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HEMATOLOGICAL STUDY OF SILYMARIN ON MONOSODIUM GLUTAMATE TOXICITY IN RABBITS

Ahmed Nezar and *Ahmed Husam Al-Deri

*Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq

*Corresponding author Email: ahmednezar8491@gmail.com

Abstract

This study was conducted to examine the ameliorative Impact of (Silymarin) on Monosodium Glutamate Toxicity. Twenty-eight local male rabbits aged between 3-4 months were divided randomly into four equal groups (7 rabbits of each) housed in experimental cages of the animal house in College of Veterinary Medicine, University of Baghdad. From period begin 18/11/2019, up to 19/1/2020. The animals in first group were fed basal diet as control group (C), second group (T1) was fed basal diet and supplied Monosodium glutamate (10 mg/100g of BW) orally, third group (T2) was supplied with water containing Monosodium glutamate (10 mg/100 g of BW) and Silymarin(0.6 mg/100 g of BW) orally and fourth group (T3) was supplied containing Monosodium glutamate (10 mg/100 g of BW) dissolved in water and Silymarin(1.2 mg/100 g of BW) orally.

The results showed that, Hemoglobin concentration was increased with time progress in (T3) group , but the treated MSG groups recorded significant ($P< 0.05$) lower values than the control group along must studied period. lymphocyte percentage in the T1 and T2 groups showed significant ($P< 0.05$) higher value than other groups, Monocyte percentage showed that T3 group significantly ($P<0.05$) lower than control, T1, T2 groups during last period of this study. On other hand, The T1 group showed significant ($P< 0.05$) reduction in cholesterol concentration in all time of this study compared with other groups. While T3 group showed significant ($P< 0.05$) higher than other groups in all time of this study. Triglyceride concentration in T1 group showed significantly ($P < 0.05$) higher values than control group at 63 days. The concentration of Alanine Aminotransferase (ALT) of rabbits in T1 group (MSG) showed significant ($P\leq 0.05$) higher value compared with other groups in 63 day period, While showed significantly ($P\leq0.05$) lower value of C , T3 groups compared with the T1, T2 groups in all time of this study. The concentration of Aspartate transaminase enzyme (AST) showed significant increases ($P\leq0.05$) in T1 group (MSG) at all time of the study compared with the other groups .While T3 was recorded significant ($P\leq0.05$) lower value compared with other groups in last stage of the study period. Creatinin concentration in T2 group showed significantly ($P < 0.05$) higher value than the other groups at the 63 days studied period.

In conclusion, it was conducted that there were toxic effects of MSG on male rabbits through increase the damage in the liver and Kidney and oxidative stress through elevation in ALT, AST enzymes, creatinine and cholesterol. Also, with adding Silymarin daily in a dose of (1.2mg/100g BW) showed ameliorative Impact of (Silymarin) on Monosodium Glutamate Toxicity.

Keywords **:** Silymarin, creatinine, Monosodium Glutamate, rabbit

Introduction

Animal health depends on many factors and recently it has been respected that diet plays essential role in health and prevention of many diseases (Finkel and Holbrooke, 2000).

Feed additive are important materials that can develop the efficiency of feed use and animal performance. However, the use of chemical products mostly those of antibiotics and hormones may cause adverse effects. Many challenges in the field of animal nutrition are being done to achieve an rise in animal production and thus profit ability (Jouany and Morgavi, 2007).

In recent years, the fruits of *Silymarin* have been promoted in Europe and in the USA as a nutritional addition in different forms: whole seeds, seed powder, cut herb, herb powder, tea bags, alcohol-based seed extract, oil-based seed extract, capsules and soft gels (Tournas *et al.*, 2013).

Silymarin preparations are used as feed supplement, intended to improve animal health and production, or for therapeutic purposes (Radko and Cybulski, 2007). Rapid growth rate of modern animal strains and high milk efficiency can lead to significant metabolic and oxidative stress, which can decrease feed conversion efficiency and can affect growth performance as well as milk and meat quality (Radko and Cybulski, 2007).

Monosodium Glutamate (MSG) is one of the most widely used food-additives in commercial foods. Its request has increased over time and it is found in many different components and processed foods available in every market or grocery store. This taste sensation is also called “savoury” (Xiong *et al*., 2009).

