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# COMBINED EFFECTS OF RIBAVIRIN AND DIAZINON ON HEPATIC, PANCREATIC AND KIDNEY BIOMARKERS IN FEMALE ALBINO RATS

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## ABSTRACT

The interaction between chemicals such as drugs and pesticides within ecological system is a complex one as it occurs among environment, host and chemicals. The present study was conducted to evaluate the combined effects of ribavirin at 30 mg/kg bw and 1/10$LD_{50}$ of diazinon, at 30mg/kg bw, on liver, pancreas and kidney functions of female albino rats. Twenty four female albino rats were equally divided into four groups and were orally given 0.5 ml corn oil (served as a control), ribavirin, diazinon and ribavirin plus diazinon for 5 consecutive days/week up to 4 weeks. The results revealed that ribavirin alone produced no significant differences in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total, direct and indirect bilirubins, albumin (Alb.), A/G ratio, α-feto protein (AFP), amylase, lipase, insulin, urea, calcium (Ca) and inorganic phosphorus (P) compared to the control group. However, there was significant increase in alkaline phosphatase (ALP), uric acid, creatinine, potassium (K) and sodium (Na), whereas, final body weights, total proteins (TP) and globulins (Glob.) were significantly decreased compared to the control groups. The results also showed that treatment with diazinon induced significant increases in the activities of ALP, AST, ALT, levels of total, direct and indirect bilirubins, AFP, amylase, urea, uric acid, creatinine, K and Na. However, significant decrease in final body weights, TPs, Alb, and Globs were observed compared to the control groups. In conclusion, ribavirin had a efficacy in ameliorating the liver impairment and pancreatic inflammation induced by diazinon, on the contrary this antiviral drug caused additional burden on the kidney leading to more deterioration in the renal function.

**Key words:** Diazinon, Ribavirin, Biochemical parameters, Female rats.

## INTRODUCTION

Humans exposure to various chemicals such as drugs, pollutants, food additives is continuously increasing. In Egypt, Hepatitis C virus is the most widespread disease in both rural and urban areas (Lavancy, 2011). The Egyptian Ministry of Health has recently recommended effective treatment of the triple course of ribavirin, interferon and sovaldi up to 3 or 6 months in order to overcome hepatitis C. Ribavirin is a nucleoside analog used as monotherapy for respiratory syncytial virus (Hall *et al*., 1983), Lassa fever virus (Robert *et al*., 2000) and foot and mouth disease virus (Saad and Fawzy 2004). Tam *et al.* (1999) stated that ribavirin has multiple biologic properties that are favorable for treating viral diseases. It can directly inhibit the replication of many DNA and RNA viruses. It can also act as an immunomodulator and thus promots T- cell mediated immunity against viral infection (Ning *et al.*, 1998; Tam *et al*., 1999). In most studies, ribavirin as monotherapy, improve liver enzymes levels without significant effects on HCV viraemia (Hoofnagle *et al.* 2003). However, pancreatitis has been reported with the combination of ribavirin and interferon alfa (da Silva *et al*., 2009).

The use of pesticides to control pests and increase food production is a common practice that adversely affects the environment and poses a great danger to many non-target species including Humans. Diazinon is one of the most selective organophosphorus compounds used indoors to control insects and outdoors to control the pests of vegetables, field crops, fruits, domestic and farm animals. It may contaminate soil and ground water. The half- life of diazinon persistence in soil is 204 weeks and its breakdown in water depends on the water acidity. In neutral water, the half-life can reach 6 months (Kamrin 1997). Toxicity of diazinon is due to the inhibition of acetyl-cholinesterase activity (Kamanyire and Karalliedde 2004), and it may induce oxidative stress which leads to imbalance in the free radicals production/elimination processes with consequent induction of cellular damage (Cakici and Akat, 2013). Liver and kidney are the most sensitive organs to organophosphorus compounds because of their roles in biotransformation and elimination (Kalender *et al.*, 2004). The mild structural and functional changes in the liver as well as in the kidney were observed in mice after 14 days of intra-peritoneal administration of 1/4 and 1/2 $LD_{50}$ diazinon (El-Shenawy *et al*., 2009). Also, El-Demerdash and Nasr (2014) showed a significant increase of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Little is known about the combined effects of ribavirin and diazinon. The objective of the present study is to measure risk of combined effects of ribavirin and diazinon on liver, pancreas and kidney functions. The objective was fulfilled using female albino rats as an experimental model.

