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RESEARCH PAPER

Histological Impacts of L-Arginine, Vitamin C and Their Combination on Liver and Kidney of Paracetamol Treated Rats

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A B S T R A C T:

Paracetamol as analgesic drugs makes hepatotoxicity and kidney damage in overdose situations. The present study investigates the protective effects of L-arginine and vitamin c against paracetamol toxicity. Thirty male rats with age of eight weeks divided into five groups: Control group with normal diet, Model animals received 500 mg/kg of Paracetamol. The Third group received 1% of L-arginine and same dose of paracetamol. Forth group received 1% of vitamin c plus paracetamol and the last group treated with a combination of all three chemicals. All drugs administrated orally in drinking water for 28 days. Liver and kidneys of the control group rats showed increasing in the weight while non-significant result was observed in the other groups. Histological investigation of the kidney of the model group shows inflammatory cell infiltration, tubular dilation and glomerulus damage, while liver sections shows sinusoid dilation inflammatory cell accumulation hepatocytes degeneration. L-arginine and vitamin C showed some protective effects when administrated alone but their combination has no good effects when compared with control group. In conclusion, L-Arginine administration has good protective effects against toxicity of liver and kidney induced by Paracetamol.

KEY WORDS: L-Arginine; Vitamin c; hepatotoxicity; nephrotoxicity
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1. INTRODUCTION

Paracetamol (Par.), as a pain reducing drug, is considered safe at convenient doses. Par. overdose makes liver damage and necrosis in laboratory animals and humans (Kaplowitz, 2005, Jaeschke et al., 2010). Basically, Par. metabolism is done by glucuronidation and sulphation; and a small amount changes to N-acetyl-p-benzoquinoimine (NAPQI) by cytochrome P450, which is quickly neutralized by glutathione (James et al., 2003).

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However, kidney damage is not more common than hepatotoxicity, but there is a chance for the development of acute renal failure without liver damage by Par. Indications. (McGill et al., 2012).

L-arginine (L- Arg.) as a semi-essential amino acid is utilized by all types of cells (Wu and Morris Jr, 1998). L-Arg. constitutes 7–5% of the total amino acids in the human's normal food intake and absorption process is in the jejunum and ileum portions of small intestine. L-Arg. participates in protein synthesis, tissue repair, urea cycle and immunity functions (White, 1985, Hendler and Rorvik, 2008). Immune system converts L-Arg. to Nitric oxide in the presence of enzyme nitric oxide synthase that has a role in cell signaling and oxidative bactericidal actions(Popovic et al., 2007). So, L-arginine can reduces infection rates, especially in

circumstances like surgery or critical illness(Böger, 2008).

Vitamin C (Vit. C), or ascorbic acid, plays roles in many biological functions. Vit. C participates in the production of collagen by taking a role in proline and lysine hydroxylation (Rebouche, 1991). Vit. C as a co-factor is used in different reactions of hydroxylation, for example, biological synthesis of cholesterol, catecholamines, ,and some hormones(Chatterjee et al., 1975). More ever Vit. is a strong antioxidant that prevents oxidation of low-density lipoproteins, boosts iron absorption and reduces free radicals in the stomach (Buettner and Jurkiewicz, 1996).

The aims of the experiment are to study the protective effect of L. Arg., Vit. C and their combination against paracetamol toxicity in kidney and liver of rats from histological points of view.

2. MATERIALS AND METHODS

2.1 Animal Grouping and Experimental Design

This study completed with thirty healthy male rats weighted between 200–250g with 8 weeks old. They harbored in polypropylene cage in the animal house belongs to the college of medicine, Hawler Medical University. All animals were bred under room temperature 25 ^{o}C with 12 hours of light and 12 hours dark pattern. Rats have had access to standard food and water ad libitum. The rats were distributed into five groups, each with six rats. Group I treated as control and receive only tap water (Control group). Other groups were treated as experimental groups. Group II was treated by paracetamol 500mg/kg (Model Group). Group III was receiving a combination of paracetamol (500mg/kg) and L-Arg. 1%, while group (Par.+L-Arg. group). Group IV were receiving a combination of paracetamol (500mg/kg) and vitamin C 1% (Par+ Vit. C group). Group V will be treated with a combination of all three substances (Par.+ L-Arg. + Vit. C Group). all drugs were dissolved in tap water and administered orally in drinking water (Chaudhary et al, 2019). The experiment conducted for 28 days.

