Comparative study between Silymarine and L. Carnitine in hepatoprotection against intoxication in broilers

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Abstract: Although hepatorenal toxicity was recorded as aside effect of paraceamol high doses, Paracetamol was used as antipyretic drug and growth stimulator for long time in poultry. Hepatoenal toxicity by paracetamol in broilers was mirrored by increase lipid peroxidation, depletion of glutathione and increase liver enzyme or even sudden death. There for this study was designated to evaluate the effect of silymarin and L.carnitine on hepatotoxic effect induced by high doses of paracetamol. The study was applied on 100 chicks (from 1 day old till 35 days old). In special cages divided into 5 groups each one contained 20 chicks. The first group was remaine as a control one, while the 2nd,3rd and 4th groups were eopplemented by silymarine, L.carnitine and paracetamol respectively. The 5th group supplemented with silymarin, L.carnitine and paracetamol together. Serum samples were collected for measuring glutathione reductase, malondialdehyde, ALT, AST, superoxide dismutase, triglycerides and cholesterol.
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1. Introduction

Paracetamol was used for long time as an antipyretic drug and as a growth stimulator. There was unpleasant side effects of paracetamol with high doses as hepatorenal damage **(Savita, et al.2015).** Toxic effect of paracetamol is caused by its toxic metabolite N-acetyl-P-benzoquinone imine which is normally conjugated with glutathione in liver and converted into mercapturic acid which is not toxic and excreted by kidney. High doses of paracetamol, cause toxic metabolite accumulation which leads to depletion of glutathione reserve, increases liver enzymes levels in blood, lipid peroxidation and consequently leads to hepatic necrosis **(Joulideh, 2016).**

There are ongoing trends of improving poultry performance and productivity in ways that are different from antibiotics **(Gadde, *et al.*2017).** Supplementing poultry diets with different amino acids improve health status of productive animals especially in organic poultry systems by **Baker (2013).** Rapid growth, higher feed intake, lower feed conversion ratio and higher final body weight could be recently achieved by many natural supplementers to poultry diets **(Kalmar, *et al.* 2014).**

L.carnitine is the main abdominal fat lowering supplementer in poultry diets beside its growth enhancement effect **(Kitagawa, *et al*,2017).** The same results were obtained by **Wang (2013)** who found that feeding chickens with 500mg L. carnitine lead to a reduction in abdominal fat and serum and yolk cholesterol and feed conversion ratio but with increased body weight gain and poultry performance in general. L.carnitine lowers subcutaneous fat deposition through reducing of fat metabolism enzymes activity as Glucouse -6- phosphate dehydrogenase, malic dehydrogenase, isocitrate dehydrogenase and lipo protein lipase **(Xu, et al. 2003).**

It is well known that silymarin has a prominent hepatocyte protective effect in hepatic intoxication caused by ochratoxin A or Paracetamol, via its anti oxidative and antiapoptotic effect as well as through increasing superoxide dismutase (SOD) and glutathione activity and decreasing lipid peroxidation product malondialdehyde **(Yu, *et al.* 2018).** Moreover, it also has a performance enhancement effect as revealed by increasing daily feed intake and final weight gain **(Jahanian, *et al.* 2017).**

This study was planned to investigate the potential ameliorative effect of L. methionine, choline, L. carnitine and silymarin on liver intoxication induced by paracetamol high doses through measuring serum AST, ALT, lipid peroxidation product (malondialdehyde), cholesterol and triglycerides levels, SOD and glutathione activity.

Many researches were established on the development of antibiotic alternatives to improve poultry health and performance **(Gadde, *et al.*2017).** Supplementation of poultry diets with L.carnitine above 500mg/kg diet resulted in improvement of poultry performance (body weight gain and feed conversion. While, decreasing in amount of abdominal fat **(Leibetseder,2016).**

Supplementation with L.carnitine above 25mg/kg in broilers diets increased breast muscle yield decreased abdominal fat and subcutaneous fat deposition by dereasing total activity of glucose-6-phosphate dehydrogenase, malic dehydrogenase, isocitrate dehydrogenase and lipoprotein lipase **(Xu, et al. 2003).**