## MATERIALS AND METHODS

**Chemical:** Ribavirin (L - B - D ribofuranosyl - 1H - 1, 2, 4 - Triazole - 3 carboxamide) is a synthetic nucleoside with antiviral activity, manufactured by OctoberPharma S.A.E. 6- October City Egypt. The drug was daily prepared by dissolving one tablet (400mg) in 50 ml saline and orally administrated to each rat at 30 mg/Kg bw /day for 5 consecutive days / week up to 4 weeks.

Diazinon was manufactured by Nippo Kayaku Company, Ltd, Japan and kindly provided by Cairo Liaison office, Sumitomo Corporation (Sumitomo Shaji Kaisha, Ltd) in technical grade of 93%. It was diluted in corn oil to a concentration of 30 mg/ Kg bw /day (1/10 $LD_{50}$) and orally given for 5 consecutive days / week up to 4 weeks.

***Animals*:** Twenty four female albino rats (4 weeks old), weighting about 98-107 gm were supplied from the animal house of Biological Applications Department, Nuclear Research Center, Atomic Energy Authority at Inshas – Egypt. All animals were housed in stainless steel cages with wire mesh lid and allowed balanced standard rodent diet and water *ad libitum* for one week for acclimation. Rats were exposed to 12h light:12h dark cycle at a room temperature of 18-22°C. They were randomly and equally divided into four groups. Rats were weighted daily to adjust the dose given. The mean weight of animals for each cage was calculated weekly. Group 1 (G1): Served as control, female rats were given orally 0.5 ml/ day corn oil. Group 2 (G2): The rats were orally given 30 mg/kg bw/day ribavirin according to Motor *et al.* (2014). Group 3 (G3): Rats were orally administrated $1/10LD_{50}$ of diazinon, using oral tube according to Enan *et al.* (1982). Group 4 (G4): Rats were orally given ribavirin plus diazinon at the same concentration as described above.

**Samples:** Four weeks later, all animals were fasted overnight before decapitation. Blood samples were collected and centrifuged at 3000 r.p.m. for 15 min and serum samples were separated and kept frozen at -20ºC till biochemical analyses. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total and direct bilirubin, total protein (TP), albumin (Alb),globulin (Glob) urea, uric acid, creatinene, calcium (Ca), inorganic phosphorus (P), potassium (K), and sodium (Na) were estimated calorimetrically using spectrophotometer (Milton Roy Spectronic 1201) and commercial kits purchased from Biodiagnostic reagent kits, Dokki, Giza, Egypt. Indirect bilirubin concentration was calculated by subtracting the values of direct from their corresponding value of total bilirubin. Globulin concentration was calculated by subtracting the values of albumin from their corresponding value of total proteins. The A/G ratio was calculated by dividing each albumin value by its corresponding globulin value. Quantitative estimation of α-feto protein (AFP) was based on solid phase enzyme linked immune sorbent assay (ELISA) using the enzyme immune assay kit (USA) (Sell and Beckar, 1978). Lipase and amylase enzymes were photometrically measured using an Abbott ARCHITECT c16000 device. Serum level of Insulin was determined by radioimmunoassay method using special rat kits (Specteria, Finland). All animal procedure were carried out in accordance with the Ethics Committee of the Nuclear Research Center conformed to the "Guide for the care and use of laboratory animals" published by the US National Institutes of Health (NIH publication No 85-23, 1996).

**Statistical Analysis:** The data obtained were presented as means ± SD. One-way analysis of variance (ANOVA) was carried out. The statistical comparisons among the groups were performed with Duncan's test. Simple regression analyses were performed between the body weight and each of biochemical markers. The statistical significance of the correlations was confirmed by "t" test. P values less than 0.05 were considered significant. The statistical analyses were performed using a statistical package program (COSTAT).

