Ameliorative Roles of Silymarin and Nigella sativa on Hematological Parameters and Immunological Capacities of Male Mice Affected by Paracetamol

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DOI: http://dx.doi.org/10.13005/bbra/1676

(Received: 10 March 2015; accepted: 30 April 2015)

Paracetamol (APAP-Acetaminophen) has been used extensively as antipyretic drug. The purpose of this study was to evaluate the ameliorative role of silymarin or/and Nigella sativa extract against APAP-induced hematotoxicity in male mice at the hematological and immunological levels. The mice were divided into seven groups (10/group). The first group was served as control. While, the second group was treated with dose of APAP. The third and fourth groups were treated with silymarin alone and Nigella sativa extract alone respectively, the fifth and sixth groups were treated with combination of APAP with silymarin and APAP with Nigella sativa extract respectively. The seventh group was treated with a combination of both ameliorative compounds (Silymarin and Nigella sativa extract) with APAP and all animals were treated for a period of 30 days.

Exposure to APAP at the treated dose to mice led to an alteration of hematological and immunological parameters, increase W.B.Cs and TNF-α, decreased R.B.Cs and Hb levels in APAP treated group. Administration of silymarin or/and Nigella sativa extract to APAP-treated mice alleviate the hematotoxicity of APAP, and this appeared clearly by biochemical improvement of hematological picture. But, the alleviation is more pronounced with the both antioxidants. Thus, the pronounce effect of Silymarin and Nigella sativa extract is most effective in reducing the hematotoxicity and immunotoxicity induced by APAP and improving immunological capacities of male mice.

Key words: Paracetamol, Silymarin, Nigella sativa extract, Hematological parameters, Immunological parameters.

Paracetamol is an extensively used analgesic and antipyretic drug and, though safe when used at therapeutic doses, is associated with significant hepatotoxicity when taken in overdose (Ramsay et al., 1989).

Paracetamol, widely used as an analgesic and antipyretic, produces acute liver damage at higher dose. The hepatotoxicity of paracetamol has been attributed to the formation of n-acetylparbenzoquineimine (NAPQI) which causes oxidative stress and glutathione depletion (Shah and Deval, 2011). It is a well-known antipyretic and analgesic agent which produces hepatic necrosis at higher doses (Hurkadale et al., 2012). Nigella sativa(L.) is a member of family Ranunculaceae. It is an annual herbaceous plant growing in the Mediterranean countries (Saad, 1975). Nigella sativa has been used for medicinal purposes for centuries, both as an herb and pressed into oil, in Asia, Middle East, and Africa. It has been traditionally used for a variety of conditions and treatments related to respiratory
health, stomach and intestinal health, kidney and liver function, circulatory and immune system support, and for general well-being. In Islam, it is regarded as one of thegreatest forms of healing medicine available (Dwivedi, et al., 2007).

Studies in mice and rats have shown that treatment with *N. sativa* extract significantly protects from cisplatin-induced falls in leukocytes counts, hemoglobin level, mean osmotic fragility and hematocrit increase (El-Daly, 1998), influences leukocytes activities (Haq et al., 1995) and causes the death of mice lymphocytes in vitro (Salomi et al., 1992).

*Nigella sativa* has a great potential in the treatment of diabetic animal because of its combined hypoglycemic (Al-Hader et al., 1993) and immunopotentiating properties (Haq et al., 1995) it is cheap and readily available. The herbal medicine extracted from seeds of the Milk Thistle, *Silybum marianum* (Silymarin) is known to have antioxidant properties and research published in Phytotherapy research shows that this extract also can help people to lower the amount of sugar bound to haemoglobin in blood, as well as reducing fasting blood sugar levels. (Wiley and Sons, 2006).

Silymarin has been used for more than 2000 years as a natural remedy for treating hepatitis and cirrhosis and to protect liver from toxic substances. Silymarin acts by anti-oxidative, immunomodulatory and liver regenerat-ing mechanisms in experimental liver diseases (Postwhite, et al., 2007).