Treatment Albino mice given 3g and 6g per Kg of body weight of MSG showed evidence of blood analysis showed a significant increase in lymphocytes count and poisoning in the treated animals. Packed Cell Volume, hemoglobin and red blood cells count were all indicative of an anemic condition in the treated animals (Ibrahim *et al*., 2012).

Materials and Methods

This experiment was carried out at the Animal House, College of Veterinary Medicine, University of Baghdad, achieved in 63 days from 2019\11\18 to 2020\1\19.

Twenty-eight healthy local male rabbits were brought at age of about 3-4 month, with average body weight of (1400 gm). Animal were kept in cages specialized for rabbit in the animal house of College of Veterinary Medicine, Baghdad University. The animals were examined healthy and clinically free of external and internal parasites. All animals where fed of concentrate diet and green Alfalfa and tap water were offered of preliminary period for (2) weeks. Animals were divided randomly and equally into four groups (7each) body weight was considered.

I. The first group (C) will daily fed a basal diet as control group.

II. The second group (T1) will received the same control diet supplemented with orally fed of Monosodium Glutamate (10 mg/100g) of body weight daily.

III. The third group (T2) will received the same control diet supplemented with orally fed of Monosodium Glutamate 10mg/100g BW and 0.6mg/100g BW Silymarin daily.

IV. The fourth group (T3) will received the same control diet supplemented with orally fed of Monosodium Glutamate 10mg/100g BW and 1.2mg/100g BW Silymarin daily.

Blood sample measurements

Blood samples were taken 3 time after each 3 weeks. Blood samples were withdrawing (5ml) from heart after sterilization the site of blood drawn by using disposable syringes sterilized. Blood sample were taken in the morning before treatments were given. The samples were divided into two parts, first part of the blood samples kept in 5ml tubes containing anticoagulant EDTA (ethyl diamine tetraacetic acid) for measuring blood hemoglobin (Hb), white blood cell differential count (WBCs). Second part samples kept in 5ml Gel tubes for measuring cholesterol, triglyceride, Serum Alanine Aminotransferase (ALT), Aspartate transaminase enzyme activity (AST) and Creatinin. The Hb was evaluated by using the spectrophotometric method according to (Drabkin and Austin, 1932). White blood cells were differentiated and measured according to (Seirverd, 1973). Cholesterol concentration and Triglyceride were measured according to National Cholesterol Education Program (NCEP, 2001). ALT measurement and described according to (Kim *et al*., 2008). AST measurement and described according to (Reitman and Frankel, 1957). Serum creatinine concentration was measured according to Kinetic method as mentioned by (Tietz, 1986). Statistical analysis was applied (Steel and Torrie, 1980).

Results

Blood hemoglobin (Hb)

Hemoglobin concentration of T3 group showed significant increase (P<0.05) compared with other groups (control, T1, T2) during the 21 and 63 days period (Table 1).

WBCs differential count

Neutrophil percentage (%)

The Neutrophil percentage of all groups were not differ among different groups along the whole experimental period (table 2).

Lymphocyte percentage (%)

The lymphocyte percentages of the T1 and T2 groups showed significantly (P < 0.05) higher value than other two groups (control andT3) during 63 days (Table 3).

Basophil percentage (%)

The basophil percentage of T1 group showed significantly (P ≤ 0.05) higher value than control, during 42 days (Table 4).

Eosinophil percentage (%)

The eosinophil percentage of all groups were not difference significantly among period & between the groups along the whole period of the experiment (Table 5).

Monocyte percentage (%)

Monocyte percentage (Table 6) showed that T3 group significantly (P<0.05) lower than control, T1, T2 groups during 63 days period of this study.

Blood Serum Cholesterol

The serum cholesterol concentration (Table 7) showed significant (P<0.05) increase in the T3 group compared with the other groups in 42days and 63 days periods of the study. While T1 group in all periods showed significant reduction compared with other groups control, T2 and T3.

Blood Triglyceride

T1 group showed significantly (P < 0.05) higher values than control group at 63 days (Table 8).