Dietary Silymarin supplementation in broilers suppressed ileal population of Escherichia Coli, Salmonella Klebsiella and total negative bacteria in aflatoxicated birds so, improvement of poultry performance **(Jahanian, *et al.* 2017).**

Silymarin was used as hepatoprotective compound in poultry for prevention and treatment of chicken liver injuiry occurred by ochratoxin A through anti-oxidative and antiapoptosis mechanisms as increasing glutathione reductase, super oxide dismutase levels and decreasing malondialdehyde and liver enzymes **(Yu, *et al.* 2018).**

Poultry dietary supplementation of l.carnitine decreased leg fat content and improved feed efficiency especially in high energy diets. In addition to improvement of oxidative stability of leg/breast muscles **(Jahanian and Ashnagar. 2018).**

Feeding broilers with 200mg/kg L.carnitine for a period of 42 days reduced plasma concentration of malondialdehyde, packed cell volume and abdominal fat deposition. Also, supplementation of poultry diets with L.carnitine increased plasma nitric oxide and immune responsiveness **(Khajali and Khajali. 2014).**

Adding L.carnitine with 50mg/L water in one day old poultry chicks improved body weight gain and feed intake only during first three weeks, early stages of growing **(Celik, et al. 2003).**

L.carnitine supplementation at level of 100mg/kg in one day old poultry chicks diets significantly reduced malondialdehyde and serum triglyceride. Also, increasing total superoxide dismutase, glutathione peroxidase and malic dehydrogenase. so, L.carnitine can potentially reduce susceptibility and mortality due to ascites **(Wang, et al. 2013).**

2. Materials and methods

1. Materials:

L.Carnitin powder.
Silymarin 10%solution.
Paracetamol powder.

2. Expermimental design:

100 poultry chicks (one day old) divided into (5 groups each one had 20 chicks) in special cages. The First group remains as the control one, while the 2nd group was supplemented with L.carnitine as recommended to NRC. The 3rd group was supplemented with Silymarin by (1000mg/kg ration). The 4th group got their hepatic intoxication by paracetamole (650mg/kg for7 days). The last group was supplemented with Paracetamol with mixture of 2 supplementers.

The doses applied each first 3 days of each week till 33 days age then collecting serum samples from wing vein and measuring the following (glutathione reductase, superoxide dismutase, Malondialdehyde, cholestrol, triglycerides, ALT and AST levels in randomly 5 serum samples from each group.

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean, standard deviation for parametric data after testing normality using Shapiro-Wilk test. Significance of the obtained results was judged at the 0.05 level and all tests were 2 tailed.

Student-t test was used for parametric quantitative variables, to compare between two studied groups. One Way ANOVA test with post Hoc Tukey test was used to compare more than 2 groups of parametric variables.

3. Results and Discussion

Comparison of glutathione reeducates mean value on serum samples of each (*L.carnitin,silymarin* and mixed) groups with control and paracetamol groups.

The present study reported that paracetamol group showed a significant decrease (p<0.001) in glutathione reductase value as compared to the control group. While silymarin group showed a significant increase (p<0.001) respectively in glutathione reductase value compared to the control group. Moreover, chicken administrated the 2 supplementers and paracetamol showed a significant increase (p<0.02) in glutathione reductase value compared to the paracetamol group without statistical change relative to the control group **(Table 1)**). This means improvement of negative paracetamol effect on glutathione reductase value**.**

Additionally**, Kettunen, *et al.* (2012)** recorded that poultry diets supplemented with silymarin from one day old to 26 days of age led to increasing total glutathione and reduced glutathione in blood and breast muscle. Also, **Tsiagbe, *et al.* (2013)** recorded that increasing silymarin level in broiler diets showed significant increasing in glutathione concentration and decreasing both malondialdehyde and plasma triglycerides levels.

Comparison of malondialdehyde mean value on serum samples of each (L. carnitine, silymarin and mixed) groups with control and paracetamol groups.

The present study reported that paracetamol group showed a significant increase (p<0.008) in malondialdehyde value compared to the control group. While chickens administrated the 2 supplementers and

paracetamol showed a significant decrease (p<0.017) in malondialdehyde value compared to the paracetamol group without statistical change relative to the control group **(Table 1) and (Figure 1).** This means improvement of paracetamol effect on malondialdehyde value**.**

Table (1): Comparison of Glutathione reductase, malondialdehyde, ALT, AST, triglycerides, cholesterol, SOD between control group and other studied groups.

