HEPATIC AND RENAL BIOCHEMICAL RESPONSES TO THE TOXICOLOGICAL INTERACTION BETWEEN ACETYLSALICYLIC ACID AND DIAZINON IN ALBINO RATS

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ABSTRACT

The present investigation is aiming at studying the effect of administrating sublethal dose of the insecticide "Diazinon" with and without acetylsalicylic acid (Aspirin, ASA). Sixty male albino rats were given orally 1/30 LD50 of the insecticide "Diazinon", with and without the high therapeutic dose of acetylsalicylic acid at a dose of (13.5mg/ kg b.w. daily) for 3 weeks. Biochemical indices of liver and kidney functions, namely serum proteins, alanine amino transferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, cholesterol, triglyceride (TG), urea and creatinine levels were determined at the end of the experiment. The present results showed significant changes in serum ALT, AST, ALP, Bilirubin, Triglyceride levels. The changes in enzyme levels indicate the toxicity of the insecticide "Diazinon" alone or in combination with the high therapeutic dose of the anti inflammatory drug "acetyl salicylic acid". The treatments did not affect the level of cholesterol or renal excretory function.

Key Words: Diazinon - acetylsalicylic acid - liver and kidney functions -

INTRODUCTION

Exposure to different xenobiotics such as insecticides, herbicides or drugs, leads to more toxic hazards owing to the possible interactions between these different xenobiotics and with the biological systems of living organism. The literature (Casart, 1973) offers much information about the adverse effects of single toxicant, but scarcely on mixtures of toxicants, especially herbicides and insecticides with drugs. Thus, the topic of chemical toxicological interaction attracted world wide attention (European issx meeting, 1992).

Biochemical estimations have become an integral part of testes in toxicological studies (Casaret, 1973); they give an early warning of signs of toxicity particularly those concerning liver and kidney functions.

Diazinon is a contact Organophosphorus insecticide with a wide range of insecticidal activity. It is effective against adults and juvenile forms of flying insects, crawling insects, acarians and spiders. It has been used since the early 1950's. (Barabas, 1998). The toxicological effects of diazinon on acetyl cholinesterases results from the accumulation of acetyl choline at the neuronal junctions (Poe et al, 2004).

Abdelsalam and Ford (1986) found that the toxic effects of diazinon were increased by pretreatment with the hepatic microsomal enzyme inducers dieldrin and phenobarbitone, at the same time, there was a rise in the liver carboxylesterase activity.

Abdelsalam and Ford (1987) showed that diazinon induced liver and kidney damage in ruminant livestock. They added that this hepatotoxic effect was enhanced by carbon tetrachloride,while the renal toxic effect was enhanced by mercuric chloride.

Aspirin (ASA) is used as an anti-inflammatory drug in rheumatoid arthritis and as an analgesic in osteoarthritis (OA). It is also used frequently in juvenile arthritis, sero-negative arthropathies and most soft tissue lesions. At very high doses, it may be of help in the treatment of acute gout and severe ankylosing spondylitis. Aspirin combining analgesic, anti-inflammatory and antipyretic actions is still a useful agent in many clinical situations varying from influenza to Still's Disease (Axon and Huskisson, 1992).

The hepatotoxic potential of ASA in general includes transient, dose-dependent and reversibly elevated serum levels of liver enzymes, namely ALT, AST, Gamma-GT and alkaline phosphatase. Abnormalities of bilirubin and prothrombin levels may occur. Serious hepatic events during correct dosage with Non Steroidal Anti-inflammatory Drugs (NSAIDs) are uncommon; a higher risk may be attributable with the use of ASA (Bernstein et al., 1977; Prescott, 1981).
According to Meade (1998), Aspirin plays a central part in preventing the recurrence of myocardial infarction and have a place in preventing first episodes as well. Hence, its use is universally spreading. On the other hand, one of the side effects, the inhibition of platelet aggregation, has advanced ASA in another therapeutic area, where it plays an important part in today's medicine. The occurrence of adverse effects depends strongly, as with every medicine, on the dose administered. Although ASA is an old drug, more trials are needed in order to establish efficacious but safe dosages especially for its new suggested therapeutic applications.