2.2 Animal Dissecting and Tissue Processing

The animals were anaesthetized by intraperitoneal injection of combination Xylazine (12mg/Kg) and Ketamine hydrochloride (80mg/Kg). After scarification, livers and kidneys removed, weighted by digital balance then fixed in formal saline. Dehydration process was done by series of ascending concentrations of ethanol (50%, 70%, 95% and 100%), followed by clearing process by using of xylene then after paraffin infiltration and embedding completed. Samples have been sectioned by using rotary microtome in five micrometer thickness (Bright, MIC) following hematoxylin and Eosin staining protocols (H&E) (Suvarna et al., 2018). Light microscopy examination is completed by digital binocular compound microscope.

2.3. Statistical Analysis

Statistical analysis was done by GraphPad Prism program version 8.3 using one-way ANOVA analysis and Tukey as recommended post hoc test.

3. RESULTS AND DISCUSSION

3.1 Organs' weights

As shown in fig.1. A, liver weight in Model group increased significantly in compare of the control group while no significant difference between three other groups when compared with the control and Paracetamol group. Par. + L-Arg. group, demonstrated better liver weight improvement in respect of mean when compared to the model group. The hepatomegaly in Par. treated rats suggested liver tissue lesions and liver injury as a side effect for indications of Par. Accumulation of collagen and extracellular protein in hepatic tissue may be the main cause of increasing liver weight (Mahmood et al., 2014). Liver weight of rats treated with Par. + L. Arg. shown closer results to control and it's maybe due to role of arginine in decreasing liver damage(Engin et al., 2006) and lipid peroxidation, inflammation and free radicals(Butterworth and Canbay, 2019).

Both right and left kidneys weight (fig. 1 B & C) have not shown statistical change and there is a physiological increase of both kidneys weight belongs to Par. model group. There is a correlation between kidney damage and increasing kidney weight(Nematbakhsh et al., 2013). The Third

group of experimental rats that treated with L. Arg. Have shown kidneys' weight close to the control group and it's maybe due to a role of arginine in decreasing oxidative stress(Kaul et al., 2008, Suschek et al., 2003).

3.2 Kidney and liver histology

As illustrates in figure 2A, the kidney section of control group has normal histological and structural appearance while the Par. model group showed glomerular shrinkage and degeneration, destruction of bowman capsule, inflammatory cells, tubular dilation, casts, cell debris in kidney tubules (fig. B 1,2&3).

The results are similar to previous researches that have reported kidney damage by Paracetamol inductions (ROSITA, 2018, Moshaie-Nezhad et al., 2019). The nephrotoxic effects of paracetamol may due to interfering with apoptotic pathways including activation of caspases and decreasing Bcl-xL protein. It's reported that Par. Can makes oxidative stress and inflammation in the kidney (Lorz et al., 2005). Free radicals that produce as byproducts of Par. Metabolism could make glomerular damage and inflammatory cell infiltration (Singh et al., 2006). Renal tubule necrosis is one of the side effects of Par. Overdose (Goodman and Gilman, 2006).

glutathione and cytochrome p450 system have had a critical role in toxicity of Paracetamol. However toxic materials are concentrated in proximal tubule as a result of secretion and reabsorption activities, Cytochrome p450 is generally situated in the proximal tubule and in less amount in the distal convoluted tubule, glomerulus and collecting duct. (McGill et al., 2012, Mazer and Perrone, 2008).

Light microscopy examination of kidneys treated with Par.+L-Arg. Shown good improvement and histological protection but still, some tubular dilations and cell debris observed (fig. 3.A).

