Effect of sweetener and flavoring agent on oxidative indices, liver and kidney function levels in rats

Kamal A Amin1,2*, Hessa M Al-muzafar1 & Adel H Abd Elsttar2

1Chemistry Department, College of Science, University of Dammam, Saudi Arabia
2Biochemistry Department, Faculty of Veterinary Medicine, Beni Suef University, Egypt

Received 03 March 2014; revised 02 July 2014

Food additives while attract consumers, improve quality, control weight and replace sugar, may affect seriously children and adults health. Here, we investigated the adverse effects of saccharin and methylsalicylate as sweetener and flavoring agent on lipid profile, blood glucose, renal, hepatic function and oxidative stress/antioxidants (lipid peroxidation, catalase and reduced glutathione in liver tissues). Saccharin and methylsalicylate were administered orally in young male albino rats at low and high dose for 30 days. Rats were divided into 5 groups, 1st control group, 2nd and 3rd (low and high saccharin-treated groups) and 4th and 5th (low and high methylsalicylate-treated group). Serum total cholesterol, triglyceride, glucose levels and body weight gain were found decreased in saccharin high dose group compared to control. Rats consumed high dose of saccharin showed a significant decrease in serum triglycerides, cholesterol and LDL levels. Low and high doses of saccharin exhibited a significant increase in liver function marker of ALT, AST, ALP activity, total proteins and albumin levels and renal function test (urea and creatinine levels) in comparison with control group. Further, saccharin at high dose induced significant decrease in liver GSH levels, catalase and SOD activity and increase in hepatic MDA level. Overall, saccharin harmfully altered biochemical markers in liver and kidney at higher as well as lower doses. Whereas, methyl salicylates did not pose a risk for renal function and hepatic oxidative markers.

Keywords: Catalase, Food additives, Lipid peroxidation, Methyl salicylates, Oxidative biomarkers, Saccharin.

The rising concern about health and life quality have encouraged societies to exercise, eat healthy food and reduce consumption of food rich in sugar, salt and fat. With increased consumer interest in reducing sugar intake, food products made with sweeteners rather than sugar have become more common1. Stevia rebaudiana (Bert.) Bertoni (Fam. Asteraceae), a Paraguayan perennial herb, has been reported to be an economically important source of non-caloric natural commercial sweetener2. Recently, Jain et al.3 have extensively reviewed the health benefits of xylooligosaccharides (XOS) and their importance in various functional foods. The low-calorie artificial sweeteners, such as aspartame, saccharin, acesulfame-K and cyclamate, have become sugar alternatives to replace sucrose4, and have been widely used in dairy products, energy control diet and diabetes in Africa, Asia, Europe and USA. Dextranucrase from Pediococcus pentosaceus CRAG3 with its high activity and stability, has been reported to be a potential food additive, in particular for improving texture of dairy products5.

Saccharin

Saccharin is a non-nutritive, non-caloric intense artificial sweetener. It has 300-500 times the sweetness of sucrose, but has a slight bitter aftertaste6. It is still the widely used sweetener7 as it is heat-stable, and thus used in hot beverages, canned vegetables, bakery products and reduced sugar jams8. Saccharin goes directly through the human digestive system without being digested; has no food energy, and can trigger the release of insulin in rats, apparently as a result of its taste9. There are different forms of saccharin including sodium saccharin, calcium saccharin, potassium and acid saccharin. Sodium saccharin is more palatable and commonly used. In the European Union, saccharin is known under the E number (additive code) E954. The accepted daily intake of saccharin is 2.5 mg/kg body wt.10.

Absorption and metabolism— The degree of saccharin absorption occurs rapidly and dependent on food intake. After its removal from the diet, it takes 3 days for complete tissue clearance. Diets containing 7.5-10% saccharin result in elevated levels of saccharine in renal, bladder, liver and muscle tissues and plasma up to 22 days due to inability of the animal to eliminate saccharin11.
Effect on vital organs, serum biochemical assay and its health risk—The renal and hepatic effects of in vivo treatment with saccharin have been determined as increased DNA elutability in the range of 130-210% compared with controls. Consumption of large amounts of saccharin may result in hypoglycemia, reduced hyperinsulinemia, decreased insulin resistance, and improved glycemic control in hyperglycemic obese mice. In contrast, intake of foods or fluids containing non-nutritive sweeteners leads to increased food intake, body wt. gain, accumulation of body fat, and weaker caloric compensation. Saccharin consumption is correlated with increased frequency of cancer (especially bladder cancer) while some argued no such correlation. It is required to have more data to understand saccharine and its oxidative stress action that may correlate to carcinogenic effects.

