Antioxidant Capacity and Hepatoprotective Effect of Silymarin Against Salinomycin – Induced Toxicity in Adult Rabbits

Ahmed Hamdy ghonaim (✉ drcahmed91@gmail.com)  
DRC: Desert Research Center  https://orcid.org/0000-0002-6922-1798

Mai G Hopo
specialist veterinarian

tarek AboElnaga
DRC: Desert Research Center

Rania Abdelrahman Elgawish
Suez Canal University Faculty of Veterinary Medicine

RH Abdou
Suez Canal University Faculty of Veterinary Medicine

Kawther A. Elhady
Suez Canal University Faculty of Veterinary Medicine

Research Article

Keywords: Feed Additive, Salinomycin, silymarin, liver enzymes, oxidative biomarkers, rabbit

DOI: https://doi.org/10.21203/rs.3.rs-415375/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Salinomycin was evaluated for its toxicity and silymarin for prophylactic management in male rabbits. Male rabbits were randomly divided into 7 groups with 7 rabbits / each. Groups 1, 2, 3 were kept as control group, salinomycin (20 mg / kg ration) and salinomycin (40 mg / kg ration), respectively. Group 4 was fed on feed containing salinomycin (20 mg / kg ration) and silymarin (6.5 mg / kg body weight). Group 5 received feed containing salinomycin (40 mg / kg ration) and silymarin (13 mg / kg body weight). Groups 6 and 7 were nourished feed containing silymarin (6.5 and 13 mg / kg body weight), respectively. Duration of the experiment was 28 days. Weekly body weights showed a significant reduction in the 3rd group when compared with control group. The activity of Malondialdehyde and the values of aspartate aminotransferase, alanine aminotransferase, total protein, albumin, total cholesterol, triglycerides, low density lipoprotein, urea and creatinine were significantly elevated in 2nd and 3rd group while glutathione, catalase, superoxide dismutase and high density lipoprotein were significantly lowered when compared with control group. Thus, it is clarified that salinomycin toxicity is owed to oxidative damage and the usage of silymarin in feed tends to treat and prevent any accidental toxicity. Relative weight of the liver increased significantly in 3rd group. There were mild pathologic changes in liver of 2nd group while there were sever pathologic changes at 3rd group when compared with control group.

Introduction

Salinomycin sodium is authorized as a coccidiostat to be used in feeds for chickens reared for fattening with a minimum-maximum concentration of 50-70 mg / kg feed and a withdrawal period of one day (EC Regulation No 496/2007 and 500/2007). Salinomycin sodium is also authorized for chickens reared for laying at 50 mg / kg feed without a withdrawal period (EC Regulation No 1852/2003), and in rabbits for fattening at 20-25 mg / kg ration and a withdrawal period of five days (EC Regulation No 937/2001).

Silymarin is obtained from Silybum marianum. Silymarin is a polyphenolic flavonoid that has a wide range of biological and pharmacological influences including anti-oxidant activity, enhancement of protein synthesis, cell regeneration and hepatoprotective activities. The potential benefits of silymarin in the cure of liver diseases have raised many concerns. The safety and efficacy of this herbal drug had been evaluated by a systematic approach in a review by Saller et al. (2001).

Rabbit meat plays a significant role in human nutrition. Rabbit efficiency in producing meat compares favorably with most other domesticated animals and can play a role in solving a part of meat shortage in Egypt especially throughout poultry crisis as bird flu. Numerous antibiotics have been used to promote the growth of farm animals. These products enhanced feed conversion, animal growth, decreased morbidity and mortality because of diseases. The average growth enhancement was assessed to be between 4 and 8 %, and feed consumption was boosted by 2 to 5 % (Ewing and Cole 1994).

The purpose of this study was to investigate the adverse impacts of oral administration of salinomycin for 28 consecutive days on liver enzymatic activities, protein and lipid profiles, and liver oxidative
biomarkers as well histopathological alterations in liver in adult rabbits and how to reduce the risk and side effects through administration of a supportive therapy of silymarin.