Serum Alanine Aminotransferase (ALT)

The results of ALT enzyme showed that there was a significant increase (P≤ 0.05) in the group T1 (Table 9) compared with other groups in 63 days period,

Aspartate transaminase enzyme activity (AST)

The results of AST showed significant (P≤0.05) increases in T1 group at all time of the study compared with the control, T2 and T3 groups

Blood Creatinin

Creatinin concentration showed a T2 group significantly (P < 0.05) higher value than the other groups at the 63 days studied period (Table 11).

Table 1 : Effect of MSG and Silymarin orally on blood hemoglobin (Hb) concentration (g/ dl) of local male rabbits. (Means ±SE).

Groups / Days	Control	T1	T2	T3	LSD
21	10.07±0.23 b	9.38±0.07 c	10.00±0.28 b	10.67±0.11 a	0.58
42	11.21±0.26 a	9.05±0.14 b	11.41±0.07 a	11.55±0.16 a	0.51
63	9.77±0.18 b	9.06±0.25 c	9.74±0.15 b	11.14±0.22 a	0.60

The different lowercase letters refer to significant differences between groups at (P<0.05).

Table 2 : Effect of MSG and Silymarin orally on Neutrophil percentage of local male rabbits. (Means ±SE).

Groups / Days	Control	T1	T2	T3	LSD
21	52.42±0.71	53.14±0.79	52.57±0.36	52.71±0.68	1.93
42	60.00±0.48	60.42±0.29	59.71±0.42	60.57±0.36	1.16
63	52.14±0.70	52.57±0.99	54.14±0.50	53.00±0.81	2.26

Table 3 : Effect of MSG and Silymarin orally on Lymphocyte percentage of local male rabbits. (Means ±SE).

Groups / Days	Control	T1	T2	T3	LSD
21	42.14±0.85 a	39.57±0.29 b	40.28±0.56 b	42.28±0.56 a	1.76
42	32.85±0.76	32.85±0.45	34.28±0.28	33.85±0.59	1.62
63	38.85±0.40 b	41.57±0.84 a	43.42±0.48 a	39.00±1.02 b	2.13

The different lowercase letters refer to significant differences between groups at (P<0.05).

Table 4 : Effect of MSG and Silymarin orally on Basophil percentage of local male rabbits. (Means ±SE).

Groups / Days	Control	T1	T2	T3	LSD
21	1.14±0.26	1.28±0.18	1.42±0.20	1.28±0.18	0.61
42	1.28±0.18 ab	1.71±0.18 a	1.38±0.18 ab	1.20±0.22 b	0.46
63	1.14±0.14	1.42±0.29	1.28±0.28	1.26±0.18	0.69

The different lowercase letters refer to significant differences between groups at(P<0.05).

Table 5 : Effect of MSG and Silymarin orally on Eosinophil percentage of local male rabbits. (Means ±SE).

Groups / Days	Control	T1	T2	T3	LSD
21	2.00±0.00	1.57±0.20	1.71±0.18	1.71±0.18	0.48
42	2.28±0.18	2.42±0.20	2.30±0.25	2.09±0.16	0.39
63	2.14±0.34	2.13±0.26	2.28±0.18	2.11±0.20	0.68

Table 6 : Effect of MSG and Silymarin orally on Monocyte percentage of local male rabbits. (Means ±SE).

Groups / Days	Control	T1	T2	T3	LSD
21	2.42±0.20 a	1.71±0.18 b	2.14±0.14 ab	2.28±0.18 a	0.52
42	3.57±0.20 a	3.14±0.26 ab	2.85±0.26 ab	2.42±0.20 b	0.68
63	3.42±0.29 a	3.42±0.20 a	3.14±0.14 a	2.42±0.20 b	0.63

The different lowercase letters refer to significant differences between groups at(P<0.05).

Table 7 : Effect of MSG and Silymarin orally on serum blood cholesterol (mg / dl) of local male rabbits. (Means ±SE).

Groups / Days	Control	T1	T2	T3	LSD
21	24.77±0.72 a	19.47±1.02 b	25.95±0.46 a	26.81±0.62 a	1.95
42	23.40±0.28 b	18.96±0.30 c	23.23±0.37 b	24.51±0.34 a	0.96
63	23.08±0.53 c	20.54±0.62 d	26.28±0.57 b	30.51±0.41 a	1.51

The different lowercase letters refer to significant differences between groups at (P<0.05).