## RESULTS

The effect of ribavirin and/or diazinon on body weights during 4 weeks are represented in figure 1. All the treatments showed significant decrease in body weights throughout the study period compared to the control group.

Table 1 shows the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the concentration of total, direct and indirect bilirubins for all the tested groups. All treatments significantly increased the activity of serum ALP than the control group. Moreover, each treatment significantly differed from the other. Alkaline phosphatase showed the highest activity after treatment with diazinon plus ribavirin. Table1 also shows significant increase in, AST, ALT, total, direct and indirect bilirubins in G3 (diazinon group), compared to the control group. (G1). In G4, treatment with diazinon plus ribavirin significantly decreased the increased levels of AST, ALT, total and direct bilirubins to the control levels compared to G3. However, the decrease of indirect bilirubin in G4 was still significantly higher than the control group.

Table 2 illustrates the levels of TPs, Alb, Globs, A/G ratio and AFP for all the tested groups. Compared to the control groups, ribavirin significantly decreased the levels of TPs and Globs. Also diazinon alone or with ribavirin significantly decreased TP, Alb., and Glob. but, significantly increased α-fetoproteinin, comrepared to each of control(G1) and ribavirin(G2) groups however, Globs. level in G3 did not significantly differ from Glob. in G2.

Table 3 shows concentrations of amylase, lipase and insulin for all the tested groups. The results of the present study revealed significant increase ($P < 0.01$) of amylase in diazinon administered animals (G3), compared to the other groups. The increased activity of amylase in diazinon administered animals showed normal activity in animals given ribavirin plus diazinon (G4). There were non-significant changes in lipase and insulin in all the tested groups compared to each other including the control group.

Table 4 shows the levels of urea, uric acid and creatinine for all the tested groups. There was significant increase ($P < 0.01$) in uric acid and creatinine in all tested groups compared to the control groups. Moreover, diazinon + ribavirin administered animals (G4) significantly increased uric acid than both of G2 and G3. Level of urea was significant increased in diazinon and ribavirin plus diazinon groups compared to the control group and to each other.

Table 5 shows levels of serum Ca, P, K and Na for all the tested groups. There were non-significant changes in Ca and P but significant increase ($P < 0.01$) in K and Na in all tested groups compared to the control groups.

In table 6 the correlation coefficient values between the body weight and the biochemical markers are illustrated. The table revealed negative significant correlations between the body weight and each of ALP ($P<0.01$), AST ($P<0.05$), AFP ($P<0.05$), urea ($P<0.05$), uric acid ($P<0.01$), creatinine ($P<0.01$), (K) ($P<0.01$) and Na ($P<0.01$ ). Whereas the positive correlations between the body weight and TP.,Alb. and Glob were $P<0.01$ for each. The statistical significancy of the different correlations "r" were confirmed with "t" test

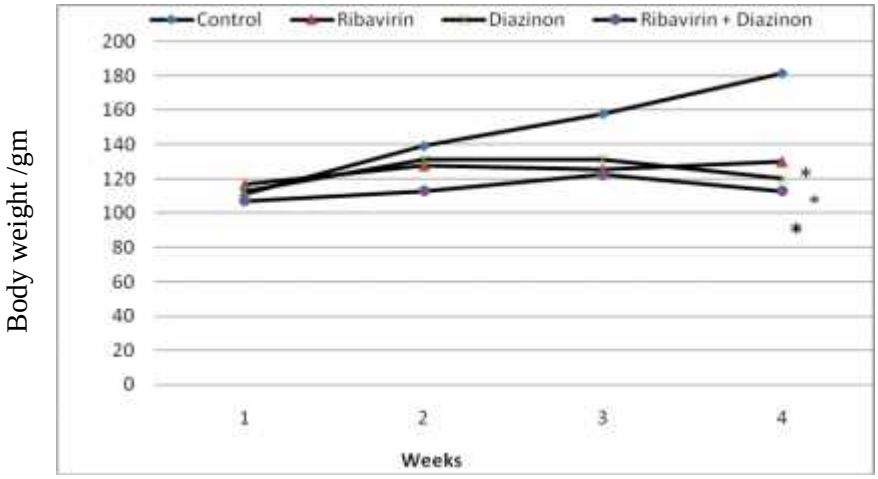


**Figure 1. Effects of ribavirin and diazinon treatments in single or combination on the means of body weights throughout 4 weeks in female albino rats.* indicate significant difference at P< 0.05 compared to control group.**

