Mansoura Veterinary Medical Journal

ROLE OF LYSINE AND METHIONINE IN CARNITINE METABOLISM IN RATS

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ABSTRACT

The present study was performed on rats for studying effect of different concentrations of lysine and methionine in carnitine metabolism in liver and muscle tissues. The animals which fed low concentrations of lysine and methionine showed significant decrease in carnitine metabolism when compared with normal control group. On the other hand the rats supplied with high concentrations of lysine and methionine showed markedly increase in carnitine metabolism. Furthermore, this study clearly appear the role of lysine and methionine concentration in anti-oxidant status of the body.

INTRODUCTION

Liver and kidneys are the mainly organs where carnitine synthesis is takenplace by the need of the essential amino acids lysine and methionine **(Borum and Broqist, 1977).** Besides ,carnitine can be synthesized by animal cells, it may be obtained from the diet especially of animal origin than plant based diet **(Borum and Broqist, 1977).** So, carnitine is considered as a Conditional health supplement **(Food and Nutritive Board of the National academy of sciences,1989)**

Lysine is the main source of carnitine carbon atoms(**Koeth et al., 2013)** .Methionine worked as a methyl donor for carnitine synthesis through its conversion to S-adenosylmethionine (**Brosnan and Brosnan, 2006**). Combination of both leading to Ne-trimethyllysine(TML) formation .TML is a carnitine precursor.

Carnitine is an important metabolite for various living cells , through its role as a fatty acids transporter . Carnitine transfers fatty acids from outside mitochondria to the inner matrix where β-oxidation is occurred , sharing in citric acid cycle to produce energy(**Mingrone, 2004)**.

From this point, this study aimed to elucidate the interaction between different doses of lysine and methionine in the biosynthesis of carnitine in hepatic and muscle tissue of rats

MATERIALS & METHODS

1)Animals:

In this study white Wistar albino rats were used . 180 to 200 grams weight and with age ranged from 16-18 weeks old thirty male rats supplied from the Animal house, Faculty of Pharmacy, Mansoura University . Before beginning the experiment, they were fed control diet with *adlibtium* amount of water. Water bottles laced with artificial teats were hanged from the cages top. The rats kept on clean plastic cages (6 rats/cage) and on a 12-h light / dark cycle in clean plastic cages with "bedding material" wood chips were changed twice /week .

2)Ration formulation:

Rats were classified into five groups according to the type of diet .

Nutrient composition will vary according to experimental objectives.

The diet formulation required for the current study was designed according to the guidelines stated in **NRC, 1995**.

3)Experimental design:

The rats grouping system and dietary requirements were represented as followed:

Group I: It was consisted of six rats kept in one cage. They were supplied by diet deprived from lysine and methionine that leads to lysine concentration 10% and methionine concentration 3.5% (low lysine &low methionine group).

Group II: It was consisted of six rats kept in one cage. They were given a control diet contains no additives. There was normal lysine concentration12 % and normal methionine concentration 5% (Control group).

Group III: It was consisted of six rats kept in one cage . They were supplied by 0.13 k.g methionine for each 100 k.g diet that lead to lysine concentration 10% and methionine concentration 5% (Low lysine & normal methionine group) .

Group IV: It was consisted of six rats kept in one cage . They were supplied by 0.26 k.g lysine for each 100 k.g diet that lead low lysine concentration 12% and methionine concentration 3.5% (Normal lysine &low methionine group).

Group V: It was consisted of six rats kept in one cage. They were supplied by 0.5 k.g lysine and0.33 k.g methionine for each 100 k.g diet that lead to lysine concentration 14% and methionine concentration 7 % (High lysine & high methionine group) .

4)Collection of blood samples:

The blood collection procedures were performed at the end of experiment (2 months). The blood samples were collected from the medial canthus of the eye of the rats after complete anesthesia with thiopental sodium. Centrifugation of blood samples were occurred at 3000 rpm for 15 minutes to separate serum which are stored at -20^{o}C for determination of the following biochemical parameters:

1- Total antioxidant capacity (TAC) according to **Koracevic .,et al (2001).**

2- Creatine phospho Kinase (CPK)according to **Chemnitz .,et al (1979) Dtscheb Ges,(1977)**.

3- Glutamate Pyruvic Transaminase (GPT) according to **Reitman , A. and Frankel , S.(1957).**

4- Glutamate Oxaloacetic Transaminase (GOT) according to **Reitman , A. and Frankel , S.(1957).**

5) **Dissection of rat:**

After collection of blood samples, rats were dissected to obtain liver and Quadriceps femoris muscle. These parts were washed by normal saline and stored for two parts:

1) The first part of liver and muscle tissues was stored in normal saline at -20ºc for determination of :
   a) Tissue Catalase activity according to **Aebi, (1984)**
   b) Tissue Nitric Oxide (NO) according to **Montgomery et al., (1961).**
   c) Tissue Glutathione Peroxidase (GPX) according to **D.E.Paglia and W. N. Valentine (1967).**
   d) Tissue Reduced Glutathione concentration (GSH) according to **Beutler, (1963)**
   e) Tissue Malondialdhyde concentration (MDA) according to **Satoh K, (1978) and Ohakawa et al., (1979)** .
   f) Enzymological determination of Total and Free Carnitine concentrations in tissue. according to **Marquis and Fritz, (1964) and Prieto et al., (2006**)
2) The second parts of liver and muscle were fully immersed in formalin 20% and stored in containers for histopathological examination.

For each 100 Kg

Food ingredients	Group I	Group II	Group III	Group IV	Group V
Wheat	19.604 k.g	19.604 k.g	19.604 k.g	19.604 k.g	19.604 k.g
Crushed Yellow Corn	47 k.g	46.61 k.g	46.87 k.g	46.74 k.g	46.17 k.g
Soya bean meal	22.945 k.g	22.945 k.g	22.945 k.g	22.945 k.g	22.945 k.g
Lime stone	0.738 k.g	0.738 k.g	0.738 k.g	0.738 k.g	0.738 k.g
Di-Calcium Phosphate	1.702 k.g	1.702 k.g	1.702 k.g	1.702 k.g	1.702 k.g
Fish meal	6 k.g	6 k.g	6 k.g	6 k.g	6 k.g
Salt	1.011 k.g	1.011 k.g	1.011 k.g	1.011 k.g	1.011 k.g
Mineral and vitamins premix	1.1 k.g	1 k.g	1 k.g	1 k.g	1 k.g
Lysine	_____	0.26 k.g	_____	0.26 k.g	0.5 k.g
Methionine	_____	0.13 k.g	0.13 k.g	_____	0.33 k.g
Total Lysine %	10 %	12%	10%	12%	14%
Total Methionine %	3.5 %	5%	5%	3.5%	7%