Table 8 : Effect of MSG and Silymarin orally on serum Triglyceride (mg/dl) of local male rabbits. (Mean ± SE).

Groups / Days	Control	T1	T2	T3	LSD
21	53.84±1.47 a	51.01±1.85 a	44.00±1.33 b	50.97±0.98 a	4.22
42	74.19±2.46 a	70.49±2.39 a	58.08±1.26 b	60.59±1.64 b	5.86
63	71.06±3.62 b	83.58±4.23 a	74.34±1.04 ab	75.31±3.15 ab	9.47

The different lowercase letters refer to significant differences between groups at (P<0.05).

Table 9 : Effect of MSG and Silymarin orally on ALT enzyme (IU/L) of local male rabbits. (means ± SE).

Groups / Days	Control	T1	T2	T3	LSD
21	32.15±0.66 b	40.12±0.99 a	40.53±0.61 a	34.48±2.65 b	2.45
42	43.38±1.08 ab	52.73±0.79 a	50.19±0.57 a	35.88±6.04 b	9.08
63	33.87±0.65 c	43.50±0.97 a	39.71±0.43 b	34.47±0.96 c	2.31

The different lowercase letters refer to significant differences between groups at (P<0.05).

Table 10 : Effect of MSG and Silymarin orally on AST enzyme (IU/L) of local male rabbits. (means ± SE).

Groups / Days	Control	T1	T2	T3	LSD
21	33.59±1.06 bc	46.50±0.89 a	35.37±1.35 b	32.08±0.53 C	2.93
42	43.30±0.93 bc	54.17±0.73 a	44.40±0.77 b	41.13±0.61 C	2.26
63	33.53±0.82 c	49.31±0.44 a	36.49±0.56 b	31.63±0.46 D	1.74

The different lowercase letters refer to significant differences between groups at (P<0.05).

Table 11 : MSG and Silymarin on blood creatinin (mg/dl) of local male rabbits. (Mean ± SE).

Groups / Days	Control	T1	T2	T3	LSD
21	0.83±0.14	1.15±0.11	0.89±0.06	1.00±0.15	0.36
42	0.98±0.02 b	1.62±0.06 a	1.44±0.17 a	0.98±0.02 B	0.28
63	0.84±0.05 c	1.17±0.05 b	1.46±0.15 a	1.08±0.38 Bc	0.26

The different lowercase letters refer to significant differences between groups at (P<0.05).

Discussion

Hemoglobin of different groups were increased with time progress. Hemoglobin concentration of T3 group showed significant increase (P<0.05) compared with other groups (control, T1, T2) during the 21 and 63 days period (table 1) The increase in the Hb of the treated groups with Silymarin may due to the effect of Silymarin to increase heme iron uptake and body status in rabbit (Jain *et al*., 2011). Silymarin is essential for maturing red blood cells and preservation of the natural characteristics of blood (Kumar *et al*., 2011). Therefore, the significant (P< 0.05) increase in the T3 group than other treated groups may related to the effect of Silymarin. Silymarin significantly improved the supply of metabolizable protein to the small intestine (Pavlova *et al*., 2018).

MSG cause increased oxidative stress which induced the formation of micro nucleated polychromatic erythrocytes (Farombi and Onyema, 2006) and (Elphick *et al*., 2008).

The Neutrophil percentage of all groups treated by silymarin and MSG were not differ among different groups along the whole experimental period in rabbit.These results were agreed with (Mohamed and Metvvally, 2009). The lymphocyte percentages of control and T3 groups showed lower values due to the Silymarin have important role in protecting the membrane of leucocyte from oxidative damage (Narendhirakannan and Hannah, 2013), while the T1 and T2 groups showed significantly (P < 0.05) higher value than other two groups (control andT3) during 63 days, indicate that MSG may cause some element of inflammation leading to disturbance in differential count. and could be attributed to an increase in immune response of the animal.