Methyl Salicylate
Salicylate esters, a chemical extensively used as flavor and fragrance additives in foods, beverages and a wide variety of consumer products, have been suspected for estrogenic activity. Exposure to salicylate esters and derivatives may result in reproductive and developmental toxicity. The salicylates are well absorbed by the oral route, and oral bioavailability is assumed to be 100%. They undergo extensive hydrolysis, primarily in the liver, to become salicylic acid that conjugates with either glycine or glucuronide and excreted in the urine as salicylylic acid and acyl/phenolic glucuronides. The expected metabolism of the salicylates does not present toxicological concerns.

The acute oral toxicity of the salicylates is moderate, with toxicity generally decreasing with increasing size of the ester R-group. The aromatic salicylates are of low to moderate acute oral toxicity (1300 to >5000 mg/kg body wt.). Differences in acute oral toxicity are related to the relative proportion of the molecular weight released as salicylic acid following hydrolysis.

From the genetic toxicity data (2 year studies of oral methyl salicylate in rats), and the metabolism of the salicylates (simple alcohols and acid metabolites), it appears that the salicylates are unlikely to be carcinogenic. The Cosmetic Ingredient Review Board in USA has concluded that use of salicylates and salicylic acid in cosmetic products would not pose a risk for reproductive or developmental effects in humans.

Prolonged use of sweeteners and flavoring agents is reported to be toxic, causing health complications such as indigestion, anemia and allergic reactions as asthma and urticaria, pathological lesions in the brain, kidney, spleen and liver, tumors and cancer, paralysis, mental retardation, abnormalities in offsprings, growth retardation and eye defects resulting in blindness.

In the absence of a clear verdict on the safety of usage of saccharides and methyl salicylates, here we tried to evaluate the largest toxic harmful effect of some synthetic additives of sweeteners and odorant or flavors on liver and metabolic biomarkers in rats.

Materials and Methods
Animals and Diet
A total of 46 young male Rattus norvegicus albino rats weighing about 70-80 g aged about 5 wk, were used in the present study. They were obtained from (National Research Center, Cairo, Egypt). Animals were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection. They were maintained in stainless steel cages at 27±5°C under good ventilation. Our work was carried out in accordance with the guidelines of Beni Suef University for animal use. Animals were fed on the standard basal diet and provided with tap water and the composition of experimental diets according to Kim et al., as follows: (fat 5%, carbohydrates 65%, protein 20.3%, fiber 5%, salt mixture 3.7%, and vitamins mixture 1%).

Drugs and Chemicals
Saccharin found in its sodium salt, is white crystalline powder, with sodium saccharin content 99.62%, arsenic l-2 ppm, and foreign substance <10 ppm was purchased from Taianjin north food Co. LTD (China) through El-Goumhoria Company, Egypt.

Methyl salicylates (food odorant) (clear colorless liquid, concentration 99.8%) was purchased from El-Goumhoria Company, Egypt. Saccharine and methyl salicylate were in a solid state, and hence were prepared into two solutions of each substance (one low and the other high) by dissolving the solid in distilled water, low and high doses of saccharin were 10 and 500 mg/kg body wt., respectively, while the low and high doses of methyl salicylates were 80 and 250 mg/kg body wt., respectively.

The instruments used in the experiment were Glass-col homogenizer with GT motor control USA, UV and visible Humalyzer 2000 spectrophotometer Jenway 6100 spectrophotometer, UK; Shaking water bath precision scientific group, USA; MLW centrifuge, GDR; IEC clinical centrifuge, USA.
Animals and treatments

Rats were divided into five groups as follows: Group I, Control (10 rats without any chemicals); Group II, saccharin low dose (8 rats administered with saccharine @10 mg/kg body wt. per day for 30 days per os using stomach tube); Group III, saccharin high dose (10 rats administered with saccharine @500 mg/kg body wt. per day for 30 days orally using stomach tube); Group IV, methyl salicylates low doses (8 rats administered with methyl salicylates @80 mg/kg body wt.; and Group V (10 rats) given the same @250 mg/kg body wt. per day for 30 days per os using stomach tube.

