups were observed. This effect may be attributed to effect of furfural on DNA of the erythropoietic cells which impairs the cell division. Furfural reacts with DNA in vitro, primarily at AT base pairs, leading to destabilization of the secondary structure of DNA and to single-strand breaks (Hadi et al., 1989; Uddin et al., 1991; Uddin, 1993; Scientific Committee on Consumer Safety [SCCS], 2012). Negative (Aeschbacher et al., 1981) or weakly positive results (Loquet et al., 1981) have been obtained for most bacterial tests for genotoxicity. In particular, positive results were obtained in three out of several assays for reverse mutation in Salmonella typhimurium at relatively high concentrations in the absence of metabolic activation. It was reported that furfural induced Sister chromatid exchange (SCE) in cultured chinese hamester ovary (CHO) cells and human lymphocytes (Galloway et al., 1985).

The present study also revealed that, eosinophils percentage was highly significantly depleted as a result of low and medium doses administration. This may be attributed to the infiltration of liver by esinophils as shown by the study of Shimizu and Kanisawa (1986) and Kaminska and Gruszecka (1977) who evidenced the presence of liver damage in the form of scattered eosinophilic globular formation and increase mitotic figures without zonal or massive necrosis observed 6 hours after exposure to furfural by gavage. Jonker (2000b and c)
in their study, found changes in the perilobular region in 5/10 of all studied rats, mainly, including cells with less coarse cytoplasm and increased clumping of eosinophils.

The liver synthesizes enzymes and structural proteins, detoxifies many internal and external products of the organism (Parkash and Caldwell, 1994). For these functions, hepatocytes use enzyme systems. Furfural is not harmful. However, its by-product, pyromucic acid, has detrimental effect ((Parkash and Caldwell, 1994)). The present study revealed an increase in the liver enzymes, total bilirubin concentration as well as plasma albumin and albumin/globulin ratio. Jonker (2000b) study on Fischer 344 rats revealed that administration of microencapsulated furfural for 13 weeks to females produced a decrease in serum alkaline phosphatase, an increase in gamma-glutamyltransferase and an increase in plasma albumin in the highest dose group. In the high dose group, there was a decrease in ALT, an increase in plasma albumin and albumin/globulin ratio. The increase in liver enzymes as a result of furfural administration can be explained by inflammation and tissue damage induced by furfural effect on the liver hepatocytes.

Irwin (1990) in a subchronic toxicity study revealed that furfural causes centrilobular necrosis and multifocal subchronic inflammation of the liver in males at 150 mg/kg.b.w. but at 300 mg/kg.b.w. per day, the same liver effects were observed both in males and females.

The increase in serum total protein and albumin levels as a result of medium and high doses of furfural in the present study may be explained on the basis liver cirrhosis induced by furfural.

In rat cirrhotic liver, there were no significant differences in levels of serum albumin or albumin mRNA expression between cirrhotic and normal liver. In primary hepatocyte culture, albumin mRNA expression, the amount of albumin secretion and the albumin promoter activity were clearly enhanced in cirrhotic hepatocytes compared to normal hepatocytes (Koura et al., 1999).

Serum globulin level, on the other hand, was significantly elevated due only to low dose. This finding was in agreement with the results of the study of Agakishiev et al. (1990) which revealed that application of furfural resulted in imbalanced levels of IgG1, IgG2, IgA, and IgM after exposure of guinea pig skin to furfural.

The use of tumour markers has become a very attractive method for the detection and diagnosis of neoplastic diseases (Pectasides et al., 1997; Yamao et al., 1999). However, their value in cancer detection has been controversial largely because no single tumour marker is sensitive and specific enough to meet strict diagnostic criteria (Attallah et al., 2006). In this
later study, Attallah et al. (2006) showed that CA 19-9 has the best sensitivity for pancreatic cancer. In hepatocellular carcinoma, AFP was the most sensitive tumour marker. The mode of action underlying the hepatocarcinogenic activity of furfural after oral exposure has not fully been elucidated. However, a genotoxic component clearly is not involved, as evidenced by the in vivo test using transgenic animals. The data do, however, point to a possible role for chronic cytotoxicity that is found in conjunction with the induction of tumours; a pathway that has also been accepted for other non-genotoxic hepatocarcinogens. In the present study, we investigated the levels of serum AFP and CA 19.9 as markers for early detection of hepatocellular carcinoma but these levels were non-significantly affected as a result of all tested doses of furfural.

In our study, the serum urea levels, was highly significantly increased as a result of all tested doses of furfural; the urea/creatinine ratio was only significantly decreased as a result of medium and high doses. However, in the study of Jonker (2000a), female rats at some doses had decreased blood urea nitrogen and creatinine concentrations, but these changes were not dose-related. This may be due to the effect of furfural on the kidney when it is eliminated through it.

In conclusion, the administration of furfural to male albino rats for 8 weeks produced macrocytic anemia, leucopenia, elevated liver enzymes and bilirubin indicating hepatic damage. Mainly these changes are dose related, but there is no evidence of carcinogenic effect by tumor markers.

5. REFERENCES

44. NTP. NTP technical report on the toxicology and carcinogenesis studies of furfural (CAS no.98-01-1) in F344/N rats and B6C3F1 mice (gavage studies). March 1990. NTP-TR 382. NIH Publication no. 90-2837; 1990.