Groups	Control	L.carnitine	Silymarin	Paracetamole	Mixed
G.reductase (U/L)	49.82± 1.6	51.14 ±0.79	60.13 ±3.16	41.25± 3.21	50.6± 6.2
Malondihyde (Nm/ml)	6.35± 0.61	5.98± 0.52	6.14± 0.47	7.21± 0.83	6.17 ±0.53
ALT (U/L)	35.67± 3.46	36.89 ±4.32	26.75 ±4.21	70.71± 3.51	39.4 ±3.89
AST (U/L)	130.75 ±3.81	135.67± 5.21	116.74 ±2.95	146.82 ±26.75	133.89± 4.21
Triglyceride (µg/dl)	16.86± 0.80	11.73 ±0.85	15.76± 0.87	23.09 ±0.71	15.95 ±0.89
Cholestrol (µg/dl)	30.27 ±3.01	24.89± 3.88	29.07 ±5.01	43.75 ±2.56	30.80 ±3.04
SOD %	63.68 ±3.52	60.9 ±3.41	71.31± 2.15	45.43 ±6.13	60.87 ±4.21

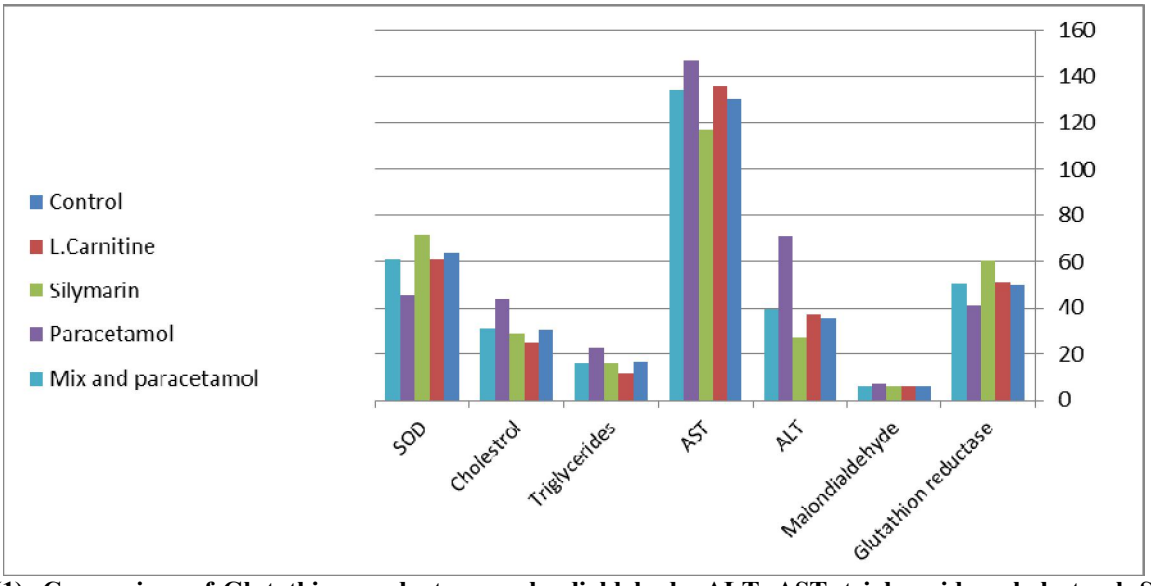

Fig (1): Comparison of Glutathione reductase, malondialdehyde, ALT, AST, triglycerides, cholesterol, SOD between control group and other studied groups.

Additionally, **Barreto, *et al.* (2011)** who reported that improving health status of poultry during first 28 days of age by organic supplementers reflected by improving the antioxidity activity of poultry as decreasing plasma malondialdehyde and increasing total plasma glutathione level. Keeping with this line **Arun (2009)** recorded that organics supplementation in broilers at 1-42days of age decreased plasma malondialdehyde level, reduced abdominal fat and plasma cholesterol level.

Comparison of ALT mean value on serum samples of each *(L.carnitine, silymarin* and mixed*)* groups with control and paracetamol groups.