**Materials And Methods**

**Rabbits**

Forty nine adult male New Zealand White bucks (1.750 to 2 kg) were purchased from EL Mohands Farm, Ismailia, Egypt. The rabbits were kept in individual cages with free water access. The diet was obtained from EL Mohands Farm, Ismailia. Daily lighting regime was adjusted to 10-12 h photoperiod/day and the rabbits were accommodated to the laboratory condition for one week before the beginning of the experiment.

**Chemicals**

Coxistac was used as a source of salinomycin and produced by Phibro Animal Health Company, Egypt. Coxistac contains 60 g salinomycin/kg. Silymarin was available as Legalon 140 capsule from Cid Pharmaceutical Company, Cairo, Egypt. Sodium Carboxymethylcellulose (CMC) comprises 40–50 % cellulose, 25-40 % hemicellulose and 15-35 % lignin on a dry basis (*Singh and Singh 2012*). CMC was obtained from Fortune Biotech, China. In this study, CMC was used as a suspending agent to dissolve salinomycin and silymarin to be given orally to the rabbits.

**Experimental design**

Rabbits were divided randomly into 7 groups (7 rabbits/each) and administrated the drugs for consecutive 28 days through the oral route. 1\textsuperscript{st} group was kept as control (received 1 ml of CMC only), 2\textsuperscript{nd} group was orally given 20 mg salinomycin/kg ration dissolved in CMC, 3\textsuperscript{rd} group was orally given 40 mg salinomycin/kg ration dissolved in CMC, 4\textsuperscript{th} group was orally given 20 mg salinomycin/kg ration dissolved in CMC+6.5 mg silymarin/kg body weight dissolved in CMC, 5\textsuperscript{th} group was orally given 40 mg salinomycin/kg ration dissolved in CMC+13 mg silymarin/kg body weight dissolved in CMC, 6\textsuperscript{th} group was orally given 6.5 mg silymarin / kg B.W dissolved in CMC and 7\textsuperscript{th} group was orally given 13 mg silymarin/kg body weight dissolved in CMC. This study was carried out in strict agreement with recommendations in the guide for the care of animals and the protocol was approved by the Ethical Committee of Animal Experiments at Suez Canal University.

**Effect on body weight gain:**

Weights of rabbits in each group were estimated at the beginning of the experiment then weekly for four successive weeks. The difference in weights of rabbits at the beginning of the experiment and after 28 days was calculated.

**Blood sampling**
At the end of the experiment, blood samples were collected from ear vein of all groups at the morning via plain tube. The blood was kept to clot and subsequently centrifuged at 3000 r.p.m for 10 min for biochemical investigations.

**Sero-biochemical parameters**

Serum was analyzed for determination of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) according to Murray *et al.* (1984) and Young (1995). Creatinine was determined according to Murray *et al.* (1984). Urea was estimated according to Kaplan (1984). Total proteins were determined according to Burtis *et al.* (2012). Albumin was determined according to Young (2002) and Burtis *et al.* (2012). Total Cholesterol was estimated according to Naito and David (1984). High and low Density Lipoprotein (HDL and LDL) were estimated according to Lopes - Virella *et al.* (1977). The previous parameters were assessed spectrophotometrically using commercially available Diamond Diagnostics kits, Germany.

**Effect on weights of Liver:**

After slaughtering of Rabbits, liver was immediately dissected out and weighed for calculation of relative organ weight.

**Oxidative stress biomarkers assay**

The liver was washed with 0.9% NaCl solution. It was frozen and stored at −20 °C for detection of Glutathione (GSH), SuperOxide Dismutase (SOD), catalase and Malondialdehyde (MDA) using commercially available BioVision's ApoGSH TM Colorimetric Assay Kit, USA.