Aim of the work:

The present investigation aims to study the effect of sublethal dose of the insecticide diazinon (1/30 LD 50) with and without higher therapeutic dose of acetyl salicylic acid (13.5 mg/kg b.wt.) on markers of liver and kidney functions. These functions were monitored through some selected biochemical parameters in the albino rats.

MATERIALS AND METHODS

Materials:

Diazinon is an Organophosphorus insecticide chemically named O, O diethyl O-2-isopropyl-6-methylpyrimidin-4-phosphorothioate. It was obtained from the market as emulsion concentrate containing 60% diazinon and 40% inactive ingredients.

Aspirin (acetylsalicylic acid) tablets containing (75 mg/tablet) as anti-inflammatory drug was purchased from the market. Aspirin was powdered and suspended in water containing 2% tween 80 and given orally to the experimental animals at a dose of 13.5 mg/kg b.wt, which is equivalent to the daily highest human antiplatelets dose calculated for rats according to surface area (Paget and Barnes, 1964).

Experimental animals:

The study was carried out on 60 adult male albino rats. Body weight ranging between (150-200 gm). Doses were administered orally in 1 ml/100 g b.wt., daily for 3 weeks treatments. The rats were divided into 4 equal groups each 15 rats as follows:

Group 1: Received 1 ml/100g b.wt., of the vehicle (water & tween 80) daily and served as control.

Group 2: Received diazinon (1/30 LD50).

Group 3: Received acetyl salicylic acid (13.5 mg/kg b.wt. higher therapeutic dose).

Group 4: Received acetyl salicylic acid (13.5 mg/kg b.wt.) and diazinon (1/30 LD50).

At the end of the experiment blood samples were taken from the retro-orbital plexus of rats for biochemical investigation.

Methods:

Serum biochemical analysis:

1. The Biuret method was used for the determination of total protein in serum as described by Henery et al. (1974).

2. Serum albumin was determined as described by Doumas et al. (1971).

3. Globulin was estimated by calculating the difference between total serum protein and albumin as indicated by Reitman and Frankel (1957).

4. ALT and AST activities were determined colorimetrically by the method of Reitman and Frankel (1957).

5. ALP was determined according to the method described by Belfeld and Goldberg (1971).

6. Bilirubin was determined as described by Michaelsson (1961).

7. The glucose oxidase method was used for the determination of serum glucose as described by Trinder, (1969).

8. Triglycerides were estimated according to the method described by Wieland (1974).

9. Cholesterol was measured enzymatically according to the method of Flegg (1973).

Statistical analysis.

Mean and standard error (SE) were calculated according to the method of Bernstein and Weatheral (1952). Student t-test described between the mean values obtained.

RESULTS

Serum biochemical analysis:

1- Serum proteins:

Acetyl salicylic acid at a high therapeutic dose [13.5mg/kg b.w] alone or combined with diazinon (1/30 LD50) after 3 weeks of treatment did not affect serum total protein, albumin, globulin or A/G ratio (table 1).

Diazinon given alone decreased serum total proteins compared to the control.

2- Serum Enzymes:

Acetyl salicylic acid did not significantly affect the level of any of the estimated serum enzymes after 3 weeks of treatment (table 2).

Diazinon alone markedly increased serum ALT, AST, & ALP levels. Also, acetyl salicylic acid combined with diazinon markedly elevated both serum ALT & AST, but did not affect ALP levels (table 2).
3- Serum bilirubin:

Both acetylsalicylic acid at [13.5 mg/kg b.w] dose level and diazinon [1/30 LD₅₀] alone or in combination caused highly significant increase in serum bilirubin levels comparing to the control group (table 3).

4- Serum triglyceride & cholesterol:

Acetylsalicylic acid given alone did not affect serum cholesterol or triglyceride levels; but diazinon alone caused a high marked elevation in serum cholesterol level, and when combined with acetylsalicylic acid caused marked elevation in serum triglyceride level (table 4).

5- Serum urea & creatinine:

Acetylsalicylic acid [13.5 mg/kg b.w] alone or combined with diazinon [1/30 LD₅₀] did not affect serum urea or creatinine levels.