**MATERIAL AND METHODS**

**Chemicals**

APAP (APAP, N-acetyl-p-aminophenol) was purchased from the Egyptian International Pharmaceutical Industries Company (EIPICO); Silymarin was obtained from “Sedeco Pharmaceutical Co-6-October city. Egypt. The *Nigella Sativa* seeds were purchased from a local herb store with a fair degree of quality assurance. Seeds were washed to remove sand and other debris and air-dried and finely powdered with an electric microniser according to traditional mode of preparation (Schleicher and Saleh., 2000). Crude extract was obtained by the maceration of 800gm of these seeds by boiling in distilled water (1200ml) for 24h and filtered through muslin (El-Daly., 1998). After 24h, the aqueous extract was filtered, concentrated at room temperature (Benhaddou et al., 2008) then the dried extract was stored at 4°C until use. Other chemicals and reagents were of the highest analytical grade and were bought from standard commercial suppliers and were purchased from Roche (Germany).

**Animals**

SWR albino male mice weighing approximately 30-35g were obtained from Animal breeding house, Faculty of veterinary medicine, Zagazig University. The animals were maintained in solid bottom shoe box, type polycarbonate cages with stainless steel wire-bar lids, using a wooden dust-free litter as a bedding material. Animals were located in air-conditioned room and were allowed free access to pelleted diet and tap water for a week before starting the experiment. The European Community Directive (86/609/EEC) and National rules on animal care have been followed. After 2 weeks of acclimation, animals were randomly divided into seven groups with 10 animals in each one as following. Groups 1 was served as untreated control (1ml/kg of physiological saline), Group 2 was treated with paracetamol (2 g/Kg; Chen et al. 2009), Group 3 was treated with Silymarin (50 mg/Kg; Hale et al., 2007), Group 4 was treated with *Nigella sativa* extract (0.25 gm/100g; Schleicher and Saleh., 2000). Group 5 was treated with Paracetamol and Silymarin respectively, Group 6 was treated with Paracetamol and *Nigella sativa* and the final 7th group was treated with Paracetamol followed by silymarin and *Nigella sativa* respectively. All the groups were treated orally for 30 successive days.

**Collection of blood samples**

At the end of the experimental period, blood samples of the fasted mice were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, MuCaps) under ether anesthesia (Boussarie., 1999) Then, the blood was centrifuged at 3000 rpm for 15 min without anticoagulant for obtaining serum and blood with anticoagulants for haemocytometer counting.

**Hematological parameters**

**Total leucocytic counts (W.B.Cs Count)**

As whole blood was mixed with gentian violet in 2% acetic acid where R.B.Cs are destroyed,
while white blood corpuscles (W.B.Cs) were left intact with deep violet stained nuclei). A 50ml of collected blood was diluted 1:20 with Turk solution. After well mixing for one min, a drop of the mixture was examined under light microscope (x10) using an improved Neubauer haemocytometer (Dacie and Lewis, 1993).

Total Erythrocytes (R.B.Cs count)

Erythrocytes count (R.B.Cs) was determined using improved Neubauer hemocytometer. Hymes solution was used in red cell dilution (Dacie and Lewis, 1993).

Determination of Hemoglobin Content (Hb)

Blood hemoglobin concentration was determined colorimetrically as cyanomethaemoglobin by using the Diamond reagent kits according to Wintrobe et al., (1981).

Tumor necrosis factor-$$\alpha$$ (TNF-$$\alpha$$)

Tumor necrosis factor (TNF, formerly known as TNF-$$\alpha$$) is a potent mediator of immune and inflammatory responses. TNF is produced by many activated cell types including monocytes, macrophages, astrocytes, granulocytes, T and B lymphocytes, NK cells, keratinocytes, fibroblasts, and certain tumor cells (Ware et al., 1998).