**Histopathology**

A part of the liver was fixed for 24 h in 4% paraformaldehyde in PBS pH 7.4 (Sigma-Aldrich Chemical). Subsequently, it was kept in 70% ethanol for histopathological examination (Bancroft et al.1996). The fixed liver samples were dehydrated throughout a series of ethanol-graded concentrations, clarified in xylene, embedded in paraffin, and sectioned at 4 μm. Hematoxylin and eosin were used to stain the liver sections for detection of histological alterations. The sections were observed by a calibrated standard digital Olympus® CX21 microscope camera (Tuscan ISH1000 digital microscope camera) with a resolution of 10 megapixels (3656 × 2740 pixel each image) and × 400 magnifications UIS optical system (Universal Infinity System, Olympus, Japan).

**Statistical Analysis**

Data were statistically investigated using the suitable analysis of variance according to Steel and Torrie (1981), using the one way analysis of variance in completely randomized design with 4 replicates for all traits. A computer program software coStat version 6.331 was used for analysis of the data of all
experiments. Least significant difference (LSD) at 5% level was used separately to assess the response of each character in each experiment.

**Results**

**Effect on body weight gain:**

Statistical analysis revealed that growth rates were significantly affected by different treatments. The use of salinomycin (20 mg / kg ration) increased the growth rate of rabbits more than control group. Use of salinomycin (40 mg / kg ration) orally for 28 successive days had significant adverse effect on weight gain when compared with that of control group. The use of silymarin (6.5 mg / kg body weight) together with salinomycin (20 mg / kg ration) or using silymarin (13 mg / kg body weight) together with double salinomycin dose (40 mg / kg ration) increased the growth rate of rabbits more than control group. The use of silymarin (6.5 or 13 mg / kg body weight) increased the growth rate of rabbits more than control group (Table 1).

**Relative liver weight**

Results revealed that administration of salinomycin (20 mg / kg ration) didn't affect the relative liver weight significantly when compared with control and other treated groups, however, using silymarin (6.5 mg / kg body weight) together with salinomycin (20 mg / kg ration) maintained the relative liver weight as control group. Prolonged administration of salinomycin (40 mg / kg ration) increased in the relative liver weight significantly than control and other treated groups, however, administration of silymarin (13 mg / kg body weight) with salinomycin (40 mg / kg ration) maintained the relative liver weight as that of control group. Use of silymarin (6.5 mg or 13 mg / kg body weight) maintained the relative liver weight as control group (Table 2).

**Liver enzymatic activities**

Daily administrations of 20 or 40 mg salinomycin / kg ration caused a significant (P<0.05) increase in AST and remarkable (P<0.01) rise in ALT activities when compared with that of control and other treated groups. However, administration of silymarin (6.5 mg) with salinomycin (20 mg) or silymarin (13 mg) with salinomycin (40 mg) improved the AST and ALT values (Table 3).

**Total protein and albumin**

Administration of salinomycin (20 or 40 mg / kg ration) caused a significant (P<0.05) decrease in total protein and albumin when compared with that of control and other treated groups. In contrast, administration of both doses of silymarin with salinomycin improved level of total protein and albumin than that of salinomycin treated group alone (Table 3).

**Urea and Creatinine**
Salinomycin (20 or 40 mg / kg ration) induced a significant (P<0.01) increase in urea and a significant (P<0.05) increase in creatinine when compared with that of control and other treated groups. Furthermore, silymarin either in 6.5 mg or 13 mg / kg body weight with salinomycin improved values of urea and creatinine (Table 4).

**Lipid profile**

Administration of salinomycin (20 or 40 mg / kg ration) induced a significant (P<0.01) increase in cholesterol, triglycerides and LDL with greatly significant (P<0.01) decline in HDL when compared with that of control and other treated groups. Administration of silymarin (6.5 mg or 13 mg) with salinomycin (20 mg or 40 mg) improved cholesterol, triglycerides, LDL and HDL (Table 5).

**Oxidative stress biomarkers**

Salinomycin (20 or 40 mg / kg ration) induced a remarkable significant (P<0.01) increase in MDA activity and a significant (P<0.01) decrease in GSH, SOD, and catalase activities when compared with that of control group. When silymarin (6.5 mg) was used in combination with salinomycin (20 mg/ kg ration), it enhanced the values of GSH, SOD and catalase activities with lower value of MDA when compared with that of salinomycin treated group alone. Administration of silymarin (13 mg / kg B.W) with salinomycin (40 mg/kg ration) orally for 28 successive days resulted in improved value of GSH, SOD and Catalase activities with lower value of MDA when compared with that of salinomycin treated group alone (40 mg/kg ration) (Table 6).