The significant (P< 0.05) decrease in the T1group may related to the effect of MSG on low density lipoprotein – cholesterol from oxidative damage and acids in degradation of cholesterol (Heinecke *et al*., 1993). These results were agreed with (Quines *et al*., 2016) who found supplementation MSG in the diet of hyper cholesterolaemic human and

animals in significant reduction in cholesterol because that MSG activates the catabolism of cholesterol. Also this study agreement with the MSG-treated rats recorded lowered serum cholesterol is believed to be partly responsible for the significantly lower serum testosterone recorded for the MSG-treated rats, as testosterone is one of the steroid hormones synthesized from cholesterol (Stoco, 1998; Hu *et al*., 2010).

The lower values of Triglyceride for T2 and T3 compared with T1 showed the Ameliorative effect of Silymarin on the effect of MSG, this is agreed with (Heidarian and Rafieian-Kopaei, 2012) who showed that Silymarin caused significant reduction in triglyceride of blood serum, which inhibit the making of triglyceride in the body due to reduce re absorption by the small intestine and excreted out. It is a fact that improvement of the lipids profile completed by decreasing the serum levels of total cholesterol, triaceylglycerol (Almeida *et al*., 2012). MSG caused elevations in serum levels of Triglyceride. This may be due to MSG is able to increase the activity of 3-hydroxyl-3-methylglutrayl-Co enzyme A reductase and also increase lipogenesis and impaired Triglyceride and total cholesterol metabolism (Egbuonu, and Osakwe, 2011; Okediran *et al*., 2014).

In this study, ALT and AST, T3 was recorded significant ($P \leq 0.05$) lower value compared with other groups in the all stage of the study. This result suggests that there was a major effect due to Silymarin additives to reduce the damage that occurs in the liver. This result was consistent with (Jain *et al*., 2011), who reported that oral supplements of Silymarin improved liver regeneration in rats, suggesting that an oral Silymarin supplement can clinically improve recovery after major liver toxicity in mice (Hamza and Al-Harbi, 2015). These results indicated that Silymarin supplementation has beneficial effects in weakening hepatic morphological and functional injury induced by MSG in rabbits.

These enzymes are released into the blood stream in the presence of hepatocellular degeneration and necrotic changes, resulting in elevation of levels of serum AST and ALT (Kang *et al.,* 2008). Silymarin has been clinically used largely as anti-hepatoxic agent due to its strong antioxidant activity (Lahiri-Chatterjee *et al*., 1999).

While MSG caused a significant elevation in the levels of AST and ALT in rabbits treated with MSG maybe indication of liver damage. The present result agrees with the reports of (Farombi and Onyema, 2006; Onyema *et al*., 2006 and Sailo, 2016) demonstrated that the activity of serum AST increased in male rats that were feed MSG probably due to the finding that MSG induced oxidative stress in the liver.

In the present study, administration of MSG resulted in weakening of some renal functions reflected by the significant ($P < 0.05$) increase in creatinine levels in (T1) group than control,T3 groups during 63 days. These results are in agreement with (Vinodini *et al*., 2010; Abass and Abd El-Haleem, 2011 and El-Nahrawy *et al.*, 2012) who showed an increase in creatinine that proved that the damages caused by MSG even compromised the kidney function. MSG might have either interfered with creatinine metabolism leading to increased synthesis or the tissue might have compromised all or part of its function which might be due to oxidative stress induced by MSG on the renal tissue (Sailo, 2016).

In the other hand, the result of T3 group showed that Silymarin caused significant reduction in the damaging effect of MSG to the renal function. That agreed with (Abdelmeguid *et al*., 2010) suggested silymarin inhibited toxic effect on glomerular and renal tubular cells morphology and agreed with (karimi *et al*., 2005) showed potective effect of silymarin on various clinical and nephrotoxicity (Behling *et al*., 2006) showed administration of silymarin cause reduction of acute tubula necrosis including. focal areas of broken basement membrane, swelling and flattening of PCT cells with brush border loss, diffuse interstitial edema and interstitial inflammatory cell infiltrate.

References

Abass, M.A. and Abd El-Haleem, M.R. (2011). Evaluation of Monosodium Glutamate Induced Neurotoxicity and Nephrotoxicity in Adult Male Albino Rats. J. Am. Sci., 78: 264-276.