Diazinon [1/30 LD₅₀] alone markedly decreased serum urea but did not affect creatinine level (table 5).

Table (1): Serum protein level of rats treated daily with acetylsalicylic acid (13.5 mg/kg b.wt.) with or without diazinon (1/30 LD₅₀) for 3 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Globulin (g/dL)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.30±0.27</td>
<td>3.43±0.52</td>
<td>4.80±0.31</td>
<td>0.76±0.07</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>9.70±0.33</td>
<td>4.04±0.17</td>
<td>5.03±0.35</td>
<td>0.80±0.48</td>
</tr>
<tr>
<td>Diazinon</td>
<td>7.01±0.05***</td>
<td>2.51±0.29</td>
<td>4.49±0.43</td>
<td>0.62±0.15</td>
</tr>
<tr>
<td>Acetyl salicylic acid+Diazinon</td>
<td>9.65±0.29</td>
<td>4.32±0.06</td>
<td>5.33±0.24</td>
<td>0.81±0.03</td>
</tr>
</tbody>
</table>

*, ** and *** denote significant difference from the corresponding control value at P<0.05, P<0.01 and P<0.001, respectively.

Table (2): Serum transaminase (ALT, AST) and alkaline phosphatase (ALP) levels of rats treated daily with acetylsalicylic acid (13.5 mg/kg b.wt.) with or without diazinon (1/30 LD₅₀) for 3 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (u/l)</th>
<th>AST (u/l)</th>
<th>ALP (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.20±0.97</td>
<td>33.60±1.22</td>
<td>64.76±1.47</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>26.60±0.78</td>
<td>32.07±1.10</td>
<td>57.50±3.17</td>
</tr>
<tr>
<td>Diazinon</td>
<td>68.40±1.96**</td>
<td>75±3.59**</td>
<td>119.70±4.51***</td>
</tr>
<tr>
<td>Acetyl salicylic acid+Diazinon</td>
<td>35.50±0.12**</td>
<td>40.00±0.75***</td>
<td>64.29±1.53</td>
</tr>
</tbody>
</table>

*, ** and *** denote significant difference from the corresponding control value at P<0.05, P<0.01 and P<0.001, respectively.

Table (3): Serum total bilirubin level of rats treated daily with acetylsalicylic acid (13.5 mg/kg b.wt.) with or without diazinon (1/30 LD₅₀) for 3 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.39±0.20</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>1.28±0.11***</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.63±0.07***</td>
</tr>
<tr>
<td>Acetyl salicylic acid+Diazinon</td>
<td>0.73±0.07***</td>
</tr>
</tbody>
</table>

*, ** and *** denote significant difference from the corresponding control value at P<0.05, P<0.01 and P<0.001, respectively.

Table (4): Serum triglyceride and cholesterol levels of rats treated daily with acetylsalicylic acid (13.5 mg/kg b.wt.) with or without diazinon (1/30 LD₅₀) for 3 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglyceride (g/dl)</th>
<th>Cholesterol (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.45±0.04</td>
<td>0.68±0.27</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>53±3.79</td>
<td>1.00±0.07</td>
</tr>
<tr>
<td>Diazinon</td>
<td>66.26±5.88</td>
<td>1.06±0.15**</td>
</tr>
<tr>
<td>Acetyl salicylic acid+Diazinon</td>
<td>78.35±1.62***</td>
<td>0.90±0.17</td>
</tr>
</tbody>
</table>

*, ** and *** denote significant difference from the corresponding control value at P<0.05, P<0.01 and P<0.001, respectively.
Table (5): Serum urea and creatinine levels of rats treated daily with acetylsalicylic acid (13.5 mg/kg b.wt) with or without Diazinon (1/30 LD50) for 3 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.88±1.25</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>18.07±2.08</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>Diazinon</td>
<td>9.64±0.57**</td>
<td>0.66±0.04</td>
</tr>
<tr>
<td>Acetylsalicylic acid+Diazinon</td>
<td>20.18±1.21</td>
<td>0.68±0.05</td>
</tr>
</tbody>
</table>

*, ** and *** denote significant difference from the corresponding control value at P<0.05, P<0.01 and P<0.001, respectively.