**Histopathological results**

Liver of rabbits treated with 20 mg salinomycin kept a preserved architecture, hepatocytes organized in thin cell trabeculae with moderate hydropic degeneration of cytoplasm and normal sinusoids While the liver of rabbits treated with 40 mg salinomycin showed disturbed architecture, marked hydropic degeneration of cytoplasm, congested vessels, and obliterated sinusoids. Marked improvement in hepatocytes with thin cell trabeculae in lobular architecture separated by thin wall blood sinusoids was observed after using silymarin in both doses (Fig. 1).

**Discussion**

In the present study, the obtained data concerning growth rate displayed that salinomycin at dose of 20 mg/ kg ration increased the growth rate of rabbits more than that of control group; however, salinomycin (40 mg/ kg ration) induced a significant reduction in growth rate. Salinomycin (40 mg/ kg ration) significantly decreased the body weight of rabbits from 3rd week post treatment. *Keshavarz and McDougald (1982)* informed that this decline in body weight may be due to the hazard influence of salinomycin in the liver and kidney and / or to a reduction in feed consumption.

AST increased significantly in rabbits administrated 40 mg salinomycin. This finding is in agreement with *Neufeld (1992)* who reported increased AST activity with salinomycin at 50 mg/kg ration and 15.5 g/Kg
feed, respectively in turkeys. However, Rizvi et al. (2008) reported that AST activity in layers was not altered significantly at 60 and 120 ppm salinomycin doses although at 180 ppm it increased significantly. ALT activity was increased significantly at groups receiving 20 and 40 mg salinomycin. Elevated levels of AST and ALT might be attributed to oxidative damage caused by free radicals generated by salinomycin resulting in hepatocellular injury. Moreover, Kamashi et al. (2004) attributed the liver damage to the influence of the toxic dose of salinomycin on hepatocytes and induction of degenerative changes in the liver. Silymarin supplementation to the rabbits alone or in combination with salinomycin improved the liver enzymatic activities which might be due to the antioxidant property of silymarin.

In the present study, salinomycin resulted in a significant hypoproteinaemia with hypoalbuminemia, in both doses (20 or 40 mg). Kamashi et al. (2004) informed that total proteins were significantly lowered in salinomycin treated broiler (120 mg/kg feed) But our result disagreed with Rajaian et al. (2009) who found that total protein concentration was not significantly altered by salinomycin. On the other hand, silymarin supplementation alone or in combination with salinomycin improved total protein and albumin toward normal level. Histopathological findings of liver confirmed the observed elevation in enzymatic activities and decrease in total protein and albumin in salinomycin treated rabbits where there is mild pathologic alterations in liver of rabbits treated with 20 mg salinomycin and sever pathologic alterations in liver of rabbits treated with 40 mg salinomycin. These results are in agreement with the results of several researchers which demonstrated destructive influences of carboxylic ionophores on the liver (Javad et al. 2014). However, administration of silymarin improved these alterations of liver due to the hepatoprotective property of silymarin. The present results are in agreement with that of many investigators who mentioned that silymarin has protective effects against lipid peroxidation and histopathological alterations (Eminzade et al. 2008; Wu et al. 2008).

Blood urea concentration significantly increased in rabbits receiving 20 and 40 mg salinomycin. Serum creatinine concentration was significantly increased in rabbits receiving 20 or 40 mg salinomycin. This is in accordance with Kamashi et al. (2004) who reported an elevation in serum creatinine and blood urea concentrations in broilers following salinomycin treatment. The obvious increase in values of creatinine and blood urea especially in salinomycin treated group may reveal a reduction in the glomerular filtration rate as well as impairment of renal blood flow. However, silymarin supplementation alone or in combination with salinomycin improved level of urea and creatinine.