Table(1): Effect of different concentrations of lysine and methionine on free carnitine, total carnitine and acyl carnitine concentrations:

" Mean ± SEM"

Groups	Hepatic free carnitine	Hepatic total carnitine	Hepatic acyl-carnitine	Muscle free carnitine	Muscle total carnitine	Muscle acyl-carnitine
I	82.36±1.66510^{b}	111.85±6.08913^{bc}	23.9±2.9^{c}	61.53±17.75245^{b}	82.34±23.73210^{b}	12.9±1.23^{c}
II	82.91 ±1.19326^{b}	125.33±3.01815^{b}	41.4±2.03^{b}	93.46 ± 6.55453^{b}	14.42±10.24794^{b}	46.9±4.09^{b}
III	81.90±1.20333^{b}	121.88±1.71853^{bc}	39.9±2.6^{b}	62.19±18.07766^{b}	74.67±18.09100^{b}	12.4±2.6^{c}
IV	67.22± 7.58421^{c}	100.19±11.24225^{c}	32.7±3.7^{b}	94.07±6.66395^{b}	139.87±9.34586^{b}	33.4±8.8^{bc}
V	134.06±5.43590^{a}	196.69±8.05518^{a}	58.2±1.48^{a}	244±22.61440^{a}	370.97±33.88432^{a}	126±14^{a}

Table(2): Effect of different concentrations of lysine and methionine on the oxidative stress(Nitric oxide and Malondialdhyde):

" Mean ± SEM"

Groups	Hepatic nitric oxide	Muscle nitric oxide	Hepatic malondialdehyde	Muscle malondialdehyde
I	26.64± .73494^{b}	25.35± .51125^{b}	19.3333± 2.96273^{b}	9.1667± 2.20479^{ab}
II	27.26± .38316^{ab}	26.54± .79478^{ab}	27.6667±1.45297^{ab}	7.5000 ± 2.88675^{b}
III	28.5± .43016^{ab}	28.35± .31466^{a}	34.3333± 9.24362^{a}	5.0000± 1.44338^{b}
IV	24.43±.79759^{c}	26.36± .79390^{ab}	16.8333±2.24227^{b}	4.1667± .83333^{b}
V	28.67± .62635^{a}	28.16± .62115^{a}	25.8333± 2.20479^{ab}	15.0000± 1.44338^{a}

Table(3): Effect of different concentrations of lysine and methionine on liver enzymes and *CreatinephosphoKinase*

" Mean ± SEM"

Groups	*Creatinephospho Kinase*	Glutamic-Pyruvic Transaminase	Glutamic-Oxaloacetic Transaminase
I	82±1.15470^{a}	111.61±5.212^{a}	179.01±2.05172^{ab}
II	7.33±1.76383^{c}	88.46 ±.56049^{ab}	166.40 ±4.03503^{b}
III	37.81±5.25793^{b}	90.5±.54590^{ab}	168.16±5.42786^{b}
IV	35.54±1.23362^{b}	87.06± 6.57301^{ab}	193.4±9.04292^{a}
V	3.2±.56862^{c}	77.39± 1.64652^{b}	175.83± 2.86860^{b}

Table(4): Effect of different concentrations of lysine and methionine on antioxidants:

" Mean ± SEM"

Groups	Hepatic catalase	Muscle catalase	Hepatic glutathione peroxidase	Muscle glutathione peroxidase	Hepatic reduced glutathione	Muscle reduced glutathione	Total Anti-oxidant capacity
I	.761±.36425^{c}	1.1537±.18823^{b}	781.07±34.72443^{c}	839.33± 24.26474^{d}	26.91±2.62218^{d}	75.44 ±.35000^{e}	2.79±.03756^{b}
II	1.71 ±.02309^{a}	1.0320±.11678^{b}	1373.72±4.71396^{b}	4373±2.64575^{b}	51.34±.50478^{c}	126.32±1.70376^{d}	2.96±.01938^{a}
III	0.153±0.05984^{d}	.8137±.09935^{b}	221.15±48.74038^{e}	235.39±53.56758^{e}	116.93±4.63529^{b}	162.72±11.55603^{c}	2.81±.04667^{b}
IV	1.04±.07654^{bc}	1.6533±.06360^{a}	421.00±16.46208^{d}	1575.51±147.27185^{c}	179.44±9.75144^{a}	194.45±1.72863^{b}	2.79±.03480^{b}
V	1.45±.08660^{ab}	1.6300±.13229^{a}	2241.72±139.43761^{a}	5031.43±230.85333^{a}	192.17±12.01650^{a}	231.62±5.09641^{a}	2.98±.04041^{a}

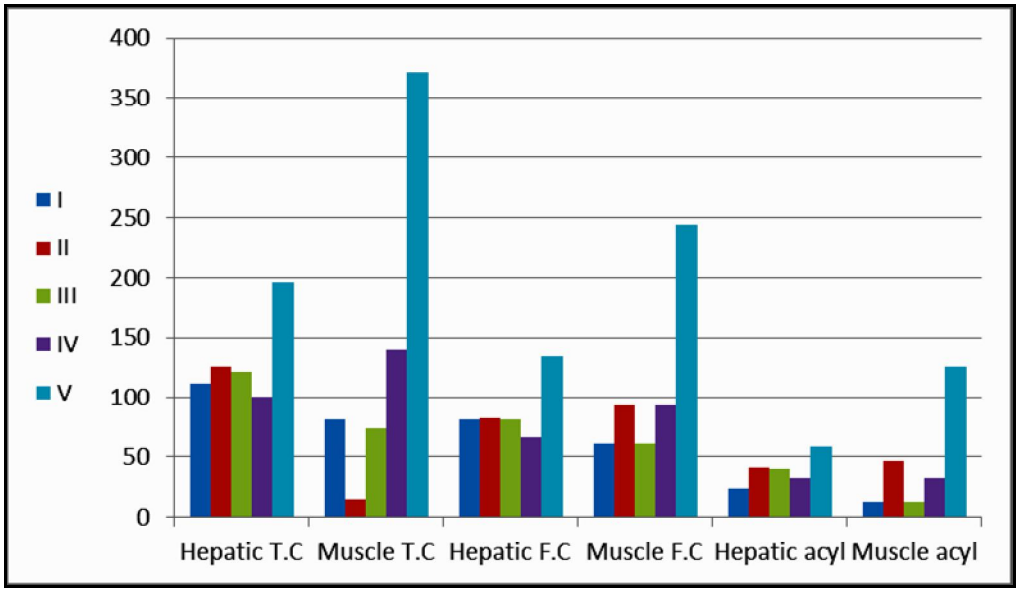


Fig.1: Effect of different concentrations of lysine and methionine on free carnitine (F.C), total carnitine (T.C) and acyl carnitine concentrations