Sampling and tissue preparation

By the end of the experimental periods, venous blood samples were collected from the orbital sinus of control, and treated rats via glass capillaries at fasting state. The blood samples were collected in dry glass centrifuge tubes and allowed to coagulate at room temperature (27±5°C) and centrifuged at 3500 rpm for 15 min at room temperature for separation of serum. Homogenates of liver tissues were prepared by dissolving 0.25 g of liver tissue in 5 mL of saline (0.9 % NaCl) in test tube, homogenized for 15 min, centrifuged for 10 min at 3000 rpm, and the supernatants were collected for determination of biomarkers of oxidative stress.

Biochemical tests of Serum and tissue samples

Serum ALT and AST activities were determined according to Reitman and Frankel\textsuperscript{25} using kits purchased from Randox Company United Kingdom. Total protein was determined according to the method of Patton and Crouch\textsuperscript{37}. Total cholesterol, triglycerides and HDL-cholesterol were estimated in serum by enzymatic colorimetric method. Glucose concentration in serum was estimated by enzymatic colorimetric method according to Trinder\textsuperscript{28}. Catalase activity was estimated in tissue homogenate by method of Cohen \textit{et al.}\textsuperscript{29}. Liver reduced glutathione content (GSH) was determined according to the procedure of Beutler \textit{et al.}\textsuperscript{30}. Determination of lipid peroxidation in tissue homogenate was determined according to the method of Presuss\textsuperscript{31}.

Statistical analysis

The statistical analysis was carried out using GraphPad Instat software (version 3, ISS-Rome, Italy). Unless differently specified, groups of data were compared with un-paired t-test and one-way analysis of variance (ANOVA) followed by Tukey–Kramer (TK) multiple comparisons post-test. Values of \( P < 0.05\) were regarded as significant.

Results

**Effect of saccharin and methyl salicylate on body weight gain and growth rate**

Rats consumed high dose of saccharin (group III) showed a significant decrease in body weight, while low dose (group II) showed non-significant changes as compared to control rats (Table 1). Rats consumed low dose of methyl salicylates (group IV) and those consumed high dose of the same food flavor (group V) showed a significant decrease in body weight when compared to control rats. Group V rats that had high dose of methyl salicylates exhibited a significant decrease in body weight than those which had low methyl salicylates dose (group IV). In body weight gain, all the groups showed decrease when compared to control. Group III had least gain preceded by group V.

Table 1—Effect of both low and high doses Saccharin (10 and 500 mg/kg body wt.) and Methyl salicylate (80 and 250 mg/kg body wt.) on body weight, serum lipid profile and glucose

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Saccharin</th>
<th>Methylsalicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>78.0±6.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.6±4.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.4±2.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>788±28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>787±26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>787±26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>96.8±4.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.7±4.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.7±4.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>143.41±4.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.13±2.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121.6±1.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>130.6±3.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.16±4.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101±3.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>87.1±1.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.51±1.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.1±2.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>34.18±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.0±1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.23±2.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data expressed as MEAN±SE. Means with the same superscript letters (s) are not significantly different. Means having different letters are significantly different (\( P < 0.05\)).
All the groups exhibited reduced growth rate compared
to control (83.85%). High dose saccharin group (Gr III)
had the lowest growth rate (30.64%) preceded by group
V, IV and II (71.28%), respectively.

**Effect of saccharin and methyl salicylate on lipid profile and serum glucose**

Rats consumed low and high doses of saccharin
groups II & III) showed a significant decrease in
serum total cholesterol and glucose level when
compared to control rats (Table 1). Group III rats showed
similar decrease in serum triglyceridesand LDL
levels as well. Serum fasting glucose was lowest
in that group. On the other hand, methyl salicylate fed
groups (IV & V) did not show any significant change
in either lipid profile (serum total cholesterol,
triglycerides, HDL and LDL–Cholesterol) nor in
fasting serum glucose. (Table 1).

**Effect of saccharin and methyl salicylate on hepatic function markers**

Groups (II-V) that had low and high dose of both
saccharin and methyl salicylate exhibited a significant
increase in serum ALT, ALP activity when compared
to control rats. High dose of saccharin (Gr III) showed
the highest level among the groups. Whereas in serum
AST activity, only saccharin fed groups showed
significant increase (Table 2). In other words,
saccharin fed groups (II & III) exhibited significant
increase in liver function markers of serum ALT,
AST, ALP activity, total proteins and albumin
concentration (Table 2). Whereas, rats fed on
methylsalicylate showed a significant decrease in
serum total protein concentration as well as serum albumin compared to control rats (Table 2).