L-Arg. supplementation effect against paracetamol induced nephrotoxicity. L-Arg. has a role in the immune system for cell signaling and oxidative action therefore it can reduce inflammation (Popovic et al., 2007, Kalil and Danner, 2006).more ever It's reported that L-Arg. could reduce oxidative stress (Dawoud and Malinski, 2020) and improve the antioxidant system in rats(Silva et al., 2017).

Paraffin sections of kidneys belong to Par.+Vit. C group have shown some protective results overall but tubular dilation, glomerular degeneration observed as well (fig. 3.B). The protective effect of Vit. C may due to its antioxidant activity(Grosso et al., 2013). Vitamin C has a role in reducing allergic reaction, boosting of the immune system and participates in infection elimination (Chambial et al., 2013).

Cross section of kidneys treated with Par. & combined L. Arg./Vit. C showed few protective effects, glomerular degeneration, tubular dilation and debris (fig. 3.C). surprisingly the combined L. Arg. And Vit. C supplementation could not show better protective result and its controversy with the previous finding that their combinations shown better anti-inflammatory and antioxidant features(Suliburska et al., 2014)

Paraffin section through the liver of control group shown normal structural and histological view (Fig. 4. A). As illustrated in Fig. 4. B 1,2 & 3, several histological alterations like inflammatory cells infiltration, central vein congestion, sinusoid dilation, hepatic tissue dissociation, necrotic cells and shading of cells have been noticed. The results are similar to previous researches(Jarsiah et al., 2017, Singh et al., 2015). In liver as a major organ of par. Metabolism , most of Par. is converted to sulfate and glucuronide, and the rest, converted to N-acetyl-p-benzoquinone imine (NAPQI) (Aycan et al., 2014). NAPQI is a reactive chemical and detoxified by enzyme glutathione peroxidase. Excessive production of NAPQI causes liver damage as a result of decline in the concentration of glutathione (Aycan et al., 2014, Omidi et al., 2014). At the time of glutathione deficiency and presence of NAPQI, the production of free radicals like hydrogen peroxide, superoxide and hydroxyl radicals has been increased and leads to oxidative stress (Merhi et al., 2019).

As shown in Fig. 5. A 1&2, the liver sections of liver treated with Par.& L.-Arg. were in approximately normal tissue structure regarding to hepatocyte and Central vein arrangements and appearance but a few inflammatory cell infiltrations have been noticed.

The observed results may due to the power of L-Arg. in the improvement of antioxidant system and reducing free radicals(De Nigris et al., 2003). L-Arg. act as a precursor for nitric oxide that plays a role in scavenging of reactive chemical species.(Ahmad et al., 2015). More ever It's been reported that L. Arginine can promotes cellular

proliferation and decreases cell death (Greene et al., 2013)

Figure 5.B 1&2 illustrated liver sections of Par.+ Vit. C treated group with inflammatory cells and congested central veins but protected from other damages. The protective results may refer to the potential of Vit. C to decreasing cell damages and its role in boosting of glutathione antioxidant.

In Liver sections of Par.+ L-Arg.+Vit. C treated group some Inflammatory cells, congested central vein and sinusoid dilations observed (Fig. 5. C1&2). Vitamin C can act as a strong antioxidant whereas at the same time it can show free radical promoter activities and produce reactive substances (Pehlivan, 2017). Chemical interactions could not be neglected as a cause for the elimination of combined Vit. C and L. Arg. effects.

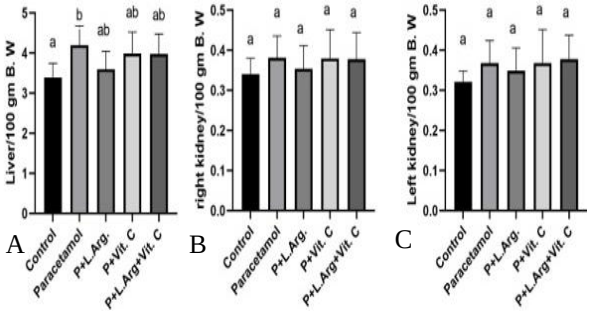

Figure 1. showing the weight of liver (A), right(B) and left kidney (C)per 100gm of body weight. Different letters on bars mean significant change and the same letters means no significant change