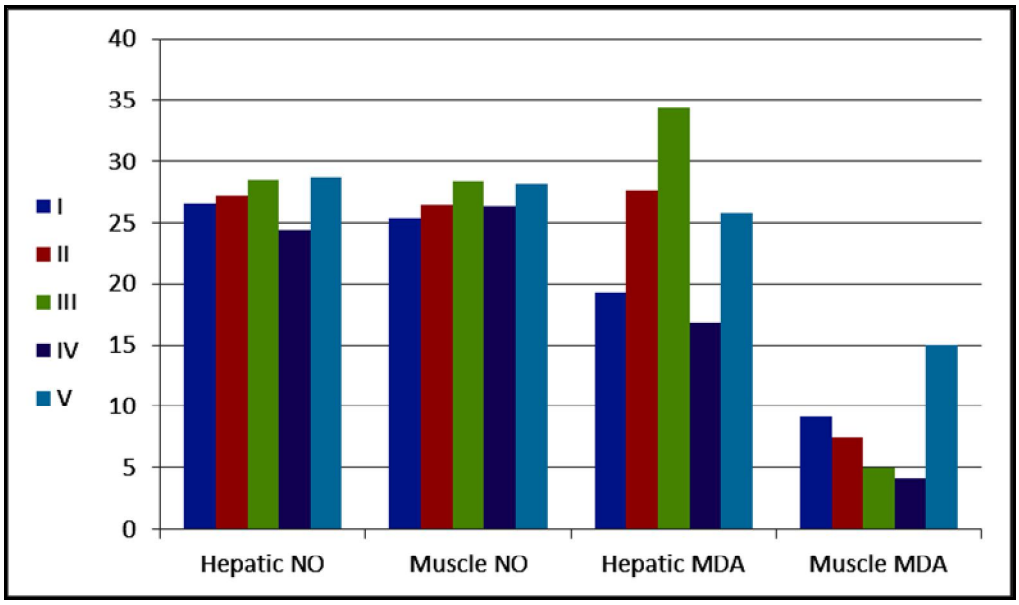


Fig.2: Effect of different concentrations of lysine and methionine on the oxidative stress (Nitric oxide and Malondialdhyde)

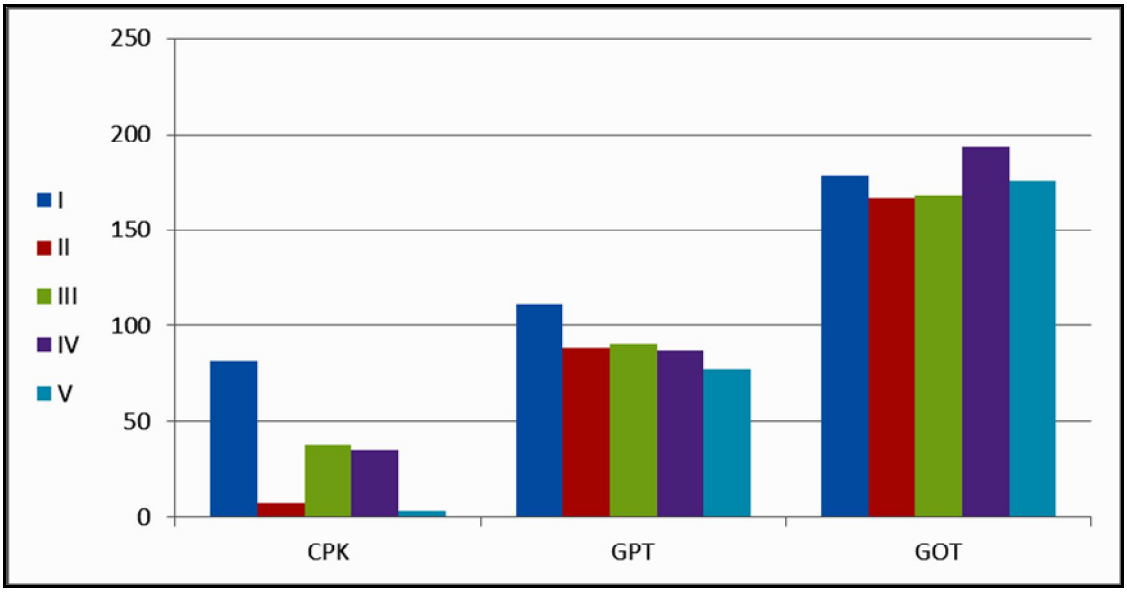


Fig.3: Effect of different concentrations of lysine and methionine on liver enzymes and *Creatinephospho Kinase (CPK)*

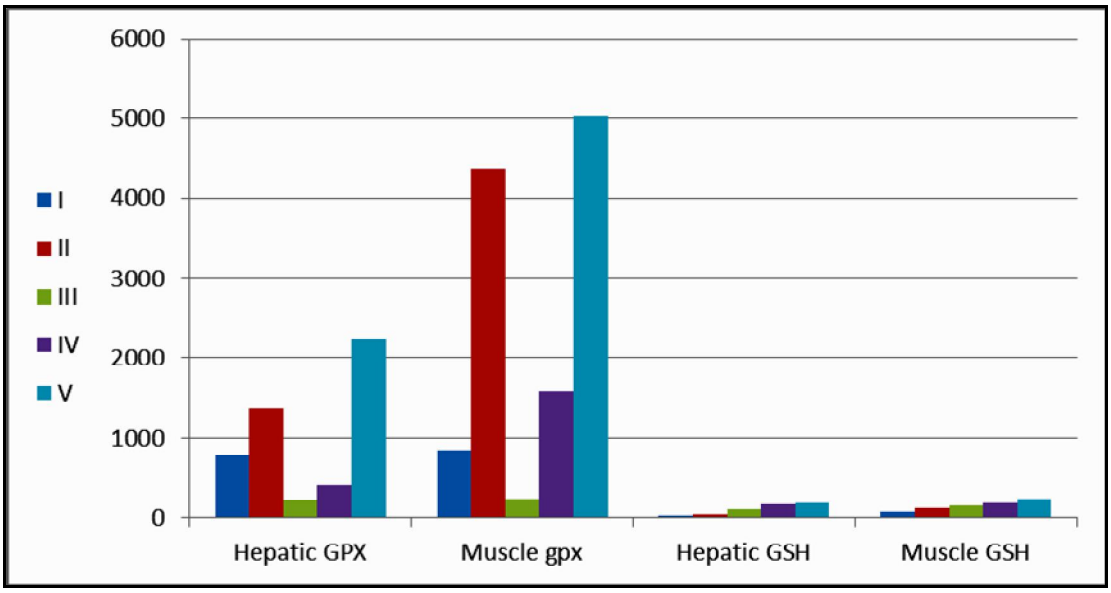


Fig.4: Effect of different concentrations of lysine and methionine on antioxidants (glutathione peroxidase& glutathione reductase)