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 17.0. Data was given in the form of arithmetical mean values ± standard error (S.E). Differences between groups were reevaluated by one-way ANOVA according to p < 0.05 and post-hoc Duncan test. For each histological parameter, a score has to be performed analyzing a number of different histological sections in each organ fore each animal and then the median value has to be calculated for each group; finally a comparison could be done by a semi-quantitative test using SPSS.

RESULTS

Hematological assessments

Effect of Acetaminophen (paracetamol), Silymarin, Nigella sativa extract and their combinations on Red blood corpuscles (R.B.Cs)

Table (1) and Fig. (1) demonstrate that the administration of Acetaminophen (paracetamol) in its recommended dose for 30 successive days afforded significant decreases ($P<0.05$) in R.B.Cs count when compared with the control group. Meanwhile, treatment of mice with Silymarin alone afforded a significant decrease in R.B.Cs count when compared with the control group. Meanwhile, Nigella sativa treated group elicited nonsignificant increase in R.B.Cs count when compared with the normal control group.

The combination of either Nigella sativa extract or Silymarin with the Acetaminophen (paracetamol) induced a significant decrease for R.B.Cs count. Whereas, the combination of Nigella sativa extract and silymarin with acetaminophen (paracetamol) elicited significant elevation in R.B.Cs count when compared to acetaminophen (paracetamol) treated group.

Effect of Acetaminophen (paracetamol), Silymarin, Nigella sativa extract and their combinations on White blood corpuscles (W.B.Cs)

Table (1) and Fig. (1) show that the administration of Acetaminophen (paracetamol) in its recommended dose for 30 successive days afforded significant decreases ($P<0.05$) in W.B.Cs count when compared with the control group. Meanwhile, treatment of mice with Silymarin alone afforded a significant decrease in W.B.Cs count when compared with the control group. Meanwhile, Nigella sativa treated group elicited nonsignificant increase in W.B.Cs count when compared with the normal control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>(R.B.Cs) ($\times 10^6$ Cell/mm$^3$)</th>
<th>(W.B.Cs) ($\times 10^6$ Cell/mm$^3$)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control group</td>
<td>5.30±0.33$^b$</td>
<td>7.72±0.75$^b$</td>
<td>13.79±0.71$^a$</td>
</tr>
<tr>
<td>2-Acetaminophen (Paracetamol)</td>
<td>3.08±0.27$^d$</td>
<td>10.82±0.19$^a$</td>
<td>9.03±0.51$^f$</td>
</tr>
<tr>
<td>3-Silymarin</td>
<td>4.75±0.24$^c$</td>
<td>6.52±1.44$^c$</td>
<td>12.70±1.01$^c$</td>
</tr>
<tr>
<td>4-Nigella sativa extract</td>
<td>5.50±0.43$^{ab}$</td>
<td>7.41±1.26$^b$</td>
<td>13.67±0.99$^b$</td>
</tr>
<tr>
<td>5-Acetaminophen + Silymarin</td>
<td>3.84±0.34$^c$</td>
<td>4.00±0.16$^e$</td>
<td>10.14±0.26$^e$</td>
</tr>
<tr>
<td>6-Acetaminophen + Nigella sativa extract</td>
<td>3.97±0.11$^{ef}$</td>
<td>4.82±0.19$^{ab}$</td>
<td>10.76±0.36$^c$</td>
</tr>
<tr>
<td>7-Acetaminophen + Silymarin + Nigella sativa extract</td>
<td>4.15±0.24$^{d}$</td>
<td>5.80±0.47$^a$</td>
<td>11.76±0.46$^e$</td>
</tr>
</tbody>
</table>

thin the same column in each category carrying different letters are significant at ($P \leq 0.05$) using Duncan’s smulte-range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically
afforded significant increases (P< 0.05) in white blood corpuscles (W.B.Cs) when compared with the control group. The administration of the *Nigella sativa* extract revealed non significant decrease in (W.B.Cs) count after four weeks post treatment compared to the control group. However, Silymarin treated group afforded slight significant decrease in (W.B.Cs) count when compared with the normal control group.