Abdelmeguid, N.E., Chmaisse, H.N. and Zeinab, N.S.A. (2010). Protective effect of silymarin on cisplatin-induced nephrotoxicity in rats. Pak J Nutr, 9(7): 624-36.

Almeida, A.F.G.; Soares, D.; Costa, C.A.S.; Gaspar, D.; Moura, E.; Ferreira, F.L.C. and Alves, N.C.C. (2012). Effects of soybean or canola oil intake on seminiferous tubules structure in young rats. Nutr Hosp, 27(5):1668-1669.

Behling, E.B.; Milena, C.S.; Heloísa, D.C.F.; Lusânia, M.G.A.; Roberto, S.C. and Maria de Lourdes, P.B. (2006). Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity! and oxidative stress in rat kidneys. Pharmacol.Rep., 58: 526-532.

Drabkin, D.L. and Austin, J.H. (1932). Spectrophotometric studies I. Spectrophotometric constants for common hemoglobin derivatives in human, dog, and rabbit blood. Journal of Biological Chemistry, 98(2): 719-733.

Egbuonu, A.C.C. and Osakwe, O.N. (2011). Effects of high monosodium glutamate on some serum markers of lipid status in male Wistar rats. J. Medi. Medical Sci., 2: 653-656.

Elphick, L.M.; Hawat, M.; Toms, N.J.; Meinander, A.; Mikhailov, A. and Eriksson, J.E. (2008). Department of Biochemistry and Pharmacy, Abo Akademi University, FIN-20521 Turku; Kass, George E. 2008-01-01.

El-Nahrawy, W.; Sanaa, A.M.; Wahba, M.R. and Ibrahim, S.E. (2012). The Potential Effects of Propolis against Monosodium Glutamate MSG Toxic Effects on Some Biochemical Aspects of Kidney, Life Sci J., 94 :4044-4054.

Farombi, E.O. and Onyema, O.O. (2006). Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and guercetin. Human Experimental Toxicol. 125: 251-259.

Finkel, T. and Holbrook, J. (2000). Oxidant, oxidative stress and the biology of aging. Nature. 408 (9): 239-247.

Hamza, R.Z. and Al-Harbi, M.S. (2015). Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and *Nigella sativa* extract supplements. Asian Pacific Journal of Tropical Biomedicine, 5(7): 521-531.

Heidarian, E. and Rafieian-Kopaei, M. (2012). Effect of silymarin on liver phoshpatidate phosphohydrolase in

hyperlipidemic rats. Bioscience Research, 9(2): 59-67.

Heinecke, J.W.; Kawamura, M.; Suzuki, L. and Chait, A. (1993). Oxidation of low density lipoprotein by thiols: superoxide-dependent and-independent mechanisms. Journal of lipid research, 34(12): 2051-2061.

Hu, J.; Zhang, Z.; Shen, W.J. and Azhar, S. (2010).Celular cholesterol delivery, intracelular procesing and utilzation for biosynthesis of steroid hormones. Nutr. Metab.(Lond).7: 47-72.

Ibrahim, O.M.; Abdulhamza, N.N. and Abbass, H.K. (2012). Some Hematological and Histological Impact of sub-acute exposure to Mono Sodium Glutamate in Mice. The Iraqi Journal of Veterinary Medicine, 36(2): 127-131.

Jain, A.; Yadav, A.; Bozhkov, A.I.; Padalko, V.I. and Flora, S.J.S. (2011). Therapeutic efficacy of silymarin and naringenin in reducing arsenic-induced hepatic damage in young rats. Ecotoxicology and environmental safety, 74(4): 607-614.

Jouany, J.P. and Morgavi, D.P. (2007). Use of 'natural'products as alternatives to antibiotic feed additives in ruminant production. Animal, 1(10): 1443-1466.

Kang, J.S.; Wanibuchi, H.; Morimura, K.; Wongpoomchi, R.; Chusiri,Y.; Gonzalez, F.J. and Fukushima, S. (2008). Role of CYP2E1 in thioacetamide-induced mouse hepatotoxicity. Toxicol. Appl. Pharmacol., 228: 295-300.

Karimi, G.; Ramezani, M. and Tahoonian, Z. (2005). Cisplatin nephrotoxicity and protection by milk! thistle extract in rats. Evid. Based ComplementAlternat. Med. 2: 383-386.