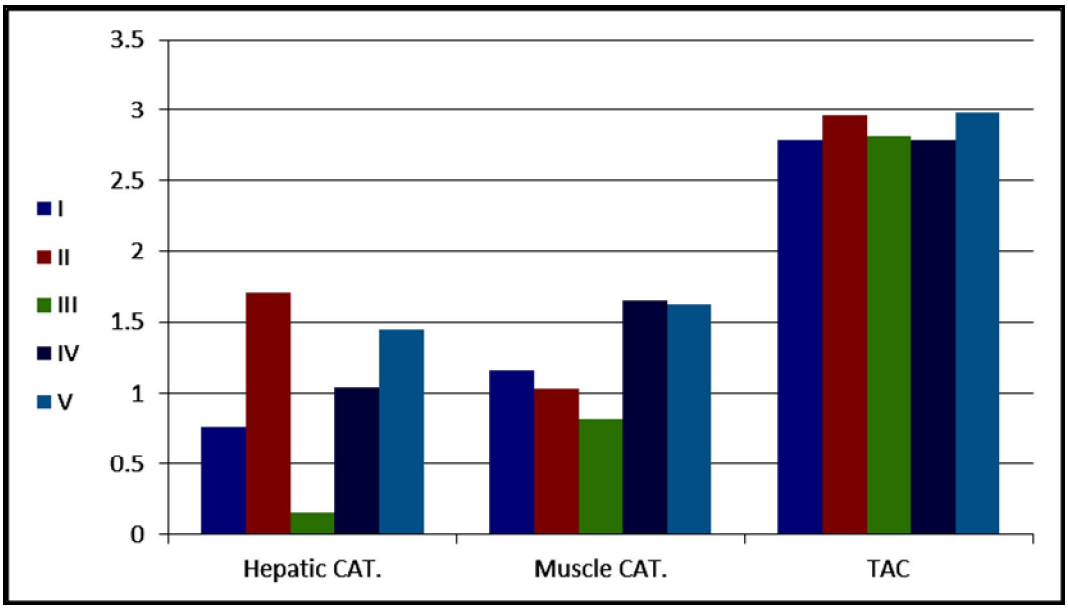
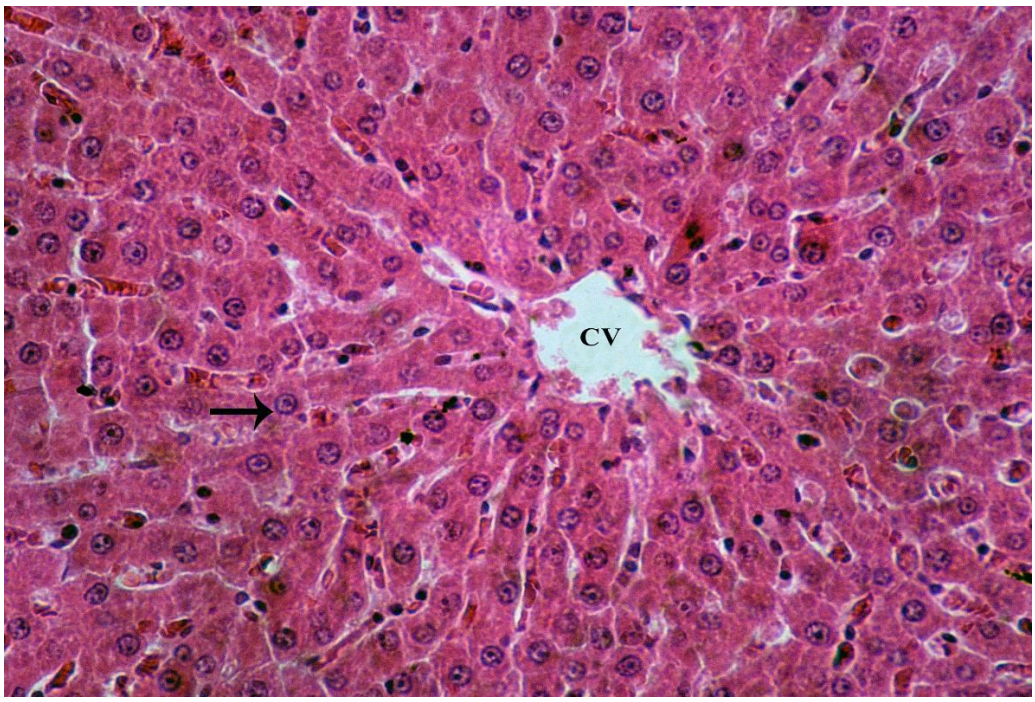
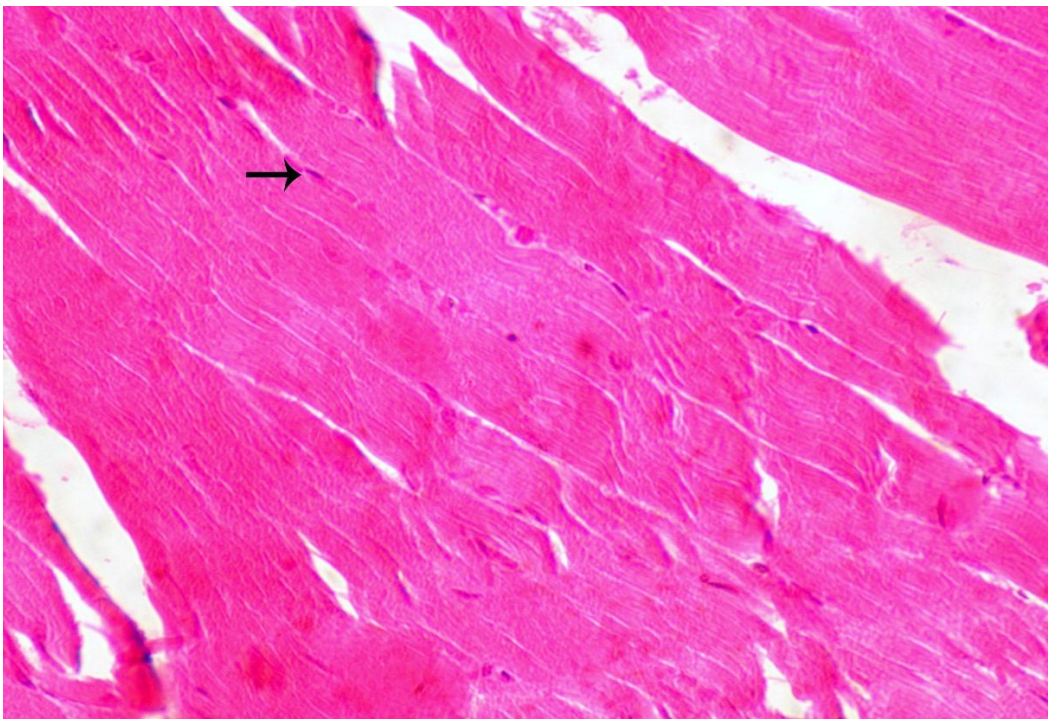


