Original article:

The Effect of *Jatropha Curcas* L Seed Extract in Oxidatve Stress and Central Vein of Liver Tissue

Putri Nurbaeti[1], Rr Ayu Fitri Hapsari[2], Endah Wulandari[3]

Abstract

Introduction*:Jatropha curcas* L is a tropical plant, besides containing antioxidants it also contains toxic compounds. This plant is believed to be anti-inflammatory, antimicrobial, antibacterial and antifertilization. It is suspected that this plant can damage tissues through oxidative stress. The liver as an organ of central metabolism and detoxification. This plant is not yet known its effect on the liver when consumed, especially on hepatocyte cells and blood flow to the central veins of the liver.***Method*:** Mice were given a dose of jatropha seed extract (0, 5, 25, 50, 250 mg/KgBW) for 28 days. Liver homogenates were measured for MDA levels, GSH activity and their correlation using a spectrophotometry. besides measuring the diameter of the central vein of the liver. ***Results*:** MDA levels after being given Jatropha seed extract: control (0); 5; 25; 50; 250 mg/KgBW were 0.818; 1,363; 2,043; 2,720; 2,518 nmol / ml and GSH activity was 0.979; 0675; 0.621; 0.463; 0.544 µg / ml. Vein diameter 169; 140; 24; 36; 30 µm and the smallest at a dose of 25 mg / BW was 23.7 µm (ANOVA, $p < 0.05$). The correlation of MDA and GSH levels was very strong negative (Pearson: -0918; $p < 0.01$). ***Discussion*:**The higher dose of jatropha seed extract, significantly increases MDA levels and decreases GSH activity. The correlation between the two is very strong negative negative correlation is very significant. Jatropha seed extract with high doses causes small central vein diameters of the liver.

Keyword: *Jatropha curcas* L, GSH, MDA, central vein, liver.

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Introduction:

The *Jatropha curcas* L is a plant that grows in the tropics, including in Indonesia. Jatropha plant. Jatropha curcas trusted by the community has various benefits in the field of health (medical). Jatropha seeds contain active compounds, such as: curcin, flavonoids, alkaloids, viteksin, isoviteksin, lectin, phorbol and esters.[1-3] The active compound can function as an anti-inflammatory, wound

The results of previous studies, it has been proven that jatropha seeds as antifertilization,[4-6] but it is still needs further research.

Another study stated that administration of Jatropha seed extract caused damage to the reproductive organs of male rats of the Sparague Dawley species, resulting in a decrease in spermatogenesis.The provision of Jatropha seed extract was able to provide a wound healing effect on liver damage.[7] The effect of tissue damage and the protective effect, researchers conducted research by measuring levels of free radicals and antioxidant activity. Jatropha seeds that also contain toxic compounds that are harmful to the body.[8] These toxic compounds will react with various compounds and form electrons without pairs in their outer orbitals, called free radicals. These free radicals can cause damage to cells in the tissues, especially in the process of combining double lipid lipids and decomposing them into malondialdehyde (MDA). Increasing MDA levels is one indicator of the occurrence of lipid peroxidation by free radicals.[9]

1. Medical Student, Student Research Committee, Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah, Jakarta. Indonesia
2. Lecturer of Histology Department, Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah, Jakarta, Indonesia
3. Lecturer of Biochemistry Department, Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah, Jakarta, Indonesia

Correspondence to: Endah Wulandari, Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah, Jakarta, Indonesia. **Email:** endah.wulandari@ uinjkt.ac.id.