However, the group treated with combinations of silymarin and *Nigella sativa* with acetaminophen afforded a significant increase in (W.B.Cs) count when compared with acetaminophen with either *Nigella sativa* or silymarin and thus recorded the best amelioration value in (W.B.Cs) count.

**Effect of Acetaminophen (paracetamol), Silymarin, *Nigella sativa* extract and their combinations on Hemoglobin level (Hb%)**

Table (1) and Fig. (2) demonstrate that the administration of Acetaminophen (paracetamol) in it’s recommended dose for 30 successive days afforded significant decreases (P< 0.05) in Hb level when compared with control group.

Meanwhile, treatment of mice with Silymarin alone afforded a significant decrease in Hb level when compared with control group. Meanwhile, *Nigella sativa* treated group elicited non significant decrease in Hb level when compared with normal control group. The combination of either *Nigella sativa* extract or Silymarin with the Acetaminophen (paracetamol) induced a significant decrease in Hb level. Whereas, the combination of *Nigella sativa* extract and silymarin with acetaminophen (paracetamol) elicited significant elevation in Hb% when compared to acetaminophen (paracetamol) treated group.

**Table 2.** Effect of Acetaminophen (Paracetamol drug) (1000 mg/kg), Silymarin (50 mg/ Kg), *Nigella sativa* extract (0.25g/100g) and their combinations on TNF-α in male mice (mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (Pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>8.51±0.31</td>
</tr>
<tr>
<td>Acetaminophen (Paracetamol)</td>
<td>21.52±0.78</td>
</tr>
<tr>
<td>Silymarin</td>
<td>6.07±0.72</td>
</tr>
<tr>
<td><em>Nigella sativa</em> extract</td>
<td>5.79±0.41</td>
</tr>
<tr>
<td>Acetaminophen + Silymarin</td>
<td>14.09±0.40</td>
</tr>
<tr>
<td>Acetaminophen + <em>Nigella sativa</em></td>
<td>16.31±0.10</td>
</tr>
<tr>
<td>Silymarin extract</td>
<td>12.99±0.09</td>
</tr>
</tbody>
</table>

Means within the same column in each category carrying different litters are significant at (P d" 0.05) using Duncan’s multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.
Tumor necrosis factor-α
Effect of Acetaminophen (paracetamol), Silymarin, *Nigella sativa* extract and their combinations on TNF-α

Table (2) and Fig. (3) showed that the administration of Acetaminophen (paracetamol) elicited a significant increase in serum Tumor necrosis factor α (TNF-α) whereas, combinations of Acetaminophen (paracetamol) with Silymarin or *Nigella sativa* extract elicited a slight significant increase in serum Tumor necrosis factor α (TNF-α). However, Silymarin elicited a nonsignificant decrease in TNF-α level, while *Nigella sativa* extract afforded significant decrease when compared with either control group or group given acetaminophen (paracetamol) alone.

DISCUSSION

The major aim of this work was to evaluate the potential benefit of silyamrin and *Nigella sativa* extract administration on APAP tissue injury, compare to silymarin or *Nigella sativa* extract treatment alone. To our knowledge, no study has been conducted on the co-effect of Silymain and *Nigella sativa* extract on APAP toxicity at the biochemical level related to Hematological picture as well as immunological capacities in male mice. This study investigated the ability of silyamrin and *Nigella sativa* extract to alleviate the APAP-induced hematotoxicity and immunotoxicity in male mice. Exposure to APAP at the recommended dosage mice led to an alteration of hematological and immunological parameters decrease R.B.Cs and Hb levels, increased W.B.Cs and TNF-α levels.