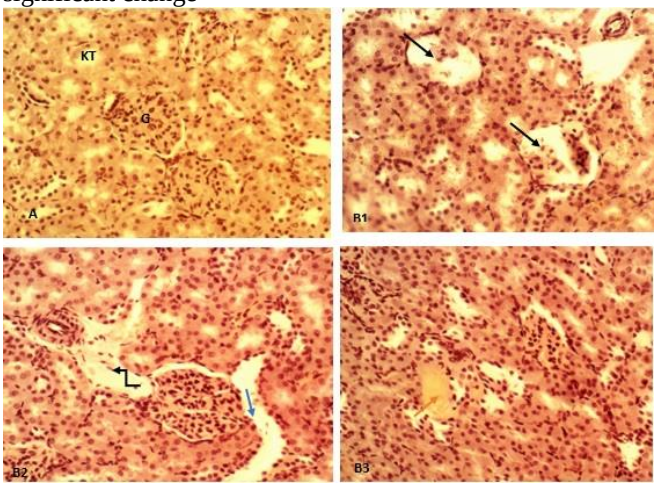

Figure 2. showing cross section through kidneys of the control group (A) and paracetamol group (B 1,2 and 3). Control group showing normal appearance in the glomerulus (G) and kidney tubules (KT). Paracetamol group showing glomerular shrinkage and degeneration (black arrow), tubular dilation (blue arrow), cellular debris (elbow arrow) and casts (red arrow). H and E stain 400 X

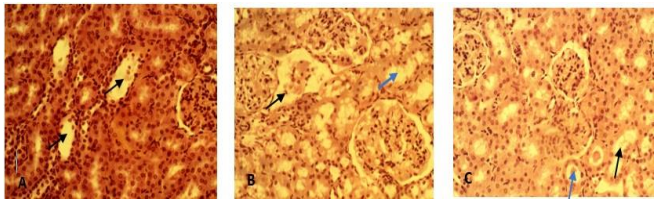

Figure 3. cross section through kidneys of rats treated with Par. & L. Arg. (A), P& vit. C (B) and P& L. Arg./Vit. C (C). Arg. group shows some tubular dilation and cell debris inside kidney tubules (black arrow) with normal glomerulus. Vit-c group shows some glomerular degeneration (black arrow) and tubular dilation (blue arrow). Arg.-Vit-c group shows some tubular dilation (black arrow) and cell debris (blue arrow) with deformed glomerulus. H&E stain, 400 X

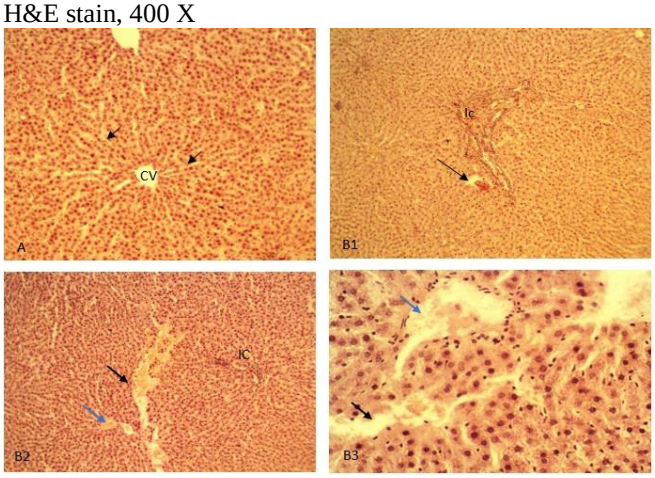

Figure 4. Figure 4 A, shows section of the liver belong to the control group with normal central vein (CV) and sinusoids (s). H & E stain. 100x. Histological sections through liver of the Paracetamol treated group (B1,2,3) show Inflammatory cells (IC), congested central veins (blue arrow) and sinusoid dilation (black arrow) filled with shaded hepatocytes. B1,2 H &E. 100x, B3 H &E 400X.