The present study reported that paracetamol group showed a significant increase (p<0.001) in ALT value compared to the control group. While silymarin showed a significant decrease (p<0.001) in ALT value compared to the control group. Moreover, chicken administrated the 2 supplementers and paracetamol showed a significant decrease (p<0.001) in ALT value compared to the paracetamol group without statistical change relative to the control group **(Table 1).** This means improvement of paracetamol effect on ALT value**.**

Additionally, **Colak, *et al.* (2016)** reported that antihepatotoxic effect of silymarin used at a concentration 600 mg/kg of twenty one -14 days old broiler and recorded improvement in ALT level which increased by Aflatoxine and improvement in feed intake and body weight gain.

Comparison of AST mean value on serum samples of each *(L. carnitine, silymarin* and mixedl) groups with control and paracetamol groups.

The present study reported that paracetamol group showed a significant increase (p<0.001) in AST value compared to the control group. While, silymarin showed a significant decrease (p<0.001) in AST value compared to the control group. Moreover, chicken

administrated the 2 supplementers and paracetamol showed a significant decrease (p<0.002) in AST value compared to the paracetamol group without statistical change relative to the control group **(Table 1).** This means improvement of paracetamol effect on AST value**.**

Additionally **Kollia, *et al.* (2016)** reported that broiler diets poor in organic matter resulted in elevation of liver enzymes and liver histopathology showed many abnormalities and fatty liver.

Comparison of triglycerides and cholestrol mean values of each (*L. carnitine, silymarin* and mixed) groups with control and paracetamol groups.

The present study reported that paracetamol group showed a significant increase (p<0.001) in triglycerides and cholestrol value compared to the control group. While L. carnitine showed a significant decrease (p<0.001) in triglycerides and cholestrol value compared to the control group. Moreover, chicken administrated the 2 supplementers and paracetamol showed a significant decrease (p<0.002) in triglycerides and cholestrol value compared to the paracetamol group without statistical change relative to the control group **(Table 1).** This means improvement of paracetamol effect on triglycerides and cholestrol value**.**

Additionally, **Jahanian and Ashnagar, (2018)** reported that diet supplemented with L. carnitine of 540 one day old Ross 308 chicks and found decreasing in feed conversion ratio and decreasing in leg fat content and total plasma lipid level.

Also, **Khajali F and Khajali Z, (2014)** reported that decreasing in total plasma cholesterol and abdominal fat deposition with using 200 mg /kg of L. carnitine on 96 day old male chicks (cob 500) till 42 day.

Comparison of SOD mean values of each (L. carnitine, silymarin and mixed) groups with control and paracetamol groups.

The present study reported that paracetamol group showed a significant decrease (p<0.001) in SOD value compared to the control group. While silymarin showed a significant increase (p<0.003) in SOD value compared to the control group. Moreover, chicken administrated the 2 supplementers and paracetamol showed a significant increase (p<0.005) in SOD value compared to the paracetamol group without statistical change relative to the control group **(Table 1).** This means improvement of negative paracetamol effect on SOD value.

Additionally, **Tsiagbe, *et al.* (2017)** reported that 120 one day old cob broilers supplemented with silymarin in diet resulted in increasing super oxide dismutase, catalase and glutathione peroxidise. Also, **Wang, *et al.* (2013)** reported that using L.carnitine at 100mgg/kg diet on a total 420 one day old Male Ross 308 broilers showed dereasing in malondialdehyde of heart tissue on day 21 and 35, decreasing in triglycerides content on day 28 and 35 and increasing in super oxide dismutase and glutathione reductase of heart tissue on day 21 and 42.

Conclusion

The present study reported that supplementation of poultry diets with L. Carnitine and silymarin causes improvement in poultry production and feed intake. Moreover, these supplementers can improve the negative effect of paracetamol by increasing glutathione and and supperoxide dismutase. Also, decreasing malondialdehyde, liver enzymes, cholesterol and triglycerides.

Conflict of interest

The present study lacks many complementary tests which can illuminate the results such as:

- Liver histopathology to confirm liver necrosis caused by paracetamol.
- Measuring glutathione and malondialdehyde in tissues as heart and liver.

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