Total cholesterol, triglycerides, and LDL were notably increased with a significant reduction in HDL in the serum of rabbits receiving 20 and 40 mg salinomycin. This could act as an indicator for cardiac damage as well as renal impairment (Kaneko et al. 1997), which might be possibly due to free radical-induced oxidative damage. The results are in agreement with Kyriakis et al. (2001) and Kamashi et al. (2004) who informed that the levels of total lipids, triglycerides, total cholesterol as well as low density lipoprotein were significantly increased in broiler chickens fed on food containing salinomycin (120 mg/kg feed). Abnormal hyperlipidemic conditions may be idiopathic or secondary to hepatic insufficiency and nephrotic syndrome (Nelson et al. 1994). Administration of silymarin resulted in improved values of
cholesterol, triglycerides, and LDL with higher value of HDL which might be due to the antioxidant activity of silymarin.

Oxidative damage is a consequence of an imbalance between oxidants and antioxidants at the cellular level and involves oxidative alteration of cellular macromolecules, cell death by apoptosis or necrosis, as well as structural tissue damage (Lykkesfeldt and Svendsen 2007). In animals intoxicated with salinomycin, tissue injury and necrosis in various organs happened; particularly myocardium and skeletal muscle (Khodakaram Tafti et al. 2008; Hosseini et al. 2013 and Hajimohammadi et al. 2014). Salinomycin (20 mg / kg ration) caused a remarkable significant increase in MDA activity and a significant decline in GSH, SOD and catalase activities when compared with that of untreated rabbits. When the silymarin (6.5 mg) was used in combination with salinomycin, it enhanced the values of GSH, SOD and catalase activities with lower value of MDA. Our result is in accordance with Hajimohammadi et al. (2015) who found significant decline in SOD, catalase and GPX in salinomycin treated sheep. Our findings showed that salinomycin intoxication increases levels of lipid peroxidation products. Simsek et al. (2006) reported that the evaluation of MDA is usually used to reveal lipid peroxidation. In accordance to the present findings, Kargin and Fidanci (2001) reported elevated levels of lipid peroxidation (MDA) in numerous diseases including kidney diseases and Hajimohammadi et al. (2015) observed an increase in MDA in salinomycin treated sheep when compared with control group.

Conclusion

This study highlighted the risk of using salinomycin in rabbit feed. Hence, it’s recommended that the use of salinomycin as growth promoter for rabbits shouldn’t exceed 20 mg/ kg ration and in homogenous way. On the other hand, duration time of use shouldn’t exceed 30 days. Also, we recommend the use of silymarin in combination with salinomycin.

Declarations

Acknowledgments:

I would like to acknowledge Mrs. Amal ELfakharany and all my family for the unlimited support and inspire.

Conflict of interest:

The authors declare that they have no conflict of interest.

1- Ethics approval and consent to participate:

This study was carried out in strict agreement with recommendations in the guide for the care of animals and the protocol was approved by the Ethical Committee of Animal Experiments at Suez Canal University with approval number (2021007).
2- Consent for publication:

Not applicable

• Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

4- Competing interests:

The authors declare that they have no competing interests

• Funding:

This research was funded by Desert Research Center

6- Authors' contributions:

AH: first and corresponding author, data collection, investigation and methodology, data curation, statistics and article writing

MG: data collection, article writing

TR: data curation, supervision

RE: investigation and methodology

RH: statistics and writing

KA: visualization and supervision

References

Due to technical limitations, the References section is not available for this version.