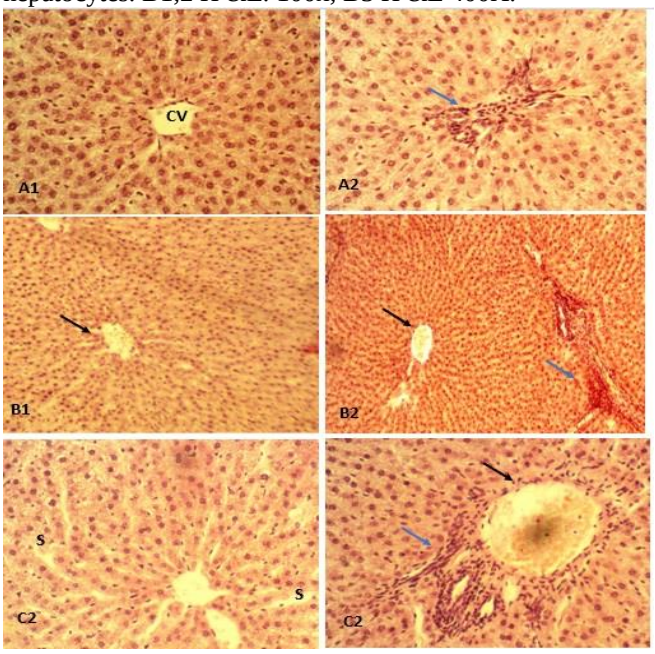

Figure 5. A1,2 show cross section of rat liver treated with Paracetamol and Arg. with approximately normal hepatic structure and central vein (CV), some inflammatory cells

(blue arrow) were seen. H& E stain 400 X. B1,2 cross section of liver belongs to Paracetamol and Vit. C group show central vein congestion (black arrow) and inflammatory cells (blue arrow) H& E stain 100 X. fig. C1,2 show liver section of rats treated with paracetamol, Arg, and Vit. C. Inflammatory cells (blue arrow), congested central vein (black arrow) and dilated sinusoids observed. H& E stain 400X

4. CONCLUSIONS

From the present study, it can be understood that L. arginine shows a good protective effect against Paracetamol toxicity. Vitamin C also shows some positive effect as well. Both Vit. C and L. Arg. show better result compared to their combination.

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References

AHMAD, A., SATTAR, M., RATHORE, H. A., HUSSAIN, A. I., KHAN, S. A., FATIMA, T., AFZAL, S., ABDULLAH, N. A. & JOHNS, E. J. 2015. Antioxidant activity and free radical scavenging capacity of L-arginine and NaHS: A comparative in vitro study. *Acta Pol Pharm,* 72**,** 245-52.

AYCAN, İ. Ö., TUFEK, A., TOKGöZ, O., EVLIYAOĞLU, O., FıRAT, U., KAVAK, G. Ö., TURGUT, H. & YUKSEL, M. U. 2014. Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. *International Journal of Surgery,* 12**,** 213-218.

BöGER, R. H. 2008. L-Arginine therapy in cardiovascular pathologies: beneficial or dangerous? *Current Opinion in Clinical Nutrition & Metabolic Care,* 11**,** 55-61.

BUETTNER, G. R. & JURKIEWICZ, B. A. 1996. Catalytic metals, ascorbate and free radicals: combinations to avoid. *Radiation research,* 145**,** 532-541.

BUTTERWORTH, R. F. & CANBAY, A. 2019. Hepatoprotection by L-Ornithine L-Aspartate in Non-Alcoholic Fatty Liver Disease. *Dig Dis,* 37**,** 63-68.

CHAMBIAL, S., DWIVEDI, S., SHUKLA, K. K., JOHN, P. J. & SHARMA, P. 2013. Vitamin C in disease prevention and cure: an overview. *Indian Journal of Clinical Biochemistry,* 28**,** 314-328.

CHATTERJEE, I., MAJUMDER, A., NANDI, B. & SUBRAMANIAN, N. 1975. Synthesis and some major functions of vitamin C in animals. *Annals of the New York Academy of Sciences,* 258**,** 24-47.