**DISCUSSION**

The present study show that acetylsalicylic acid (13.5 mg/kg b.w = 150mg/day) given alone or combined with diazinon to rats did not affect serum proteins, albumin, globulin or A/G ratio (table 1). This agrees with (Kurowski and Brune, 1992). They showed that aspirin & the salicylates are generally considered safe drugs. The lack of any changes on serum proteins indicates that the treatments had no effect on the biosynthesis of the protein as a function of the liver hepatocytes since the latter is the sole site of protein production (Grant et al., 1977). Moreover, diazinon (1/30 LD50) alone significantly decrease serum total protein and slightly decrease serum albumin, globulin, and A/G ratio.

The results in table (2) indicate that acetylsalicylic acid when administered alone did not significantly affect the levels of serum ALT, AST, or ALP. These results agree with kurowski and Brune (1992) investigation regarding aspirin and salicylates as safe drugs. On the other hand, Prescott (1980) stated that aspirin & salicylates have been recognized as potentially hepatotoxic. Also, Ebong et al (1998) showed that aspirin significantly increase the levels of ALT & AST.

The present study show that diazinon given alone or combined with ASA produced marked elevation of serum enzyme ALT, AST. According to Ingram (1993), the elevation of both transaminase enzymes are presumed to be resulted from leakage or damage or necrotic liver cells in the same time, ALP was elevated by administration of diazinon to rats. These results agree with Tiefenbich and Wichner (1985) finding where an increase in transaminases of liver was observed, These enzymes show an early rise in almost all diseases of the liver and remain elevated for 2 to 6 weeks in the presence of the disease. In addition to transaminases elevation, ALP is increased in most hepatic diseases as well as in both intrahepatic and extrahepatic obstructive diseases(Tiefenbich and Wichner, 1985). These results which match with (Kalender et al., 2005), they found that there was a statistically significance in all parameters (ALT, AST, ALP, total cholesterol, & triglyceride levels) when diazinon treated group is compared to control group.

In table (3) the results reveal that both acetylsalicylic acid and diazinon administered alone or together caused marked elevation in serum bilirubin level, this elevation of bilirubin is generally seen in drug induced liver disease or toxic liver injury (Ingram; 1993). According to Ebong et al (1998), aspirin increases total & conjugate bilirubin upon treatment of animals.

The present study found that Aspirin did not affect the lipid profiles, particularly cholesterol and triglycerides this is partially agreed with Ibrahim and El-Gamal( 2003). They reported a non significant change in cholesterol level of some experimental mammals. Diazinon alone cause a significant elevation in serum cholesterol level that may reflect limited effects on carbohydrates & lipid metabolism (Onaka, 1993). Also, these results suggest that diazinon may interfere with lipid metabolism in mammals (Ibrahim and El-Gamal, 2003), but when diazinon combined with acetylsalicylic acid caused marked elevation in serum triglycercide level, such elevation might relate to partial impairment of certain aspect of liver function (Zilva et al., 1988).

The results in table (5) showed that acetylsalicylic acid alone or combined with diazinon did not affect serum urea or creatinine level. Diazinon alone decreased serum urea level. (Morgan et al., 1977) studied the factors which lead to abnormal levels of the urea in the plasma and divided them into three main categories: prerenal, renal, postrenal, prerenal cause may be related to a degree of liver affection (Smith, 1985).

In conclusion, chronic aspirin administration in a dose that interferes with platelets aggregation only can elevate serum bilirubin level. On the other hand, exposure to diazinon showed different changes in biochemical parameters like elevated levels of ALT, AST, ALP, bilirubin, and total cholesterol and reduced levels of serum total proteins and urea. Finally, aspirin administration during exposure to diazinon can elevate serum transaminases, ALP, bilirubin, triglycerides.

The results obtained in the present work support the general opinion that aspirin (ASA), as any drug, should be used with caution especially with these exposed to diazinon and serum biochemical analysis should be carried out periodically for them.

**REFERENCES**


levamisole and diazinon in claves. J comp Pathol, 97: 619-627”.