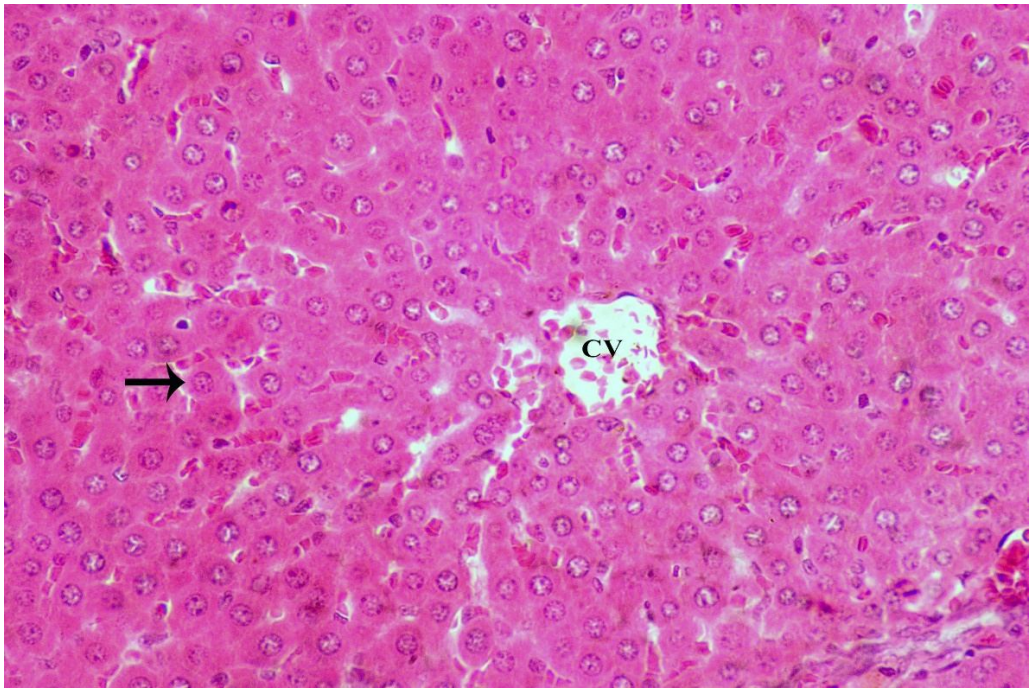
Fig.5: Effect of different concentrations of lysine and methionine on antioxidants (catalase &total antioxidant capacity)



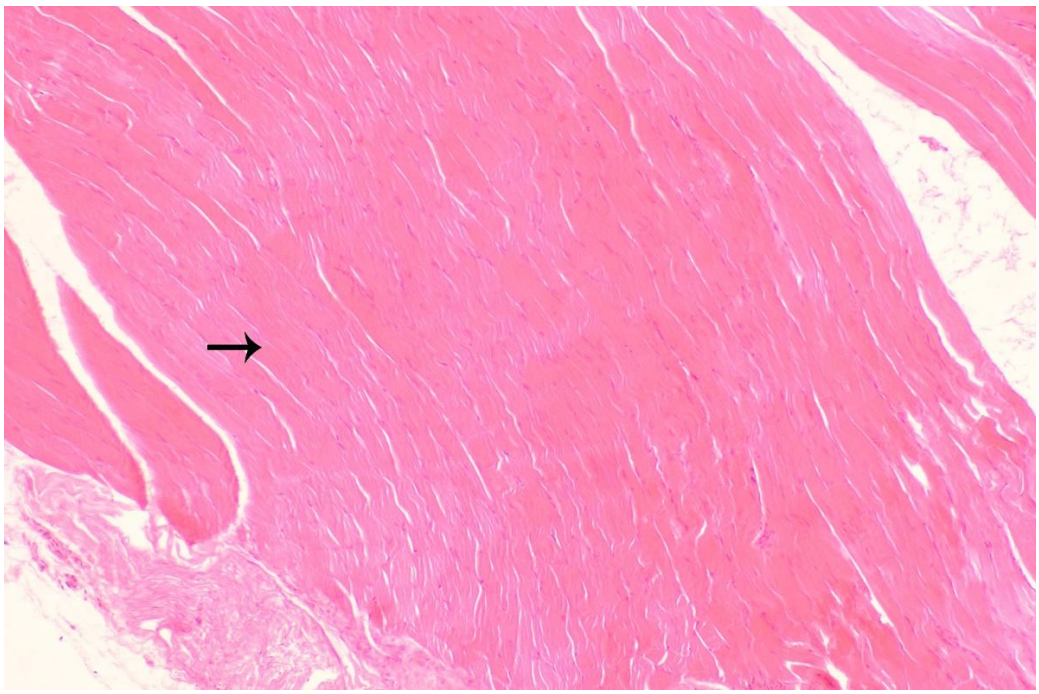
(Fig.6) Group I: Liver is showing normal hepatocytes (arrow) and normal histological architecture around central vein (CV). (HE, 400x)