**Effect of saccharin and methyl salicylate on renal function tests**

Low and high dose of saccharin (groups II & III)
produced a significant elevation in serum urea and
creatinine levels following higher level with high
dose, whereas low and high methyl salicylate (groups
IV & V) did not induce any significant change in urea and
creatinine levels (Table 2).

**Effect of saccharin and methyl salicylate on hepatic oxidative stress markers**

Rats consumed high dose of saccharin (group III)
showed a significant decrease in liver GSH levels,
catalase and SOD activity but increased in hepatic
MDA level when compared to control rats. However,
biomarkers of oxidative stress in liver homogenate
were neither significantly changed with low saccharin
dose (group II) nor with both low and high
methylsalicylates groups (Gr IV & V) (Table 3).

### Table 2—Effect of both low and high doses Saccharin (10 and 500 mg/kg body wt.) and Methyl salicylate
(80 and 250 mg/kg body wt.) on serum liver function and renal function markers

<table>
<thead>
<tr>
<th>Liver/renal function markers</th>
<th>Control</th>
<th>Low Saccharin</th>
<th>High Saccharin</th>
<th>Control</th>
<th>Low Methyl salicylate</th>
<th>High Methyl salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>8.91±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.5±1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.58±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.16±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.4±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>47.85±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.70±2.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.36±2.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.23±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.51±0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>87.56±2.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117.01±1.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117.55±0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>108.95±2.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>112.38±1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T. Proteins (g/dl.)</td>
<td>5.59±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.70±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.41±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.14±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.81±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.42±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.59±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.73±0.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.82±0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>17.54±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.95±0.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44.53±1.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.96±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.9±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.32±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.96±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.82±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.34±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.39±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as MEAN±SE. Means with the same superscript letters (s) are not significantly different. Means having different letters are significantly different (P <0.05).

### Table 3—Effect of both low and high doses of Saccharin (10 and 500 mg/kg body wt.) and Methyl salicylate
(80 and 250 mg/kg body wt.) on oxidative stress biomarkers (GSH, MDA, SOD and catalase) in liver homogenate

<table>
<thead>
<tr>
<th>Oxidative stress biomarkers</th>
<th>Control</th>
<th>Low Saccharin</th>
<th>High Saccharin</th>
<th>Control</th>
<th>Low Methyl salicylate</th>
<th>High Methyl salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (nmol/100 mg)</td>
<td>72.9±1.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.13±0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.12±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.10±1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.24±1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/g/h)</td>
<td>4.82±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.03±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.64±0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.08±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.91±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SOD (u/g)</td>
<td>70.68±1.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.58±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.33±0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.27±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.22±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Catalase (k×10&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>47.03±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.85±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.65±2.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.56±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.2±0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as MEAN±SE. Means with the same superscript letters (s) are not significantly different. Means having different letters are significantly different (P <0.05).
Discussion

In this study, we explored the impact of two food additives commonly used in Egyptian and Saudian foods by checking the toxic side effects and biochemical changes in experimental rats. We considered low dose (double of acceptable daily intake (ADI)) because young children in Egypt can consume a double of ADI (or more) daily in several products without control. In addition, we used the high dose (a much higher than ADI) to evaluate the toxicity and health hazards of these additives on biochemical assay and oxidative stress.

The loss of bodyweight with high saccharine (Table 1) could be attributed to reduced food consumption per day and also as a consequence to the hypotriglyceridemia and hypocholesterolemic effect as revealed by a decrease in total serum cholesterol, particularly with the high dose treated groups.\textsuperscript{32,33} The present results are in agreement with the observation of Dib \textit{et al.}\textsuperscript{34} who reported a significant reduction in body weight of rats (50%) after 14-day administration of sodium saccharin; butin contrary to that of Polyák \textit{et al.}\textsuperscript{35} who reported increased body weight in saccharin consumed rats. However, frequent consumers of saccharin have also been reported to be at increased risk of excessive weight gain, metabolic syndrome, type 2 diabetes, and cardiovascular disease.\textsuperscript{36}

The poor body weight gain in case of rats fed on methyl salicylate may be attributed to uncoupling of oxidative phosphorylation and inhibition of Krebs cycle enzymes (succinate and α-ketoglutarate dehydrogenase) by salicylate, and thereby inhibition of amino acid synthesis.\textsuperscript{37}

The hypoglycemia observed in saccharin dosed rats (Table 1) are in accordance with Abdallah\textsuperscript{33} and Horwitz \textit{et al.},\textsuperscript{13} who observed that consumption of large amounts of saccharin might reduce blood glucose concentration. Moreover, as said earlier, saccharin has no food energy, and it can trigger the release of insulin apparently as a result of its taste, and thereby reduce blood glucose level.\textsuperscript{7} Further, Bailey \textit{et al.}\textsuperscript{14} also reported a reduction of hyper-insulinemia, decreased insulin resistance and improved glycemic control during saccharin consumption in hyperglycemic obese mice.