The liver as the center of metabolism, therefore easily accumulates free radicals. Liver parenchymal cells also have an important role in regulating free radicals and handling oxidative stress through various mechanisms of antioxidant formation. Glutathione S-Transferase (GSH), an endogenous antioxidant whose role is to detoxify which regulates counteracting toxic compounds from conditions of liver oxidative stress.[10,11]Selenium was an important trace element due to as a component of antioxidants enzymes (selenoproteins), including glutathione peroxidase for protection against free radical.There was no correlation between blood selenium level and plasma glutathione peroxidase activity.[12]When free radical production increases or the system of culling is not effective, there will be a buildup of free radicals, oxidative stress arises. Increased levels of MDA that are not offset by the amount of antioxidant GSH that is formed will cause hepatocyte damage. Hepatocytes play an important role in protein synthesis in the rough endoplasmic reticulum (RE) while refined RE plays a role in the oxidation, methylation, and conjugation process for inactivation or detoxification of various substances before being excreted. Damage to the hepatocytes will cause the detoxification process to not go well, dangerous substances carried by the bloodstream in the liver system, this will lead to central vein.[13-16] In this study this study also measured the diameter of the liver central vein. Based on the background above, this study was conducted to determine the effect of Jatropha curcas L extract on the oxidative stress and image of the liver central veins.

Methods and Materials:

The study performed analytical experimental study. Liver tissues were obtained from 25 rats with 5 group given doses of extract jathropha seed (0, 5, 25, 50 and 250 mg/KgBW) for 28 days. The study had been approved by the Medical and Health Research Ethic Committee, Faculty of Medicine, University of Indonesia (SK No 68/UN2.F1/ETIK/2015). The study was conducted in Faculty of Medicine, State Islamic University SyarifHidyatullah, Jakarta. The evaluation of GSHactivity and MDAlevels using spectrofotometry was performed at the Laboratory of Biochemistry and evaluation histology of liver at laboratory of histology.

Materials utilized in the study were as followed: rat of Sprague-Dawley, extract *Jatropha curcas L* seed from BALITRO institution (Bogor), the materials were measured GSHactivity (trikloroasetat TCA 5%, DTNB, and phosphate Buffer of pH 8,0) and MDA levels (TCA 10%, and 2-thiobarbituric acid (TBA) 0,67%), phosphate buffered saline (PBS) pH 7.4, terraced alcohol, toluene, paraffin, Hematoxylin-Eosin (HE), canada balsam.

MDA Level Measurement.The prepare 0.125 ml of liver tissue homogenate in a centrifugation tube. Then add 10% TCA solution as much as 0.25 ml. After that, it is centrifuged at 5000 rpm for 5 minutes. Then take the supernatant, transfer it to the test tube and add 0.75% TBA solution as much as 0.375 ml. Incubate in a boiling water bath for 10 minutes then remove and cool. The absorbance results were read using a spectrophotometer with a wavelength of 532 nm. The determine MDA level by comparing the MDA standard curve

Measurement of GSH Activity. The prepare 25 µL homogenate liver tissue in a micro tube. then add 100 µL of 5% TCA, to precipitate the protein, and shake until homogeneous. Then add 875 µL buffer phosphate pH 8.0. Next centrifuged at 3200 rpm for 10 minutes. The supernatant was taken and transferred to another micro tube and added 12.5 µL DTNB in a phosphate buffer of pH 7.0 (1: 1) and then incubated for one hour. Absorption is measured at a wavelength of 412 nm with a spectrophotometer. The determine GSH level by comparing the GSH standard curve.

Preparing Histological Slides.The liver tissue obtained was immersed in cold 0.9% NaCl; then, it was cut in 3-5 mm thickness. Furthermore, a fixation was performed by transferring it into 10% formalin solution. Next, dehydration was performed by immersing the specimen in increasing concentration of 70% alcohol incubated for 24 hours, 80% alcohol incubated for 24 hours, 95% alcohol incubated for 24 hours, 100% alcohol for 2 x 24 hours. Afterward, clearingwas performed by immersing the specimen in xylol 2 x 24 hours. Then, the embedding was performed, i.e. by infiltrating the specimen with liquid paraffin. After the tissue specimen was ready, it was cut into sections with microtome in 4-5 µm thickness. The sections were then taken using a brush and were transferred to a water bath so that they were allowed to widen. The sections were carefully transferred to a warm water bath at 40-46°C. At this point, the sections were trimmed and transferred into a slide that had been smeared with eiwit (egg white and glycerin), which served as an adhesive. The slide and tissue specimen on it were set in a special shelf and transferred into

incubator at 40-60°C for 24 hours or until the slide was ready for staining.Histologicstainingis donewithHematoxylin-Eosin. Cell qualification was performed in 5 high power field (HPF) for each
slide of liver tissue. High power field was determined as 20x magnification. Then measure the average diameter of the liver central vein, through cross sectional cuts.