Effect on blood picture parameters

The obtained decrease in RBCs count in Acetaminophen (Paracetamol)treated group could be possibly attributed to degenerative changes induced in haemopoietic system. Our results were supported with that reported by Abbas, (2009). She reported degenerative changes in the liver represented by swollen hepatic cells with cytoplasmasis of their cytoplasm (severe hydropic degeneration) beside hyperplastic kuffer’s cells. Some portal areas revealed leucocytic aggregations mainly lymphocytes beside hyalinized wall of the hepatic arterioles and proliferation of bile duct epithelium. In addition to a possible depressing of bone marrow and other haemopoietic organs.

In accordance with our results (Oyedeji *et al.*, 2013) showed that the haematological study has shown that treatment of rats with paracetamol caused significant decrease in RBC count which could indicate that there were destruction of matured RBC and reduction in the rate of erythropoiesis. This could also imply that paracetamol has the potential to inhibit erythropoietin release from the kidneys. Also the indicated that Paracetamol caused significant decrease in haemoglobin (Hb) concentration which suggest a reduction in the oxygen-carrying capacity of blood and the amount of oxygen-carrying capacity of blood and the amount of oxygen delivered to the tissues.

The non-significant decreased WBCs count induced by *Nigella sativa* in normal rats agree with Diab *et al.*, (2010). They reported that total WBCs in rats treated with *N. sativa* increased due to impairment of the immune system in diabetic rats. This discrepancy might be attributed to differences in doses, route and duration of drug administration.

A result which disagree with our obtained results were obtained by (Oyedeji *et al.*, 2013) as they reported that Paracetamol caused non-significant changes in total WBC and lymphocyte counts, which suggest that the immune system have not been compromised.

Acetaminophen (Paracetamol) elicited a significant decrease in WBCs count along the entire course of the experiment when compared with control group. This might be due to inflammatory changes induced in the liver induced in Acetaminophen treated mice.

In accordance with our results, (Yousef *et al.*, 2010) showed that Paracetamol also caused an increase in total leukocytic count (TLC), while induced a decrease in total erythrocytic count (TEC), haemoglobin (Hb) and packed cell volume (PVC). These findings also were in agreement with results obtained by Wershana *et al.* (2001) and Bhaumik and Sharma (2002), who attributed the reduction in total erythrocytic count to the inability of the damaged hepatic parenchyma to produce erythropoietinogen.

Additionally, the disintegration of erythrocytes in the circulation might have resulted in the reduction of hemoglobin content, which was
in turn associated with decreased PVC. Meanwhile, the increase in total leukocytic count value might be due to stress coupled with inflammatory changes in body tissue responsible for phagocytosis of toxic substances (Bhaumik and Sharma, 2002).

**Effect on Immunological parameters (TNF-α)**

Paracetamol overdose is known to be associated with inflammation, marked by an increase in the inflammatory cytokines; tumor necrosis-a (TNF-a) which is greatly in accordance with our results by showing increment in TNF-α in paracetamol treated group, interleukin-1α and interleukin-1β (James et al., 2003). Such cytokines produced during inflammation shunt amino acids to increase the synthesis of proteins important to the inflammatory process, thus decreasing albumin synthesis as it is not essential to inflammation (Rothschild et al., 1972).

In accordance with our results, Ahmad et al., 2013 showed that expression of TNF-α was markedly reduced in Silymarin treated group as compared with the normal control group and this is greatly in agreement with our finding as TNF-α was reduced in Silymarin treated group and Paracetamol + Silymarin treated group which indicate the ameliorative role of Silymarin in alleviating the toxicity induced by paracetamol.

In agreement with our results Dietzmann et al. (2002) reported a significant thiol deficiency in patients with end stage diabetic nephropathy that correlated directly to a significant diminished T-cell activation and an elevated synthesis of TNF-α which facilitates the production of ROS by neutrophils (Beutler et al. 1985) and they reported that Silymarin treatment could reverse these effects.

**REFERENCES**

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