Kim, W.R.; Flamm, S.L.; Di Bisceglie, A.M. and Bodenheimer, H.C. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology, 47(4): 1363–1370.

Kumar, T.; Larokar, Y.K.; Iyer, S.K.; Kumar, A. and Tripathi, D.K. (2011). Phytochemistry and pharmacological activities of *Silybum marianum*: a review. apex, 10: 12.

Lahiri-Chatterjee, M.; Katiyar, S.K.; Mohan, R.R. and Agarwal, R. (1999). A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. Cancer Res., 59: 622.

Mohamed, A.M. and Metvvally, N.S. (2009). Antiaflatoxigenic Activities of Some Plant Aqueous Extracts Against Aflatoxin-B1 Induced Renal and Cardiac Damage. Journal of pharmacology and toxicology, 4(1): 1-16.

Narendhirakannan, R.T. and Hannah, M.A.C. (2013). Oxidative stress and skin cancer: an overview. Indian Journal of Clinical Biochemistry, 28(2): 110-115.

NCEP (2001). National Cholesterol Education Program, Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II) JAMA June 16, 269, : 23.

Okediran, B.S.; Olurotimi, A.E.; Rahman, S.A.; Michael, O.G. and Olukunle, J.O. (2014). Alterations in the lipid profile and liver enzymes of rats treated with monosodium glutamate. Sokoto journal of veterinary sciences, 12(3): 42-46.

Onyema, O.O.; Farombi, E.O.; Emerole, G.O.; Ukoha, A.I. and Onyeze, G.O. (2006). "Effect of Vitamin E on Mono- sodium Glutamate Induced Hepatoxicity and Oxidative Stress in Rats," Indian Journal of Biochemistry & Bio- Physics, 43(1): 20-24.

Pavlova, I.; Lukanov, H.; Ivanov, V.; Petrova, Y. and Genchev, A. (2018). Simultaneous administration of silymarin and doxycycline in Japanese quails suggests probable herb-drug interaction. Bulgarian Journal of Agricultural Science, 24(1): 126-131.

Quines, C.B.; Rosa, S.G.; Chagas, P.M.; Da Rocha, J.T.; Dobrachinski, F.; Carvalho, N.R. and Nogueira, C.W. (2016). Homeostatic effect of p-chloro-diphenyl diselenide on glucose metabolism and mitochondrial function alterations induced by monosodium glutamate administration to rats. Amino acids, 48(1): 137-148.

Radko, L. and Cybulski, W. (2007). Application of silymarin in human and animal medicine. Journal of Pre-Clinical and Clinical Research, 1(1).

Reitman, S. and Frankel, S.J. (1957). Acolorimetric method for determination of serum oxaloacetatic acid and *Pyruvic transaminases*. Am. J. Clin. Pathol., 28: 56-63.

Sailo, L. (2016). Ameliorative Role of Carnitine on Monosodium Glutamate Induced Testicular Toxicity in Wistar Albino Rats.

Seirverd, C.E. (1973). Hematology for Medical Technologies 4th Lea and Febiger (ed). Philadelphia: 17-120.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics: a biometrical approach.

Stoco, D.M. (1998). Testosterone biosynthesis. In: Neil, J.D. and Knobil, E. (eds) Encyclopedia of Reproduction. Academic Pres, New York. 4: 784-789.

Tietz , N.W. (1986). Text book of Clinical Chemistry .WB. Saunders, Philadelphia, 1271-1281.

Tournas, V.H.; Calo, J.R. and Sapp, C. (2013). Fungal profiles in various milk thistle botanicals from US retail. International journal of food microbiology, 164(1): 87-91.

Vinodini, N.A.; Nayanatara, A.K.; Ramaswamy, C.; Ranade, A.V.; Kini, R.D.; Damadara, G.K. M.; Ahamed, B.; Shabarinath, and Ramesh, B. (2010). Study on evaluation of monosodium glutamate inducedoxidative damage on renal tissue on adult Wistar rats. J.Chin. Clin. Med., 53: 144-147.

Xiong, J.S.; Branigan, D. and Li, M. (2009). Deciphering the MSG controversy. International journal of clinical and experimental medicine, 2(4): 329.