Our observations that high and low saccharin induced hypocholesterolemia and high saccharin induced hypotriglyceridemia (Table 1) are in agreement with results obtained by Osfor & Elias.\textsuperscript{38} Cholesterol is a soft waxy substance in the blood stream and in the body’s cells. It is used to form cell membranes and to produce certain hormones. Its total body content depends on the balance between synthesis by the body as well as that absorbed from the diet. The intestinal cholesterol pool comes from dietary cholesterol and the majority from biliary excretion. Approximately, 50% of the intestinal cholesterol pool is reabsorbed by the intestines and recirculated via enterohepatic circulation, with the remainder excreted in feces. The deviation from normal serum cholesterol levels is considered as symptoms of liver diseases.\textsuperscript{40} In the present study, the decreased cholesterol level implies liver damage which is in accordance with the increased alkaline phosphatase level.

The mechanism of hypocholesterolemic and hypolipidemic effect may be attributed to reduced total cholesterol synthesis by the saccharin suppressed in vivo liver enzymatic activity of acetyl-CoA synthetase, citrate lyase, and mitochondrial citrate exchange leading to a reduction of available cytoplasmic acetyl-CoA, which is required for the synthesis of cholesterol and fatty acids.\textsuperscript{41} Moreover, liver acetyl-CoA carboxylase, phosphatidate phosphohydralase, and glycerol-3-phosphate acyl transferase activities were markedly reduced by the saccharin analogues. Suppression of these enzymes would lead to a reduction of triglyceride synthesis.

Both oral dose of low and high methyl salicylate (wintergreen odour) reduced serum albumin significantly and as a result the total serum protein level also fell down (Table 2). Salicylates inhibit protein synthesis by inducing phosphorylation of the subunit of eukaryotic translation initiation factor 2, which results in the inhibition of mRNA translation in the cells.\textsuperscript{42} Thus, the food odour methyl salicylate induced a significant hypoproteinemia when given orally for 30 days. This decrement may be due to loss of protein formation from the alimentary tract or decreased formation of protein in the liver (impaired ability of the liver to form albumin).

Low and high dose of saccharin exhibited a significant increase in serum ALT, AST and ALP activities as compared to control rats (Table 2). Our results correlate well with Abdallah.\textsuperscript{33} In addition Osfor & Elias\textsuperscript{38} also reported that saccharin treated rats showed a significant increase in ALT activity after both 6 and 12 weeks of administration. AST levels were significantly higher in saccharine treated group and that chronic saccharin intake reflects...
various metabolic, hormonal and neural responses in males and females. The elevation in serum aminotransferase activities could be due to drastic effects caused by free radicals interaction with cellular membranes or related to breakdown of liver parenchyma. The changes in liver function could be attributed to hepatocellular impairment which subsequently caused leakage and the release of greater than normal levels of intracellular enzymes into the blood. Elevation in the activities of aminotransferases indicated an early diagnosis of hepatotoxicity and considered as tissue damage biomarkers.

In the present study, both low and high doses of methyl salicylate showed a significant elevation in serum ALT and ALP activities in agreement with Humphreys who reported enlargement of the liver of dogs after oral administration of methyl salicylate. The elevation in serum alkaline phosphatase (ALP) in low and high saccharin and methyl salicylate may be an evidence of obstructive damage in the liver tissue due to saccharin and methyl salicylate. The liver cells play an important role in both synthesis and secretion of ALP into the bile. Therefore, the alterations in ALP activity caused by saccharin may be attributed to early cholestatic liver damage which primarily affects the liver parenchyma, thus making ALP a sensitive index in the diagnosis of infiltrative diseases. After ingestion of salicylates, salicylic acid is formed, and readily absorbed in the stomach and small bowel. Salicylate poisoning is manifested clinically by disturbances of several organ systems, including the CNS and the cardiovascular, pulmonary, hepatic, renal, and metabolic systems.