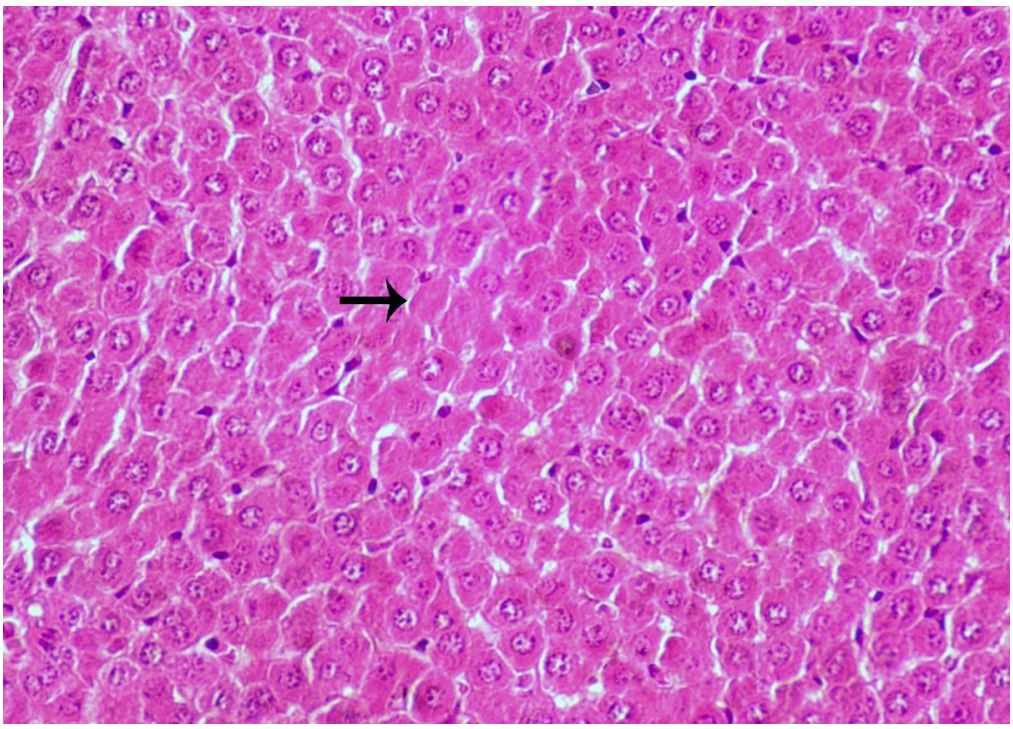
(**Fig.7) Group I**: Muscle is showing normal muscle fibers with normal sarcoplasm and normal nucleus (arrow). (HE, 400x)



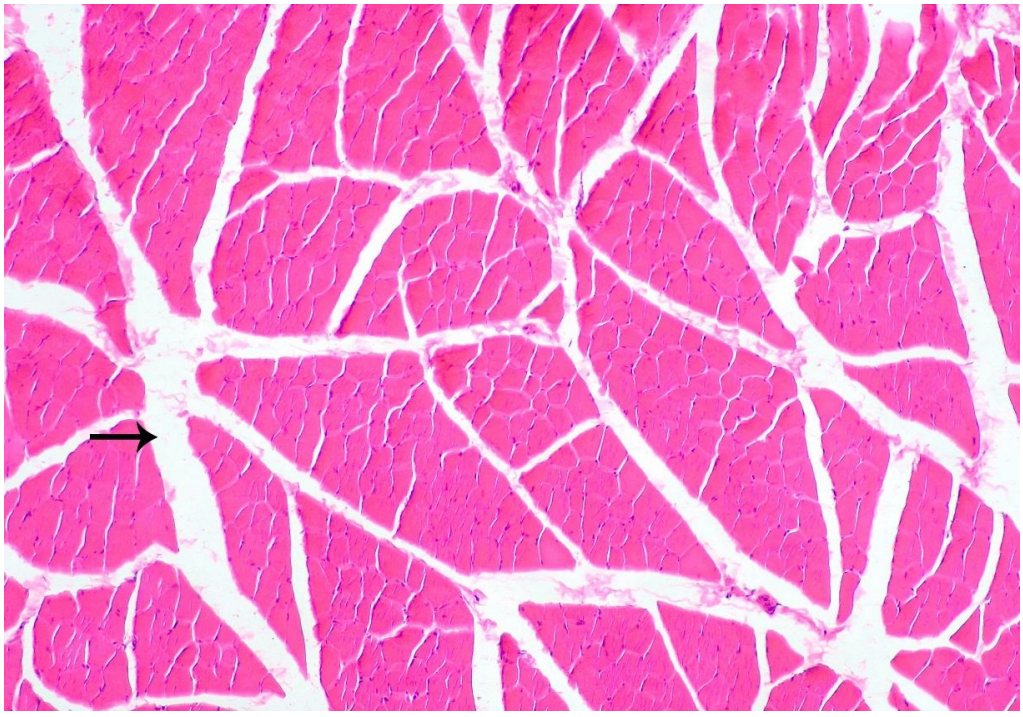
(Fig.8) Group II: Liver is showing normal hepatocytes (arrow) and normal histological architecture around central vein (CV). (HE, 400x)



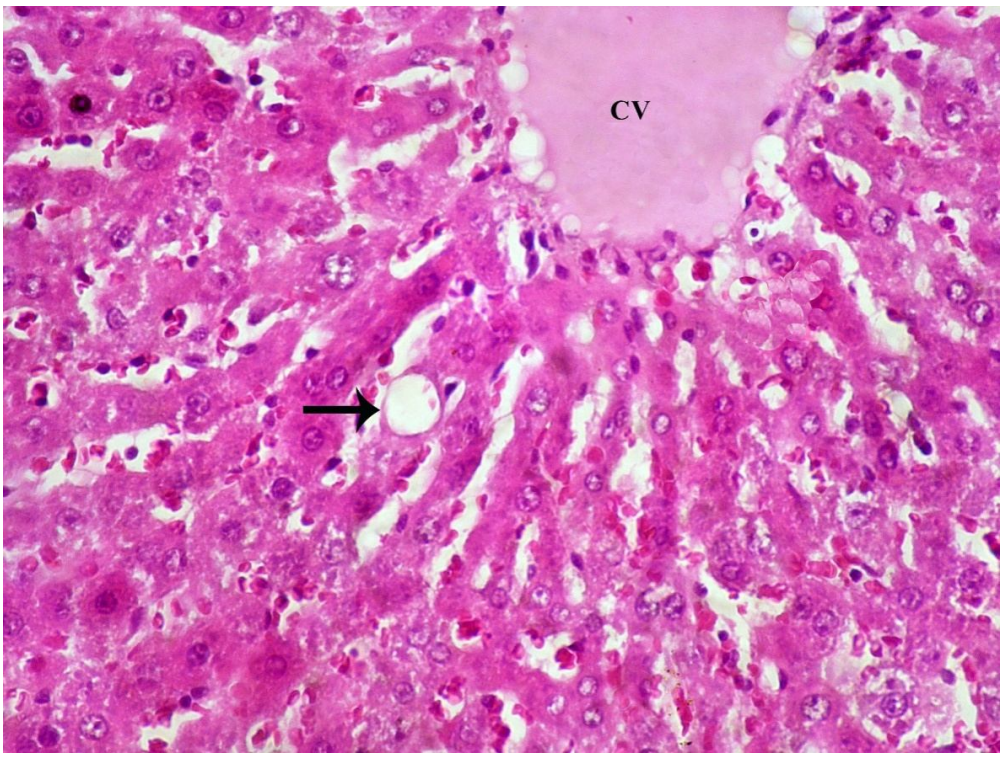
(Fig.9) Group II: Muscle is showing normal muscle fibers with normal sarcoplasm and normal nucleus (arrow). (HE, 400x).