Tables

Table 1. Body Weight Gain (kg) of control and treated rabbits (Mean ± SE)
Different letters are significantly different at $P < 0.05$

SAL means salinomycin and SILY means silymarin

Table 2. Relative Liver Weights of control and treated rabbits (Mean ± SE)

| Groups | Dose                              | Initial Weight | 1<sup>st</sup> week | 2<sup>nd</sup> week | 3<sup>rd</sup> week | 4<sup>th</sup> week | weight gain |
|--------|-----------------------------------|----------------|----------------------|----------------------|----------------------|----------------------|-------------|
| I      | Control                           | 1.956 ± 0.033  | 1.978 ± 0.058        | 2.168 ± 0.047        | 2.241 ± 0.065        | 2.316 ± 0.091        | 0.360 ± 0.102<sup>ab</sup> |
| II     | 20 mg SAL./kg ration              | 2.036 ± 0.095  | 2.144 ± 0.084        | 2.381 ± 0.076        | 2.469 ± 0.068        | 2.530 ± 0.080        | 0.494 ± 0.065<sup>a</sup> |
| III    | 40 mg SAL./kg ration              | 2.053 ± 0.121  | 2.073 ± 0.107        | 2.244 ± 0.116        | 2.390 ± 0.107        | 2.319 ± 0.106        | 0.266 ± 0.059<sup>b</sup> |
| IV     | 20 mg SAL./kg ration +6.5 mg Sily. / kg B.W | 2.040 ± 0.034 | 2.166 ± 0.032 | 2.420 ± 0.031 | 2.557 ± 0.043 | 2.591 ± 0.063 | 0.551 ± 0.042<sup>a</sup> |
| V      | 40 mg SAL./kg ration +13 mg Sily. / kg B.W | 2.004 ± 0.058 | 2.175 ± 0.070 | 2.358 ± 0.076 | 2.498 ± 0.070 | 2.511 ± 0.056 | 0.507 ± 0.079<sup>a</sup> |
| VI     | 6.5 mg Sily. / kg B.W             | 1.941 ± 0.102  | 2.047 ± 0.063        | 2.150 ± 0.072        | 2.277 ± 0.071        | 2.423 ± 0.073        | 0.481 ± 0.114<sup>a</sup> |
| VII    | 13 mg Sily. / kg B.W              | 1.934 ± 0.030  | 1.987 ± 0.044        | 2.050 ± 0.040        | 2.243 ± 0.055        | 2.463 ± 0.026        | 0.529 ± 0.017<sup>a</sup> |
Liver (gm) | Group
--- | ---
2.65 ± 0.06\(^b\) | Control
2.74 ± 0.08\(^b\) | 20 mg SAL / kg ration
3.14 ± 0.04\(^a\) | 40 mg SAL / kg ration
2.63 ± 0.05\(^b\) | 20 mg SAL / kg ration + 6.5 mg SILY / kg BW
2.53 ± 0.20\(^b\) | 40 mg SAL / kg ration + 13 mg SILY / kg BW
2.56 ± 0.14\(^b\) | 6.5 mg SILY / kg BW
2.64 ± 0.09\(^b\) | 13 mg SILY / kg BW

Different letters are significantly different at \(P < 0.05\)

SAL means salinomycin and SILY means silymarin

---

Table 3. Liver enzymatic activities, total protein and albumin of control and treated rabbits (Mean ± SE)

| Albumin (g/dl) | Total protein (g/dl) | ALT (U/L) | AST (U/L) | Group |
|---|---|---|---|---|
| 2.0 ± 0.1\(^{a,b}\) | 6.8 ± 0.3\(^{a,b,c}\) | 5.5 ± 0.5\(^c\) | 8.5 ± 0.87\(^b\) | Control |
| 1.7 ± 0.2\(^b\) | 6.5 ± 0.4\(^{b,c}\) | 7.1 ± 0.8\(^{a,b}\) | 10 ± 1.2\(^{a,b}\) | 20 mg SAL / kg ration |
| 1.7 ± 0.2\(^b\) | 5.7 ± 0.5\(^{b,c}\) | 8.3 ± 0.6\(^a\) | 12.3 ± 0.8\(^a\) | 40 mg SAL / kg ration |
| 2.1 ± 0.1\(^a\) | 7.9 ± 0.5\(^a\) | 5.9 ± 0.4\(^{b,c}\) | 9.3 ± 0.8\(^b\) | 20 mg SAL / kg ration + 6.5 mg SILY / kg BW |
| 2.02 ± 0.1\(^{a,b}\) | 7.4 ± 0.6\(^{a,b}\) | 6.3 ± 0.2\(^{b,c}\) | 9.3 ± 0.8\(^b\) | 40 mg SAL / kg ration + 13 mg SILY / kg BW |
| 2.2 ± 0.1\(^a\) | 7.0 ± 0.3\(^{a,b,c}\) | 5.4 ± 0.5\(^c\) | 8.5 ± 0.9\(^b\) | 6.5 mg SILY / kg BW |
| 2.2 ± 0.1\(^a\) | 6.9 ± 0.3\(^{a,b,c}\) | 5.6 ± 0.2\(^{b,c}\) | 9.3 ± 1.4\(^b\) | 13 mg SILY / kg BW |
Different letters are significantly different at $P < 0.05$