Results:

The MDA Level and GSH Activity After administration of Jatropha Seed Extract.In Figure 1.A shows that the more doses of jatropha seed extract, the more increased MDA levels in rat liver (ANOVA; p <0.05). The highest MDA level is at a dose of 50 mg / BBKg of Jatropha seed extract. In contrast to Figure 1.B, there was a purification of GSH activity after administration of Jatropha seed extract (ANOVA ; p <0.05). The lowest GSH activity was highest at a dose of 50 mg/BBKg of Jatropha seed extract. The results of this study indicate the occurrence of oxidative stress where MDA levels are higher and GSH activity is low compared to controls.

In figure 1.C. Results Correlation was very significantly negative between MDA levels and GSH activity (Pearson Correlation; -0.918; p <0.01).

At a dose of 250 mg / BBKg Jatropha seed extract there is an improvement in liver oxidative stress due to the removal of toxic resin compounds in the extract.Figure 2 shows the hepatic central vein after being given various doses of castor bean extract. In the jatropha seed extract dose of 5 mg/ KgBW, the central vein vein was still in good condition, still the same as the control. Whereas the dose of 25-250 mg/KgBW of liver central vein vein appears small, the average yield of liver central vein diameter 24-36 µm compared to 169 µm control, this is significantly different.

Discussion:

The toxic compounds in jatropha seed extract, causing low GSH activity. This results in being unable to suppress the amount of free radicals formed. Imbalance of antioxidant and prooxidant production causes the accumulation of free radicals, causing oxidative stress. This is indicated by increasing levels of MDA as a biomarker of oxidative stress conditions.[17]

These oxidative stress conditions can cause cell damage through various reactions, such as lipid peroxidation, cross reactions and changes in protein, and DNA damage.[17] This condition is