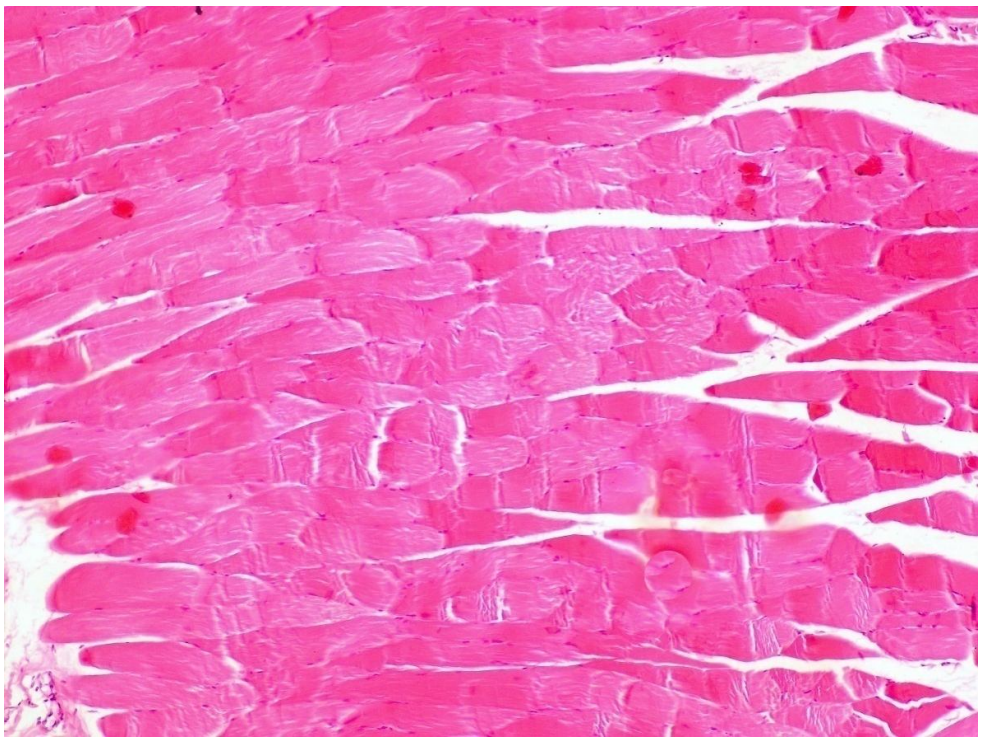
(Fig.10) Group III: Liver is showing mild degeneration hepatocytes (arrow) and normal histological architecture around central vein (CV). (HE, 400x).



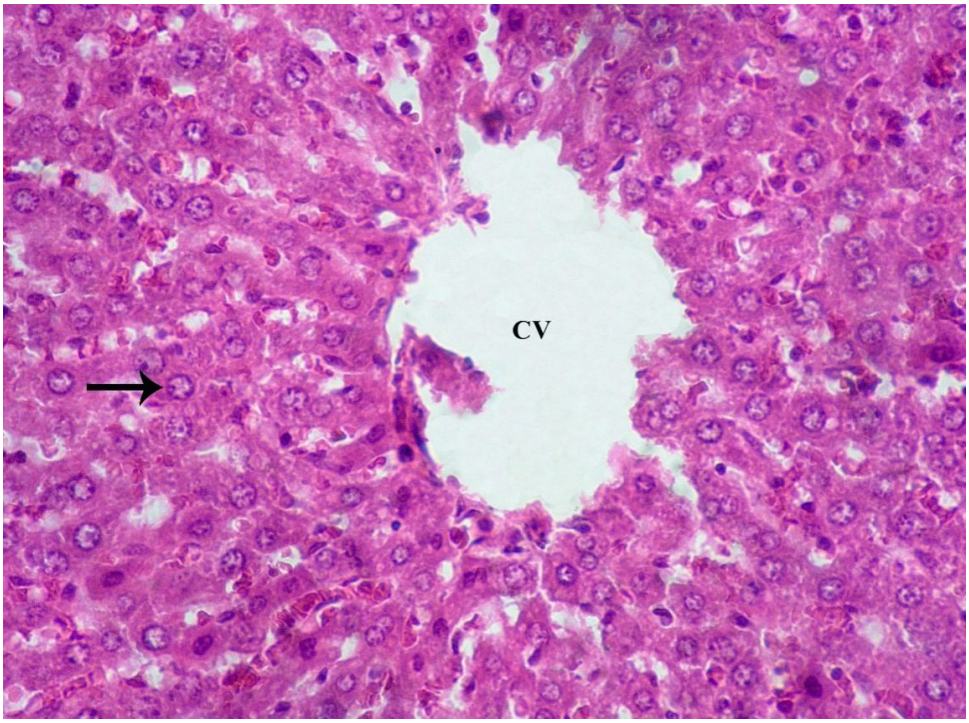
(Fig.11) Group III: Muscle is showing normal muscle fibers with mild widening of interstitial tissue (arrow). (HE, 400x).