**Table 1. Effects of ribavirin and diazinon treatments in single or combination on liver function tests in female albino rats.**

| Groups<br>Parameters | G1<br>(Control) | G2<br>(Ribavirin) | G3<br>(Diazinon) | G4<br>(Diazinon+ Ribavirin) | P Value |
|---|---|---|---|---|---|
| **ALP U/l** | 106.31 ± 6.3$^{d}$ | 128.70± 13.3$^{c}$ | 144.75± 8.5.$^{b}$ | 209.22±15.2$^{a}$ | < 0.05 |
| **AST U/l** | 126.62± 4.7$^{b}$ | 139.33± 5.4$^{b}$ | 170.02± 19.7$^{a}$ | 129.34±12.3$^{b}$ | < 0.01 |
| **ALT U/l** | 49.10± 3.6 $^{b}$ | 47.01± 1.8$^{b}$ | 61.21± 5.4 $^{a}$ | 50.11± 2.4 $^{b}$ | < 0.01 |
| **Total billirubin (mg/dl)** | 0.05 ± 0.01$^{b}$ | 0.08± 0.01$^{b}$ | 0.22± 0.05$^{a}$ | 0.09±0.04$^{b}$ | < 0.01 |
| **Direct billirubin (mg/dl)** | 0.02 ± 0.01 $^{b}$ | 0.027± 0.02$^{b}$ | 0.063± 0.03$^{a}$ | 0.017±0.01$^{b}$ | < 0.05 |
| **Indirect billirubin (mg/dl)** | 0.03 ± 0.01 $^{c}$ | 0.05± 0.01$^{bc}$ | 0.16± 0.01$^{a}$ | 0.06±0.02 $^{b}$ | < 0.01 |

Values are shown as means ± SD of n = 6.

Values with small different letters in rows shown that are significantly different and similar letters are not significantly different ($p<0.05$) in various groups.

**Table 2. Effects of ribavirin and diazinon treatments in single or combination on total proteins, albumin, globulin, A/G ratio and α-fetoprotein in female albino rats.**

| Groups Parameters | G1 (Control) | G2 (Ribavirin) | G3 (Diazinon) | G4 (Diazinon + Ribavirin) | P Value |
|---|---|---|---|---|---|
| **TP g/dl** | 8.43 ±0.5$^{a}$ | 7.82± 0.1$^{b}$ | 7.11± 0.1$^{c}$ | 6.90±0.2$^{c}$ | < 0.01 |
| **Alb. g/dl** | 4.40± 0.1$^{a}$ | 4.11± 0.3$^{a}$ | 4.02 ± 0.2$^{b}$ | 3.90± 0.2$^{b}$ | < 0.01 |
| **Glob. g/dl** | 4.01 ±0.4$^{a}$ | 3.60± 0.4$^{b}$ | 3.21± 0.1 $^{bc}$ | 2.87±0.14 $^{c}$ | < 0.01 |
| **A/G ratio** | 1.03 ± 0.2$^{b}$ | 1.10±0.3$^{b}$ | 1.21± 0.2$^{b}$ | 1.53±0.05$^{a}$ | < 0.05 |
| **AFP IU/ml** | 2.46 ± 0.09$^{b`}$ | 2.53± 0.05$^{b}$ | 3.23± 0.6$^{a}$ | 3.81±0.8$^{a}$ | < 0.01 |

Values are shown as means ± SD of n=6.
Values with small different letters in rows shown that are significantly different and similar letters are not significantly different ($p<0.05$) in various groups.