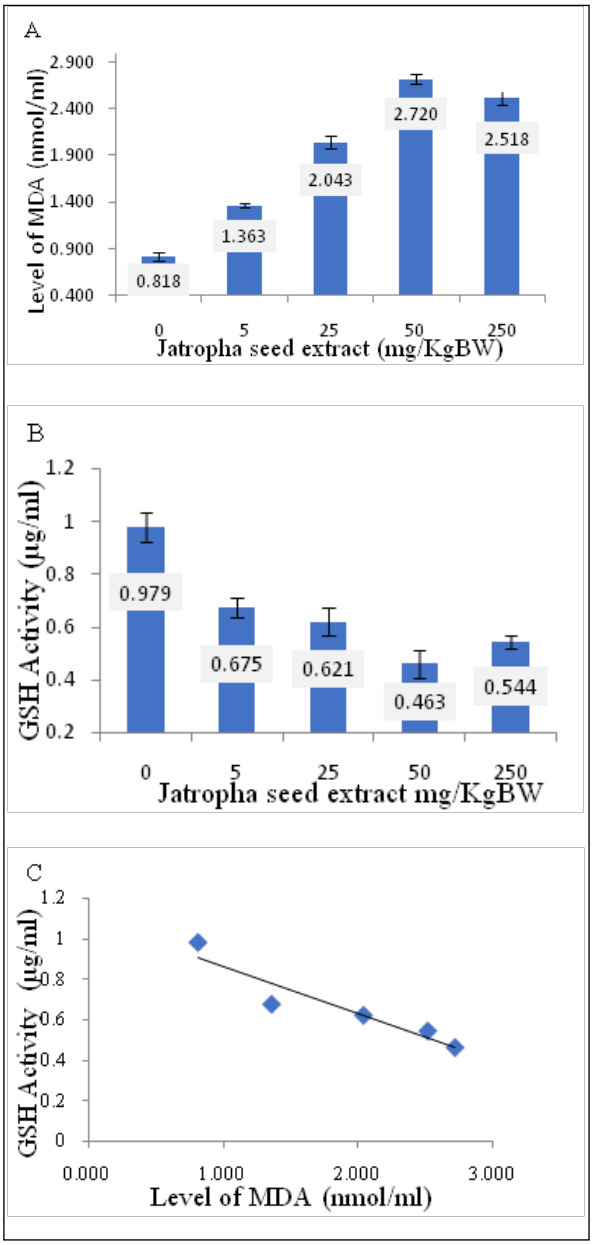
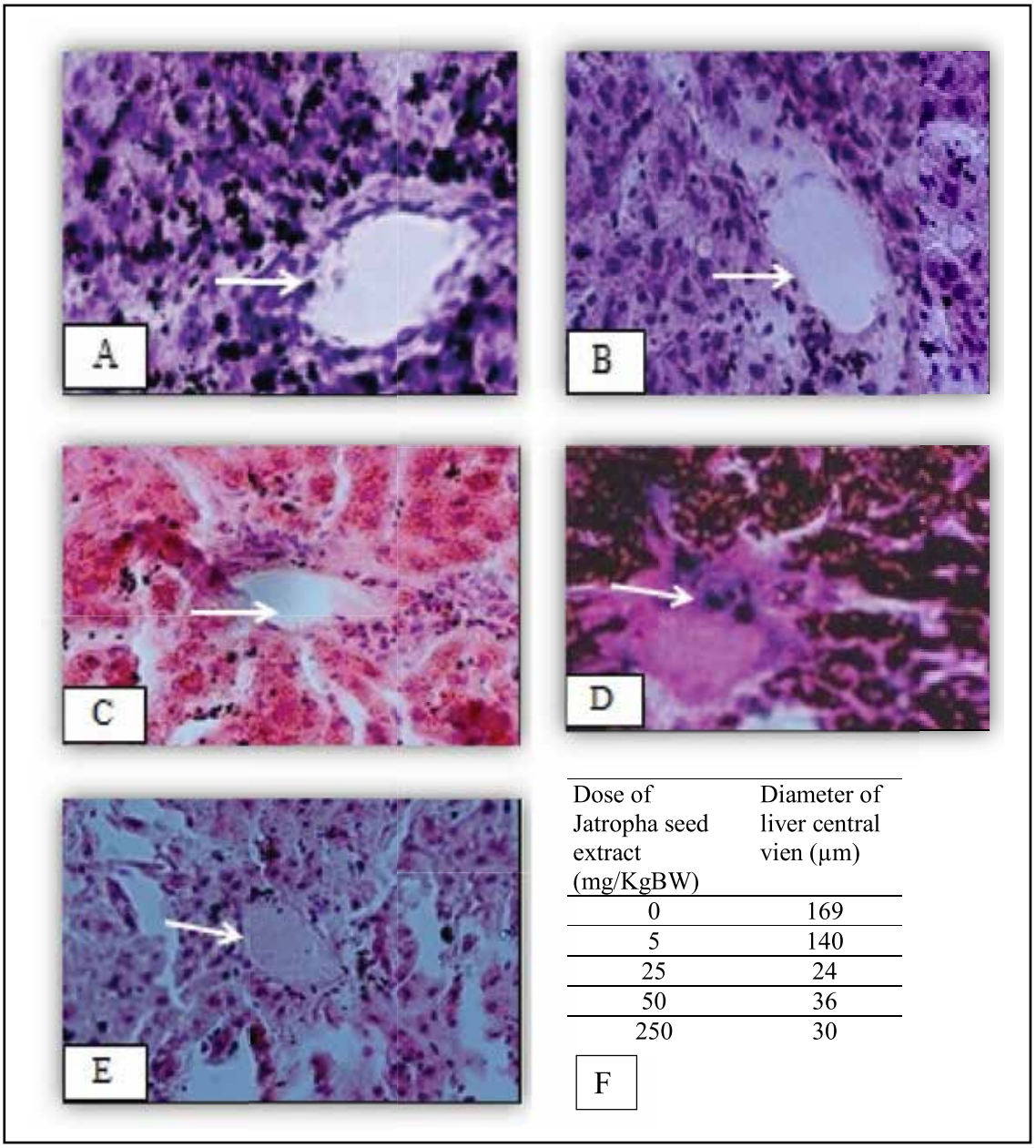