When the acetates administered orally, they hydrolyze rapidly in liver into acetic acid and their corresponding alcohol (in case of isoamylacetates it is isopentanol) which can affect the liver cells by increasing the cytotoxicity and thereby increase the release of hepatic enzymes like ALT, and ALP into circulation. Thus, saccharin and methyl salicylate, have a risky effect on liver and alter hepatic function by elevating serum ALT and ALP in both low and high doses as observed here, and this effect could be more appreciable at high doses.

Significantly elevated serum creatinine and urea with high and low dose of saccharin (Table 2) could be due to the toxic effects of saccharin on the kidney especially with high dose that can lead to disorders in the renal function, and hence reduced glomerular filtration rate followed by retention of urea and creatinine in the blood. Saccharin administration caused a reduction in the renal accumulation of p-aminohippurate and tetraethylammonium, and at 60 days of age, increased urine volume, decreased urine osmolality, and increased potassium excretion were also observed. A combination of saccharin and aspirin had a high incidence of renal papillary necrosis and calcification. These toxic effects of aspirin and saccharin are independent responses, and administration of both greatly accentuates these responses. From the obtained results, we postulate that saccharin affects the kidney and cause disturbed renal function and increased serum creatinine and urea levels.

Oxidative stress is an imbalance between oxidants and antioxidants in favour of the oxidants, potentially causing damage to cells or cellular components. Particularly, destructive aspect of oxidative stress is the production of reactive oxygen species (ROS), which include free radicals, H₂O₂ and peroxides that can cause extensive cellular damage. Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and damage on DNA.

Our study revealed that high dose of saccharin induced a significant reduction in liver catalase, SOD, and GSH, but increased liver MDA level when compared to control rats, while methyl salicylate dosed rats didn’t show any significant changes (Table 3). We attribute the oxidative stress induced by high dose of saccharin to the inflammation of the liver cells; this opinion is supported by observation of Abdallah who stated that livers of rats administrated with saccharin showed a portal infiltration with mononuclear inflammatory cells mainly lymphocytes and macrophages, as observed by Hassanin too. Consequently, inflammatory processes induce oxidative or nitrosative stress and lipid peroxidation, thereby generating excess ROS, reactive nitrogen species, and DNA-reactive aldehydes. Stimulated inflammatory cells undergo a respiratory burst and release ROS such as superoxide anion, hydrogen peroxide and numerous secondary oxidants as well as the arachidonic acid cascade.

 Liberation of ROS by inflammatory cells induces lipid peroxidation by depletion of GSH content and decrease catalase and SOD activities and increase the
formation of malondialdehyde as a product of lipid peroxidation by high dose of saccharin. These free radicals can also damage the hepatic cells and increase the liberation of AST and ALT enzymes into serum as observed in saccharin treated rats particularly those consumed high dose (500 mg/kg body wt.). Increased oxidative markers in saccharine group may not be due to lipid profile elevation but liver function disturbances.

Regarding hepatic antioxidant system there was a significant inhibition of the antioxidant defense system during saccharin administration, specifically decreased catalase and SOD activities and equivalent fall in GSH content (Table 3) which prevented the cell death by the toxic radicals. Hence, their levels in the tissue homogenate were decreased where GSH was consumed for ROS removal and changed into oxidized GSSG. On the other hand, MDA level was increased as a product of lipid peroxidation by the ROS action on lipids of cellular membrane.

Conclusion
Frequent consumption of saccharine have the counterintuitive effect of inducing metabolic derangements that affect body weight, glucose and lipids levels. These results question the effect of saccharine on weight-maintenance or growth rate. Saccharin induces changes in hepatic and renal function in a dose dependent manner and becomes more risky at higher doses because of its ability to induce oxidative stress by formation of free radicals. Food flavoring agent methyl salicylate has a damaging effect on liver function and induce oxidative stress by formation of free radicals. More risky at higher doses because of its ability to function in a dose dependent manner and become significant inhibition of the antioxidant defense system during saccharin administration, specifically decreased catalase and SOD activities and equivalent fall in GSH content (Table 3) which prevented the cell death by the toxic radicals. Hence, their levels in the tissue homogenate were decreased where GSH was consumed for ROS removal and changed into oxidized GSSG. On the other hand, MDA level was increased as a product of lipid peroxidation by the ROS action on lipids of cellular membrane.

References
18. EMEA/MRL— The European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit, (1999), Committee for veterinary medical products salicylic acid, sodium salicylate, aluminium salicylate basic and methyl salicylate basic and methyl salicylate summary report.