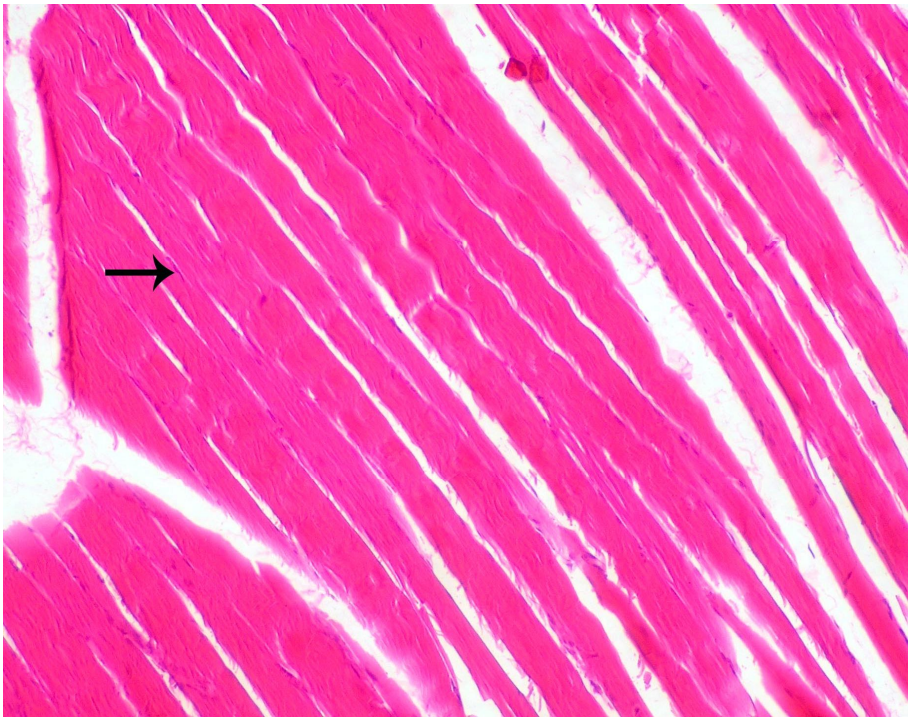
(Fig.12)Group IV: Liver is showing vacuolation of hepatocytes (arrow) and normal histological architecture around central vein (CV). (HE, 400x)



(Fig.13) Group IV: Muscle is showing normal muscle fibers with normal sarcoplasm (arrow). (HE, 400x)



(Fig.14) Group V: Liver is showing normal hepatocytes (arrow) and normal histological architecture around central vein (CV). (HE, 400x)



(Fig.15) Group V: Muscle is showing normal muscle fibers with normal sarcoplasm and normal nucleus (arrow). (HE, 400x).

RESULTS & DISCUSSION

1) Total&Free Carnitine concentration:

In contrast of **Wolf and Berger,(1961)** study which proposed that lysine couldn't be a precursor of carnitine in rat .Our study showed the importance of lysine and methionine in carnitine synthesis in the liver. With a notice of high concentration of lysine and methionine combination showing markedly increase in carnitine level. Subsequently , low methionine concentration leading to significant decrease in carnitine synthesis as showen in group IV

Based on **COX and HOPPEL**,**(1973)** methionine is a source of methyl group to forming trimethyllysine (methyl donor)

According to **Lindstedt and Lindstedt (1970)** rat muscle has no ability to hydroxylate γ –butyrobetaine so, muscle can't produce carnitine. Only liver and testis have the ability to form carnitine. **2)Acylcarnitine concentration**

According to, **Sewell and Bohles , (1995)** both free and total carnitine and acyl carnitine affected by each other. So , acylcarnitine has the same scientific indices with the concentration of free and total carnitine. Our study results showed the crucial role of lysine and methionine in carnitine synthesis in the muscle and liver that clearly appeared in group with high concentration of lysine and methionine combination which showed markedly increase in carnitine level .

3)Antioxidant status

a) Tissue Catalase activity:

In agreement with **Al-Malki, (2015)** our results showed that low and normal levels of lysine in diet leading to significant decrease in catalase activity. On the other hand **Mori and Hirayama, (2000)** suggested that methionine has a role in CAT increase which was due to long term supplementation of methionine **.**

b)Tissue Nitric Oxide (NO)

According to **White (1985) and Bogle et al.,(1992)** lysine is a competitive inhibitor of arginine which considered as a rate limiting step in NO synthesis .In our study results it was clearly that significant increase in nitric oxide occured in case of lysine and methionine combination either in low or high percent .With a notice of low lysine and methionine combination was markedly decrease than the high combination.

c)Tissue Glutathione Peroxidase (GPX)

In rabbits with methionine rich diet supplementation for 6 month there is markedly antioxidant enzyme elevation**(Toborek et al., 1995)**. It was indicated from the result of this study that low concentration of lysine and methionine either alone or in combination would result in a significant reduction in GPx activity.

d)Tissue Reduced Glutathione(GSH)

In our study result methionine showed an important role in increasing glutathione level. Although, the dietary supplementation of lysine and methionine either together or alone lead to a significant increase in reduced glutathione concentration.

Wang et al., (1997) proposed that the supplementation of methionine in diet increased the concentration of reduced glutathione by two fold due to the stimulation of γcystinyl glutamate synthetase

In a similar study, **JordaoJúnior et al., (2009)** mentioned that the methionine overload increased the concentration of cellular GSH.

e)Tissue Malondialdhyde(MDA)

MDA in muscle showed a significant elevation in MDA in Group V when compared with other Groups II, III and IV which was interacted with muscle membrane sensitivity and oxidative stress and lipid peroxidation due to its tendency to muscle dystrophy **(Murphy and Kehrer, 1989)**.

Liver and muscle lipid peroxidation level didn't change when rats supplied with normal level of lysine and methionine(**Heincinger et al., 2011)**.In contrast , adding excess lysine and methionine to the diet leading to increased LDL concentration in tissues and plasma **(Giroux et al., 1999)** . Moreover , it produced lipid peroxide when oxidized**(Princen et al., 1995)**.

f)Total antioxidant capacity (TAC)

Rats which were supplied with a diet contained both methionine and lysine showed a significant increase total antioxidant capacity concentration .On the other hand , rats which supplied with lysine only or methionine only or both but in low concentration showed a significant reduction in TAC. The same result was found in poultry**(Abd El-Wahab et al., 2015)** and in quails**(Abdel-Fattah et al., 2014)** also.

g) serum Creatin Kinase (CPK)

Rats in group I which supplied with low concentration of both lysine and methionine showed the highest creatin kinase activity.

High levels of cpk indicate myocardial damage**(Elberry et al .,2010)**.

h) Glutamic-Pyruvic Transaminase (GPT) & Glutamic- Oxaloacetic Transaminase (GOT) in serum:

Increasing levels of AST and ALT activities reflect liver injury which related with excess methionine concentration in a diet(**Ronald et al .,1992) .** The same found in rabbits , when supplied with high methionine concentration **(Taravati et al ., 2013)**

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