Figure 1. The Results Measurement of MDA Level and GSH Activity. (A) The results of measurement of MDA levels after administration of Jatropha seed extract (ANOVA, P <0.05), (B) Results of measurement of GSH activity after administration of Jatropha seed extract (ANOVA, P <0.05). (C) Results correlation of MD levels with GSH activity (Pearson correlation: -0,918; P <0.01).

in accordance with previous studies, the effect of jatropha seed extract reduces VEGF protein levels and tubular damage seminiferus. Decreased VEGF protein levels correlates with increased cell apoptosis. This is due to decreased cell protection against VEGF expression. The activation process of Caspase-3 increases, and causes apoptosis.[18,19] Jatropha seeds as antifertilization, can experience



Dose of Jatropha seed extract (mg/KgBW)	Diameter of liver central vien (µm)
0	169
5	140
25	24
50	36
250	30

Figure 2. The central veins are shown with white arrows (magnification 20x) (A) Jatropha seed extract dose of 0 mg/KgBW/control, (B) 5 mg/KgBW (C), 25 mg/KgBB (D), 50 mg/kgBB (E) 250 mg/kgBB. (F) The average diameter of liver centralvien.

damage such as the liver in this study, the tubular organs of the semiferus suffered damage causing obstructed the process of spermatogenesis. This condition results in atrophy and necrosis of the seminiferous tubules and damage to spermatozoa production.

The high GSH activity can be caused by the response of cells to protect themselves by increasing the production and antioxidant activity against free radicals. The GSH will reduce free radicals through electron donors so as to prevent lipid peroxidation.[20,21] Prevention of lipid peroxidation will suppress the formation of MDA.[21,22]

Removal of resin compounds (toxic) as at a dose of 250 mg / KgBB of Jatropha seed extract can reduce the effect of toxicity. The oxidative stress level decreases. As a suggestion from further research, by removing resin compounds in jatropha seed extract, in order to reduce the level of oxidative stress.

This contradicts previous research that jatropha seeds contain several major antioxidant enzymes, such as superoxide dismutase, glutathione,

ascorbate peroxidase, and catalase to prevent oxidative stress conditions due to free radical production, such as MDA. GSH can conjugate with endogenous and xenobiotic free radical components so as to protect from cell damage in the body.[18] High levels of antioxidants have the potential to prevent the progression of steatosis into steatohepatitis and fibrosis in liver fat. Antioxidant can reduce TGF-β concentrations and improve inflammation.[20]

A very strong negative correlation between GSH activity and MDA level, shows that the relationship between GSH activity and MDA is inversely proportional. If there is an increase in GSH activity, MDA levels decrease, and vice versa.High antioxidants will be conjugated with endogenous or exogenous free radicals therebyreducing the concentration of free radicals in the tissues.High antioxidant activity in jatropha seed extract should protect cells from free radicals. However, these plants also have toxic compounds,

then the dose can increase the production of free radicals, and the activity of a number of antioxidants in cells. When giving high doses of castor bean extract, MDA levels are greater than GSH activity, resulting in oxidative stress and cell damage.[23]

At low doses of castor bean extract, does not show cell damage, oxidative stress has not occurred that triggers changes in central vein structure. This condition is demonstrated by low MDA levels and high GSH activity at (dose 5 mg / KgBW). Large diameter size will reduce blood vessel resistance thereby reducing the potential for obstruction of blood flow in the central vein of the liver.GSH activity is higher, this condition causes a protective effect on liver tissue. Hepatocyte cells are still able to suppress oxidative stress so that the process of detoxification of toxic substances in the blood runs optimally. Thus, blood flowing into the central vein undergoes a detoxification process so as to prevent oxidative damage in the central vein.[13]

VEGF protein plays an important role in increasing the proliferation of hepatocytes in response to cell injury due to oxidative stress.28 Factors that can cause this condition are the presence of toxic compounds, such as phorbol esters, curcin, saponins, phytate, protease inhibitors and curcalonic acid which are also present in seed plants jatropha.