DAWOUD, H. & MALINSKI, T. 2020. Vitamin D₃, L-Arginine, L-Citrulline, and antioxidant supplementation enhances nitric oxide bioavailability and reduces oxidative stress in the vascular endothelium – Clinical implications for cardiovascular system. *Pharmacognosy Research,* 12**,** 17-23.

DE NIGRIS, F., LERMAN, L. O., IGNARRO, S. W., SICA, G., LERMAN, A., PALINSKI, W., IGNARRO, L. J. & NAPOLI, C. 2003. Beneficial effects of antioxidants and L-arginine on oxidation-sensitive gene expression and endothelial NO synthase activity at sites of disturbed shear stress. *Proceedings of the National Academy of Sciences,* 100**,** 1420-1425.

ENGIN, A., ZEMHERI, M., BUKAN, N. & MEMIS, L. 2006. Effect of nitric oxide on the hypoglycaemic phase of endotoxaemia. *ANZ J Surg,* 76**,** 512-7.

GOODMAN, L. S. & GILMAN, A. 2006. *The pharmacological basis of therapeutics.*, McGraw-Hill.

GREENE, J. M., FEUGANG, J. M., PFEIFFER, K. E., STOKES, J. V., BOWERS, S. D. & RYAN, P. L. 2013. L-arginine enhances cell proliferation and reduces apoptosis in human endometrial RL95-2 cells. *Reproductive Biology and Endocrinology,* 11**,** 15.

GROSSO, G., BEI, R., MISTRETTA, A., MARVENTANO, S., CALABRESE, G., MASUELLI, L., GIGANTI, M. G., MODESTI, A., GALVANO, F. & GAZZOLO, D. 2013. Effects of vitamin C on health: a review of evidence. *Front Biosci (Landmark Ed),* 18**,** 1017-29.

HENDLER, S. S. & RORVIK, D. M. 2008. *PDR for nutritional supplements*, Thomson Reuters.

JAESCHKE, H., WILLIAMS, C. D., MCGILL, M. R. & FARHOOD, A. 2010. Herbal extracts as hepatoprotectants against acetaminophen hepatotoxicity. *World journal of gastroenterology: WJG,* 16**,** 2448.

JAMES, L. P., MAYEUX, P. R. & HINSON, J. A. 2003. Acetaminophen-induced hepatotoxicity. *Drug metabolism and disposition,* 31**,** 1499-1506.

JARSIAH, P., NOSRATI, A., ALIZADEH, A. & HASHEMI-SOTEH, S. M. B. 2017. Hepatotoxicity and ALT/AST enzymes activities change in therapeutic and toxic doses consumption of acetaminophen in rats. *International Biological and Biomedical Journal,* 3**,** 119-124.

KALIL, A. C. & DANNER, R. L. 2006. L-Arginine supplementation in sepsis: beneficial or harmful? *Current opinion in critical care,* 12**,** 303-308.

KAPLOWITZ, N. 2005. Idiosyncratic drug hepatotoxicity. *Nature reviews Drug discovery,* 4**,** 489-499.

KAUL, D. K., ZHANG, X., DASGUPTA, T. & FABRY, M. E. 2008. Arginine therapy of transgenic-knockout sickle mice improves microvascular function by reducing non-nitric oxide vasodilators, hemolysis, and oxidative stress. *American Journal of Physiology-Heart and Circulatory Physiology,* 295**,** H39-H47.

LORZ, C., JUSTO, P., SANZ, A. B., EGIDO, J. & ORTIZ, A. 2005. Role of Bcl-xL in paracetamol-induced tubular epithelial cell death. *Kidney international,* 67**,** 592-601.

MAHMOOD, N., MAMAT, S., KAMISAN, F., YAHYA, F., KAMAROLZAMAN, M., NASIR, N., MOHTARRUDIN, N., TOHID, S. & ZAKARIA, Z. 2014. Amelioration of paracetamol-induced

hepatotoxicity in rat by the administration of methanol extract of Muntingia calabura L. leaves. *BioMed research international*, 2014.

MAZER, M. & PERRONE, J. 2008. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. *Journal of Medical Toxicology*, 4**,** 2-6.