**Table (3). Effects of ribavirin and ribavirin diazinon treatments in single or combination on pancreatic function in female albino rats.**

| Groups Parameters | G1 (Control) | G2 (Ribavirin) | G3 (Diazinon) | G4 (Diazinon + Ribavirin) | P Value |
|---|---|---|---|---|---|
| **Amylase (U/l)** | 366.8 ± 39.5$^{b}$ | 400.9± 52.8$^{b}$ | 450.9± 44.7$^{a}$ | 359.5±19.6 $^{b}$ | < 0.01 |
| **Lipase(U/l)** | 114.1± 17.1$^{a}$ | 123.7 ± 7.8$^{a}$ | 120.1 ± 14.8$^{a}$ | 121.5± 8.2$^{a}$ | 0.475 |
| **Insulin (µU/ml)** | 6.74± 0.92 $^{a}$ | 7.26± 0.67$^{a}$ | 7.12± 1.07$^{a}$ | 6.34±1.13$^{a}$ | 0.469 |

Values are shown as means ± SD of n=6.
Values with small different letters in rows shown that are significantly different and similar letters are not significantly different ($p<0.05$) in various groups.

**Table 4. Effects of ribavirin and diazinon treatments in single or combination on kidney function tests in female albino rats.**

| Groups Parameters | G1 (Control) | G2 (Ribavirin) | G3 (Diazinon) | G4 (Diazinon +Ribavirin) | P Value |
|---|---|---|---|---|---|
| **Urea (mg/dl)** | 34.43 ± 1.8$^{c}$ | 33.11± 5.2$^{c}$ | 49.70± 3.6$^{a}$ | 41.13±4.3$^{b}$ | < 0.01 |
| **Uric acid (mg/dl)** | 3.71± 0.3$^{c}$ | 5.56 ± 0.6$^{b}$ | 5.80± 0.7$^{b}$ | 6.91 ± 0.1$^{a}$ | < 0.01 |
| **Creatinine(mg/dl)** | 0.32± 0.03$^{b}$ | 0.58± 0.03$^{a}$ | 0.53±0.06$^{a}$ | 0.61± 0.09$^{a}$ | < 0.01 |

Values are shown as means ± SD of n=6.
Values with small different letters in rows shown that are significantly different and similar letters are not significantly different ($p<0.05$) in various groups.

**Table 5. Effects of ribavirin and diazinon treatments in single or combination on electrolytes in female albino rats.**

| Groups Parameters | G1 (Control) | G2 (Ribavirin) | G3 (Diazinon) | G4 (Diazinon +Ribavirin) | P Value |
|---|---|---|---|---|---|
| **Ca mg/dl** | 12.38 ±1.3$^{a}$ | 11.71± 1.5$^{a}$ | 12.40± 0.67$^{a}$ | 12.62±0.61$^{a}$ | 0.649 |
| **P mg/dl** | 6.41± 0.9$^{a}$ | 6.24± 0.7$^{a}$ | 7.30 ± 0.2$^{a}$ | 7.11± 0.8$^{a}$ | 0.248 |
| **K mmol/l** | 6.64 ±0.7$^{b}$ | 15.52±1.1$^{a}$ | 13.95± 1.2$^{a}$ | 14.35±1.0 $^{a}$ | < 0.01 |
| **Na mmol/l** | 154.20 ±4.8$^{b}$ | 347.53±25.5$^{a}$ | 354.81±27.7$^{a}$ | 358.01±20.7$^{a}$ | < 0.01 |

Values are shown as means ± SD of n=6.
Values with small different letters in rows shown that are significantly different and similar letters are not significantly different ($p<0.05$) in various groups.

**Table 6. Correlation coefficient between body weights and biochemical markers.**

| | | | **Livers biomarkers** | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | ALP | AST | ALT | Total Billirubin | Direct Billirubin | | Indirect Billirubin |
| Body weight | -0.5568** | -0.42075* | -0.27025 | -0.37531 | -0.30683 | | -0.34473 |
| | | | **Total protein, Albumin, Globulin,A/G ratio and AFP** | | | | |
| Parameters | TP | ALB. | Glob. | A/G ratio | AFP | | |
| Body weight | 0.6555** | 0.542894** | 0.57631** | -0.3778 | -0.4056* | | |
| | | | **Pancreatic biomarkers** | | | | |
| Parameters | Amylase | Lipase | Insulin | | | | |
| Body weight | -0.06735 | 0.182155 | 0.1375 | | | | |
| | | | **Kidneys biomarkers** | | | | |
| parameters | Urea | Uric acid | Creatinine | Ca | P | Na | K |
| Body weight | -0.46533* | -0.6505** | -0.67605** | -0.08838 | -0.33859 | -0.74049** | -0.58039** |