SAL means salinomycin and SILY means silymarin

### Table 4. Urea and creatinine of control and treated rabbits (Mean ± SE)

| Creatinine (mg/dl) | Urea    (mg/dl) | Group                                      |
|--------------------|------------|--------------------------------------------|
| 4.2 ± 0.03<sup>b</sup> | 73.4 ± 1.8<sup>de</sup> | Control                                    |
| 5.1 ± 0.4<sup>ab</sup>  | 81.1 ± 1.03<sup>b</sup> | 20 mg SAL / kg ration                       |
| 5.5 ± 0.3<sup>a</sup>   | 94.4 ± 1.5<sup>a</sup> | 40 mg SAL / kg ration                       |
| 4.7 ± 0.2<sup>abc</sup> | 77.0 ± 1.6<sup>bcd</sup> | 20 mg SAL / kg ration + 6.5 mg SILY / kg BW |
| 4.3 ± 0.3<sup>c</sup>   | 78.2 ± 1.7<sup>bc</sup> | 40 mg SAL / kg ration + 13 mg SILY / kg BW  |
| 4.5 ± 0.2<sup>bc</sup>  | 76.6 ± 1.3<sup>cd</sup> | 6.5 mg SILY / kg BW                         |
| 4.2 ± 0.4<sup>b</sup>   | 72.8 ± 0.8<sup>de</sup> | 13 mg SILY / kg BW                          |

Different letters are significantly different at $P < 0.05$

SAL means salinomycin and SILY means silymarin

### Table 5. Cholesterol, triglycerides, HDL and LDL of control and treated rabbits (Mean ± SE)
| LDL (mg/dl) | HDL (mg/dl) | Triglycerides (mg/dl) | Cholesterol (mg/dl) | Group |
|------------|------------|----------------------|---------------------|-------|
| 118.4 ± 1.2<sup>c</sup> | 14.3 ± 0.7<sup>de</sup> | 31.1 ± 0.4<sup>c</sup> | 138.9 ± 1.1<sup>e</sup> | Control |
| 136.1 ± 0.5<sup>b</sup> | 12.8 ± 0.4<sup>ef</sup> | 33.2 ± 0.5<sup>b</sup> | 155.6 ± 1.4<sup>c</sup> | 20 mg SAL / kg ration |
| 153.7 ± 1.3<sup>a</sup> | 11.4 ± 0.6<sup>f</sup> | 35.8 ± 0.8<sup>a</sup> | 172.2 ± 1.2<sup>a</sup> | 40 mg SAL / kg ration |
| 121.02 ± 1.1<sup>c</sup> | 17.1 ± 0.8<sup>bc</sup> | 31.6 ± 0.4<sup>bc</sup> | 144.4 ± 1.6<sup>d</sup> | 20 mg SAL /kg ration + 6.5 mg SILY / kg BW |
| 136.3 ± 0.8<sup>b</sup> | 18.5 ± 0.5<sup>b</sup> | 31.6 ± 0.7<sup>bc</sup> | 161.1 ± 0.7<sup>b</sup> | 40 mg SAL /kg ration + 13 mg SILY / kg BW |
| 111.3 ± 0.8<sup>d</sup> | 15.7 ± 0.5<sup>cd</sup> | 31.6 ± 0.8<sup>bc</sup> | 133.3 ± 0.9<sup>f</sup> | 6.5 mg SILY / kg BW |
| 96.1 ± 1.4<sup>e</sup> | 25.7 ± 0.8<sup>a</sup> | 30.0 ± 0.7<sup>c</sup> | 127.03 ± 1.2<sup>g</sup> | 13 mg SILY / kg BW |