Repeated exposure of toxic compounds, until a process of ongoing inflammation, this will cause changes in the structure of blood vessels and increased activity of liver stellate cells to repair damaged cells and replace them with connective tissue. Such conditions are called fibrosis. Fibrosis can cause withdrawal of cells in the surrounding structure.[24] In addition, conditions of oxidative stress can also cause hepatocellular hypertrophy which results in reduced sinusoidal circulation, hypoxia, and necrosis.[25,26]

Hepatocyte cells have crude RE which plays a role in protein synthesis and fine RE which plays a role in the detoxification process. Decreasing the role of crude RE in protein synthesis can be correlated with low VEGF protein concentration in the process of repairing vascular tissue. A decrease in VEGF concentration indicates that the cell cannot protect due to high cell apoptosis. In addition, the decrease in refined RE function causes the detoxification process to not work optimally, toxic substances continue to carry liver blood flow to the sinusoid space and reach the central vein. The accumulation of toxic compounds along the bloodstream can cause oxidative stress in blood vessels to leave cell lesions. Cell damage continues, tissue recovery is hampered by the regeneration process, but is followed by cell turnover into connective tissue. Migration and proliferation of these fibroblasts will change the structure of the liver tissue. Liver tissue will stiffen and cause traction. The central veins of the liver thicken and the ducts become small.[10,13,27]

Limitations and Problems:

The limitation and problem of this study is that the histological slides staining results are less than the maximum, but we can still identify the central vien diameter of the liver.

EthicalApproval:

The study had been approved by the Medical and Health Research Ethic Committee, Faculty of Medicine, University of Indonesia (SK No 68/ UN2.F1/ETIK/2015).

Conflictof interest: None declared

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Author's Contributions:

Data gathering and idea owner of this study: PN
Study design: PN
Data gathering: RAF
Data analysis and consultation: EWWriting and submitting manuscript: PN

References:

1. Agbogidi OM, Akparobi SO, Eruotor PG. Health and environmental benefits of Jatrophacurcas Linn. AppSciRep.2013;1(2): 36-39.
2. Sharma S, Dhamija HK, ParasharB."Jatrophacurcas: Areview".AsianJ Res Pharm Sci. 2012; 2(3): 107-111.
3. Prasad DMR, IzamA, Khan MR. "Jatropha curcas: Plant of medical benefits". JMed PlantsRes. 2012; 6(14): 2691-2699.
4. Devappa RK, Makkar PSM, and Becker K. Jathropha toxicity-a review. J Toxicol Environ Health. B Crit Rev. 2010;13(6):476-507.
5. Salim MN, Masyitha D, Harris A, Balqis Ummu, Iskandar DC, Hambal M, and Darmawi. Anti-inflamatory activity of Jathropa curcas Linn latex in cream formulation on CD68 expression in mice skin wound. Vet Worl. 2018;11(2):99-103.
6. Jain S, Choudhary GP, and Jain DK. Pharmacological evaluation and antifertility activity of jathropha gossypifolia in rat. Biomed Res Int. 2013:125980.
7. SumitNL, SurendraS, Mruthunjaya K,Sachin BZ and Manjunath SM. "Jatropha curcas : ASystemic Review on Pharmacological, Phytochemical, Toxicological Profilesand Commercial Applications". RJPBCS. 2013;4(1): 989-1010.
8. Igbinosa OO, Igbinosa IH, Chigor VN, Uzunuigbe OE, Oyedemi SO, Odjadjare EE et al. Polyphenolic Contents and antioxidant potential of stem bark extracts from Jatropha curcas (Linn). Int J Mol Sci. 2011;12(5):2958-71.
9. Vipin K, Singh SP, Amit S and Chaudhary JK. Evaluation of subacute toxicity of Jathropa curcas seeds and seed oil toxicity in rats. Indian J of veterinary Pathology. 2014;38(2):88-93.
10. ShaL,HorYouT,etal."The Role of Oxidative Stress And Antioxidants in Liver Disease". Int JMol Sci. 2015; 16(11): 26087-26124.
11. EditaB, BiruteK, et al. "Glutathione andGlutathione S-Transferase LevelsinPatientswith Liver Metastases of Colorectal Cancer and Other Hepatic Disorder". TurkJ Gastroenterol. 2016; 27:336-341.
12. Kirana AN, Prafiantini, Hardiany NS. Correlation between age, body mass index, and blood selenium level with glutathione peoxidase activity among elderly in South. IJHHS. 2020;4(02):89-93.
13. AnthonyL Mescher. Junqueira's Basic Histology, Twelve Edition. US: The McGraw-Hill. 2010.
14. Elpek GO. Angiogenesis and liver fibrosis. World J Hepatol. 2015;7(3):377-391.
15. Maiers JL, and Malhi H. Endoplasmic reticulum stress in metabolic liver diseases and hepatic fibrosis. Samin Liver Dis. 2019;39(2):235-248.
16. Malhi H and Kaufman RJ. Endoplasmic Reticulum stress in liver disease. J Hepatol. 2011;54(4):795-809.
17. Yonny ME, Garcia EM, Lopez A, et al. "Measurement of Malondialdehydeas Oxidative Stress Biomarker in Goat Plasma by HPLC-DAD". Michrochemical Journal. 2016; 129:281-285.
18. Silva, LJD et al."Lipid Peroxidation and AntioxidantEnzymes ofJatropha curcasL Seed Storesat Different Maturity Stages". Acta Sci.2018;40:1-10.
19. Walter TJ, Cast AE, Huppert KA and Huppert SS. Epithelial VEGF signaling is required in the mouse liver for proper sinusoid endothelial cell identity and hepatocyte zonation in vivo. Am J Physiol Gastrointest Liver Physiol. 2014;301(10):G849-62.
20. Feng X, Tian L, Zhang Z, Yu Y, Cheng J, Gong Y, at al. Caspase 3 in dying tumor cells mediates post-irradiation angigenesis. Oncotarget. 2015;6(32):32353-67.
21. Valko,Metal."Free Radicals and Antioxidantsin Normal Physiological Functionand Human Disease". IntJ Biochem CellBiol.2007;3(1):44-84.
22. Moreno MG, Oramas DR, Avila ZM, Torres TA, Falcon D, De la tijera FH, at al. Bahvior of oxidative stress Markers in alkoholic liver cirrhosis patient. Oxid Med Cell Longev. 2016:9370565.
23. Y Chen, H Dong, etal."Glutathione Defense Mechanismin LiverInjury: Insights From Animal Models". Food Chem Toxicol. 2013; 60: 38-44.
24. Zachary, JF, Mc Gavin MD. Pathologic Basis of Veterinary Disease Expert Consult 5th ed. Missouri: Elsevier. 2012.
25. Maronpot, RLet al. Hepatic Enzyme Induction: Histopathology.Toxicologic Pathology. 2010; 38(5): 776-795.
26. Li S, Hong M, Tan H-Y, Wang N, and Feng Y. Insight into the role and interdependence of oxidative stress and inflammation in liver diseases. Oxidative Medicine and cellular longevity. 2016;21.
27. Osada S, Imai H, Tomita H, Takuyma Y, Okumura N, Sakashita F and Sugiyama Y. Vascular endothelial growth factor protects hepatoma cells against oxidative stress-induced cell death. J Gastroenterol Hepatol. 2006;21(6):988-93.