MCGILL, M. R., WILLIAMS, C. D., XIE, Y., RAMACHANDRAN, A. & JAESCHKE, H. 2012. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. *Toxicology and applied pharmacology*, 264**,** 387-394.

MERHI, Z., GARG, B., MOSELEY-LARUE, R., MOSELEY, A. R., SMITH, A. H. & ZHANG, J. 2019. Ozone therapy: a potential therapeutic adjunct for improving female reproductive health. *Medical gas research*, 9**,** 101.

MOSHAIE-NEZHAD, P., HOSSEINI, S. M., YAHYAPOUR, M., IMAN, M. & KHAMESIPOURE, A. 2019. Protective effect of ivy leaf extract on paracetamol-induced oxidative stress and nephrotoxicity in mice. *Journal of Herbmed Pharmacology*, 8.

NEMATBAKHSH, M., ASHRAFI, F., NASRI, H., TALEBI, A., PEZESHKI, Z., ESHRAGHI, F. & HAGHIGHI, M. 2013. A model for prediction of cisplatin induced nephrotoxicity by kidney weight in experimental rats. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, 18**,** 370.

OMIDI, A., RIAHINIA, N., TORBATI, M. B. M. & BEHDANI, M.-A. 2014. Hepatoprotective effect of Crocus sativus (saffron) petals extract against acetaminophen toxicity in male Wistar rats. *Avicenna journal of phytomedicine*, 4**,** 330.

PEHLIVAN, F. E. 2017. Vitamin C: An antioxidant agent. *Vitamin C***,** 23-35.

POPOVIC, P. J., ZEH III, H. J. & OCHOA, J. B. 2007. Arginine and immunity. *The Journal of nutrition*, 137**,** 1681S-1686S.

REBOUCHE, C. J. 1991. Ascorbic acid and carnitine biosynthesis. *The American journal of clinical nutrition*, 54**,** 1147S-1152S.

ROSITA, Y. 2018. Nephroprotective Activity of Ethanol Extract of Curcuma Mangga val. In paracetamol-Induced Male Mice.

SILVA, E. P., BORGES, L. S., MENDES-DA-SILVA, C., HIRABARA, S. M. & LAMBERTUCCI, R. H. 2017. l-Arginine supplementation improves rats' antioxidant system and exercise performance. *Free Radical Research*, 51**,** 281-293.

SINGH, A., KUMAR, G. R., GUPTA, S. S., SINGH, S., RAWAT, A. & RAO, C. V. 2015. Hepatoprotective Potential of Ziziphus oenoplia (L.) Mill Roots against Paracetamol-Induced Hepatotoxicity in Rats. *AJPCT*, 3**,** 64-78.

SINGH, D., KAUR, R., CHANDER, V. & CHOPRA, K. 2006. Antioxidants in the prevention of renal disease. *Journal of medicinal food*, 9**,** 443-450.

SULIBURSKA, J., BOGDANSKI, P., KREJPCIO, Z., PUPEK-MUSIALIK, D. & JABLECKA, A. 2014. The effects of L-arginine, alone and combined with vitamin C, on mineral status in relation to its antidiabetic, anti-inflammatory, and antioxidant properties in male rats on a high-fat diet. *Biological trace element research*, 157**,** 67-74.

SUSCHEK, C. V., SCHNORR, O., HEMMRICH, K., AUST, O., KLOTZ, L.-O., SIES, H. & KOLB-BACHOFEN, V. 2003. Critical role of L-arginine in endothelial cell survival during oxidative stress. *Circulation*, 107**,** 2607-2614.

SUVARNA, K. S., LAYTON, C. & BANCROFT, J. D. 2018. *Bancroft's theory and practice of histological techniques E-Book*, Elsevier Health Sciences.

WHITE, M. F. 1985. The transport of cationic amino acids across the plasma membrane of mammalian cells. *Biochimica et biophysica acta*, 822**,** 355-374.

WU, G. & MORRIS JR, S. M. 1998. Arginine metabolism: nitric oxide and beyond. *Biochemical Journal*, 336**,** 1-17