*P<0.05 ,** P<0.01

## DISCUSSION

In toxicological studies, body, organ and relative organ weights are important criteria for evaluation of toxicity (Heikal *et al*., 2011). In the present study, the animals administrated ribavirin and diazinon in single or combination showed that the body weights were significantly lower than the control animals, which might be attributed to decreased appetite resulted in decreased food and water intake. Moreover, the treatments could affect lipid and protein degradation. Narayana *et al*. (2005) linked the decrease in body and organ weights, excluding testis and epididymis, of rats orally given 200 mg/kg/day ribavirin for 14 days with decreased food and water in-take. Garcia-benayas *et al.* (2002) and Perez-Olmeda *et al*. (2003) suggested that ribavirin might potentiate mitochondrial damage in subcutaneous adipose tissue, leading to lipoatrophy and weight loss. Alam *et al*.(2013) attributed the weight loss or changes in other body composition such as skeletal muscle mass, body fat mass, waist-hip ratio, body cell mass and total body water in HCV patients under pegIFN plus ribavirin treatment to decrease in serum leptin levels. Putz-Bankuti *et al.* (2010) revealed a significant reduction of leptin levels and an increase of the adiponectin to leptin ratio during antiviral treatment with pegIFN plus ribavirin.

In the current study, reduced body weight of animals administrated diazinon could be linked with metabolic disturbances due to cytotoxic effect of diazinon and loss of appetite. Mossa *et al*. (2011) proved that the mixture of four organophosphorus insecticides (chlorpyrifose, profenose, diazinon and malathion) reduce the body weight gains in rats as well as diazinon in the study of Sayed (2007) and Shah, (2011). Stromborg (1981) found that dietary level of diazinon above 50 mg/kg is associated with reduced food consumption, weight loss, in northern bobwhites. Low body weight gain is indicative to loss of appetite, decrease food intake, metabolic and hormonal disturbance (Abd El-Ghaney, 2002; Al-Shinnawy 2008). The reduction in body weight attributed also to the combined action of cholinergic and oxidative stress (Saafi *et al*., 2011) and due to the overall increased degradation of lipids and proteins as a result of the direct effects of diazinon (Goel *et al*. 2005). The pronounced depression of the body weight gain in the group treated with ribavirin plus diazinon might prove an additive catabolic effect of each other.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities and bilirubin level are largely used as common biochemical markers to evaluate liver injury (Casillas *et al.,* 1983). In the present study, ribavirin did not affect the activities of AST and ALT in addition to the levels of total, direct and indirect bilirubin that might reflect that ribavirin did not harm the hepatic cells. Johnson *et al*., (2002) stated that ribavirin is a guanin analogue that is phosphorylated into its most active form, ribavirin- triphosphate. This compound competes with adenosine triphosphate and guanine triphosphate for the binding sites at the polymerase resulted in inhibiting transferase enzymes. Therefore, in patients receiving ribavirin monotheraby, serum ALT is reduced in a considerable proportion. Motor *et al. (*2014) found non-significant changes in ALT and AST in rats administrated 30 mg/kg/day ribavirin for 30 days. Fang *et al*. (2003) reported that 8-day dosing of ribavirin (120 mg/kg/day), levovirin (2000 mg/kg/day) and viramidine (120 mg/kg/day) do not induce any of the Cytochromes P450 system (CYPs) (including CYP1A, 2B, 3A and 4A) at the protein level and had no remarkable effect on the mRNA expression of hepatic toxicology genes in rats. However, administration of ribavirin in female rats in the present study (G2) showed significant increase in the activity of serum ALP which might be due to the inflammatory tissue response of the host. This results are consistent with a study of Ibrahim *et al*.(2005) who

showed that ALP significantly increased in ribavirin treated rats.