Different letters are significantly different at $P < 0.05$

SAL means salinomycin and SILY means silymarin

Table 6. Oxidative stress biomarkers in rabbits of control and different treated groups (Mean ± SE)
| MDA (nmol/gram tissue) | Catalase (U/gram tissue) | SOD (U/gram tissue) | GSH (mg/gram tissue) | Group |
|------------------------|--------------------------|---------------------|----------------------|-------|
| 0.2 ± 0.01<sup>e</sup> | 6.3 ± 0.3<sup>b</sup>   | 5.2 ± 0.1<sup>a</sup> | 37.9 ± 0.4<sup>ab</sup> | Control |
| 0.3 ± 0.01<sup>b</sup> | 4.8 ± 0.1<sup>c</sup>   | 4.3 ± 0.1<sup>bc</sup> | 35.2 ± 0.5<sup>c</sup> | 20 mg SAL / kg ration |
| 0.4 ± 0.01<sup>a</sup> | 3.8 ± 0.1<sup>d</sup>   | 4.2 ± 0.1<sup>c</sup> | 29.7 ± 0.8<sup>d</sup> | 40 mg SAL / kg ration |
| 0.2 ± 0.01<sup>d</sup> | 5.1 ± 0.1<sup>c</sup>   | 4.4 ± 0.1<sup>b</sup> | 35.8 ± 0.9<sup>bc</sup> | 20 mg SAL / kg ration + 6.5 mg SILY / kg BW |
| 0.3 ± 0.01<sup>c</sup> | 6.8 ± 0.1<sup>a</sup>   | 4.2 ± 0.04<sup>bc</sup> | 34.4 ± 0.8<sup>c</sup> | 40 mg SAL / kg ration + 13 mg SILY / kg BW |
| 0.2 ± 0.01<sup>e</sup> | 6.7 ± 0.1<sup>ab</sup>  | 35.8 ± 1.0<sup>bc</sup> | 6.5 mg SILY / kg BW |
| 0.2 ± 0.01<sup>e</sup> | 6.9 ± 0.1<sup>a</sup>   | 5.2 ± 0.1<sup>a</sup> | 38.8 ± 1.0<sup>a</sup> | 13 mg SILY / kg BW |

Different letters are significantly different at $P < 0.05$

SAL means salinomycin and SILY means silymarin

**Figures**
Figure 1

(A) Liver of control rabbits with hepatocytes showing abundant cytoplasm and small nuclei (black arrow) arranged in thin cell trabeculae separated by thin wall blood sinusoids (green arrow). (B) Moderate hydropic degeneration (black arrow) of cytoplasm and normal sinusoids (green arrow) in liver treated with 20 mg salinomycin. (C) Hydropic degeneration (black arrow) of cytoplasm and congested vessels (red arrowhead) and obliterated sinusoids (green arrow) in liver treated with 40 mg salinomycin. (D)
Abundant cytoplasm with residual mild hydropic degeneration (black arrow), cell trabeculae in lobular architecture with slightly narrowed sinusoids (green arrow) in liver treated with 20 mg salinomycin and 6.5 mg silymarin. (E) Hepatocytes (black arrow) arranged in thin cell trabeculae in lobular architecture separated by thin wall blood sinusoids (green arrow) in liver treated with 40 mg salinomycin and 13 mg silymarin. (F and G) Hepatocytes (black arrow) showing abundant cytoplasm and small nuclei arranged in thin cell trabeculae separated by thin wall blood sinusoids (green arrow) in liver treated with 6.5 and 13 mg silymarin. (H&E stain, X400).