The obtained data recorded significant increases in the activities of ALP, AST and ALT and the levels of total, direct and indirect bilirubin in serum of the rats treated with diazinon. The increase in the activities of these enzymes might be due to damage of the liver cells and increased permeability of plasma membrane. Similar results were reported by Gokcimen *et al*. (2007); Al-Attar and. Abu Zeid, (2013); El-Demerdash and Nasr (2014). The authors reported that insecticides cause increase in serum AST and ALT in different species of animals. In support with the present results, the author attributed the rise serum AST, ALT, total and direct bilirubins in mice treated with 1/50 and 1/20 $LD_{50}$ cyolane (organophosphorus insecticide) to liver damage and impaired liver function (Hassanin, 2004). Irshadahmad and Gautam (2014) reported significant increase in serum billirubin level in the freshwater teleost fish exposed to various concentrations of Nuvan (organohposphorus pesticides) for 7,15, 30 and 60 days.

The current study demonstrated improved liver function in the animals administrated ribavirin + diazinon, compared to the diazinon group. These could be attributed to the competition of phosphorylated active form ribavirin- triphosphate for the binding sites at the polymerase resulted in inhibiting the transferase activities. Abdel Salam *et al*. (2007) found that ribavirin treatment in the model of CCl4-induced liver injury results in improved liver damage.

Levels of the total proteins, albumin and globulins are used to monitor the course of diseases in immune disorders, liver dysfunction and impaired kidney activity (Banaee *et al*., 2011). In fact, the changes of TPs were due mostly to change in Alb. and Globs. The decrease in serum total proteins and globulins in group 2 might be postulated to the effect of ribavirin on the kidney that might increase passage of globulins across the glomerulus into renal tubules. Pfeifer and Weber (1979) attributed the depressed serum proteins to kidney dysfunction and passage proteins into urine. Since albumin is synthesized in the liver the non significant change of albumin after ribavirin intake might confirm intact liver function.

Diazinon might affect the synthesis of albumin and globulins in liver, plasma and endothelial cells. Moreover, diazinon might also dysregulate the passage of the plasma proteins across the glommerulus into the renal tubule resulted in loss of proteins via urine. These results are in accordance to those of Al-Attar and. Abu Zeid, (2013); El-Demerdash and Nasr (2014). Sary *et al*. (2003) reported that significant decreases of total proteins and globulins were observed in rats exposed to different concentrations of two organophosphorus pesticides for one month. Devi (1981) recorded reduction in total proteins due to the effect of endosulfan (organochlorine insecticide) on the nucleic acids . He attributed decreased proteins to the requirement for high energy which obtained from stimulation of protein catabolism in the tissues. Singh *et al*. (1996) attributed the reduction in protein contents of the exposed animals to an organochlorine insecticide to either inhibition of RNA synthesis at the transcriptional level or to impaired incorporation of amino acids into polypeptide chains.

As mentioned by Garg *et al*. (2004) the observed decrease in serum globulins is attributed to reduction in synthesis of globulins by the plasma cells. The combined effect of ribavirin plus diazinon in the present study showed non significant decreases in total proteins, albumin and globulins than diazinon group which might reflect no additive effect of ribavirin to diazinon. The significant increase of A/G ratio in G4 might be explained as the significant decrease of globs. in animals under stress of both ribavirin and diazinon (G4) was more than the decreased Globs, in the animals under stress of only ribavirin(G2).

Abnormal serum level of AFP has been reported in patients with liver cirrhosis and hepatocellular carcinoma (Gupta *et al*., 2003). In this study the significant increase in AFP was observed after diazinon and ribavirin+ diazinon treatments that might be referred to role of diazinon on liver injury. However, ribavirin failed to modulate the deleterious effect of diazinon on the liver. In the present study, ribavirin did not affect the pancreatic functions. These results are in agreement with Motor *et al*. (2014) who found that ribavirin does not cause any pancreatic dysfunction or hepatotoxicity. On the contrary the present study revealed significant increase in amylase in the diazinon administered animals, that might pointed to the organophosphate intoxication which either cause excessive cholinergic stimulation of the pancreas or decreased the metabolic clearance of amylase. These results are in agreement with Gokcimen *et al*. (2007) who stated that 24 hours after rats administrated different doses of diazinon the amylase enzyme was significantly increased compared to the control group. Kamath *et al*. (2008) reported that rats administrated daily dose of dimethoate at 40 mg/kg/d b.w. for 2 months increased amylase. Alp *et al*. (2011) showed that female rats given malathion (200 mg/kg) reflected significant increase in amylase activity, after 24 hours later. The present study showed that ribavirin ameliorated the harmful effect of diazinon on amylase enzyme. Huang *et al*. (2011) stated that interferon / ribavirin therapy ameliorated pancreatic β-cell function in chronic hepatitis -C patients.

In the present study, the significant increases in serum uric acid, creatinine, K and Na in response to each of ribavirin, diazinon and ribavirin plus diazinon treatments and the significant increase in serum urea in response to diazinon and ribavirin plus diazinon reflected the deleterious effect of these two components on the

kidney. The studies of El-Shenawy *et al.* (2009); AbdElmonem (2014); El-Demerdash and Nasr (2014) are in similar with the present findings. AbdElmonem (2014) reported significant increase in urea and uric acid of rats administrated 1/10 LD50 diazinon for ten days.

Sodium and potassium are essential mineral micronutrients. Sodium is the most abundant electrolyte in the extracellular tonicity (Rose and Port, 2001) whereas potassium is the main intracellular ion for all types of cells. Maintaining fluid and electrolyte balance in the body of humans and animals is necessary for the function of all living cells(Pohl *et al.*, 2013; Clausen and Poulsen, 2013). In the present study, both ribavirin and diazinon treatments resulted in hypernatremia and hyperkalemia. Hypernatremia might reflect disruption in water balance mechanism. Both treatments might impair water intake and increase water loss resulted in cellular dehydration. The water-balance protective mechanism is necessary to maintain normal plasma osmolality. Androgue and Madias (2000) stated that hypernatremia denotes hypertonic, hyperosmolality and always cause cellular dehydration. Moreover, Waite *et al.* (2013) reported that hypernatremia developed following intensive care unit admission in 4.3% of patients. It was independently associated with a 40% increase in risk for hospital mortality.

Hyperkalemia is a potentially life threatening metabolic problem caused by inability of the kidneys to excrete potassium, impairment of the mechanisms that move potassium from the circulation into the cells, or a combination of these factors (Hollander-Rodriguez and Calvert, 2006). Hyperkalemia in the present study was introduced by ribavirin and diazinon treatments single or combined, that might affect potassium homeostasis. Conte *et al.*(1990) proved that Hyperkalemia is independent of acid- base or hormonal mechanisms know to regulate extra renal homeostasis of potassium but strictly correlated with rise in plasma osmolality. Moreover Hessels *et al*.(2015) stated that Hyperkalemia, Hypokalemia and potassium variable are independent associated with increased mortality. In addition, Rocha-Filho *et al*. (2009) suggested that potassium is an early sign of acute ischemic insult severity and early distinguish potential survivors from non-survivors. Adedeji (2010) reported significant increase in concentrations of plasma K and Na in African catfish (Clarias gariepinus) exposed for 96 hours to 6.6 ppm of diazinon. Zannan *et al*. (2008) found that changes in blood electrolytes are an indicative to renal impairment.

The relationships between the two variables of the body weights and each of the studied biochemical markers were accomplished through use of the sample correlation coefficient (r) in order to indicate the strength of association. The results reflected strong negative association between the body weight and kidney function whereas the association between body weight and liver function was the least strength. The present finding is in accordance with the study of Pappas and Quresh (1988). They stated that total liver enzyme activity of AST decreases in several species, including mice and cattle, as the body weight increase. However, Lee *et al*.(2001) proved that slight to moderated gains in the body weight are associated with increase in AST and ALT activities.

**Conclusion:** The results obtained indicated, relative safety of ribavirin insofar as liver and pancreatic functions. Potential modulatory effect of ribavirin on the possible harmful effect of diazinon concerning liver function and amylase activity. Ribavirin did not has a significant role on the changed AFP, creatinine, K and Na. On the other hand, ribavirin by himself disturbed ALP, globulin and most of the kidney`s biochemical markers. Moreover, ribavirin augmented the deleterious effect of diazinon concerning ALP, urea and uric acid.

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