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Research Article

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Prophylactic Impact of Beta vulgaris L in Ameliorating Cyclosporine A-Induced Hepatotoxicity in Rats

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ABSTRACT

Background: Hepatotoxicity induced by Cyclosporine A (CsA) remains one of the major side effects. The aim of this study was to determine the protective effects of beet root (Beta Vulgaris L) extract and silymarin against hepatotoxicity induced by Cyclosporine A in rats. Methods: Sixty male albino rats, were divided into 6 groups (n=10). Group I control group. Group II CsA-treated and received (50mg/kg weight, orally). Group III received (500mg/kg b.wt) beet root extract orally. Group IV received beet root extract and CsA as in group II and III. Group V was received (100 mg/kg b.wt) silymarin orally. Group VI received CsA and silymarin as in group II and V. Serum levels of (ALT, AST, ALP) and bilirubin (Total and Direct) were measured. Oxidative stress biomarkers, antioxidant status, damage to DNA, apoptosis and inflammatory mediators were measured in the tissues of the liver. Result: CsA administration significantly increased serum levels of the liver enzymes ALT, AST, ALP and bilirubin. In addition, significant increase in MDA, Nitrite, 8-OHdG, caspase3, NF-κB, TNF-α and significant decrease of GST in liver tissues was noticed. Furthermore, histopathological changes occurred in CsA treated rats exhibited disruption of normal liver architecture, congested central vein, vacuolated cytoplasm and inflammatory cells infiltration. Co-administration of beet root extract or silymarin +CsA ameliorated all these parameters. Conclusion: The present study suggests that beet root extract and silymarin have beneficial effect in reducing hepatotoxicity induced by CsA via decreasing oxidative stress, inflammation, DNA damage, apoptosis and repairing the histopathological changes.

Key words: *Cyclosporine A, Hepatotoxicity, Beta Vulgaris L, Silymarin.*

INTRODUCTION

The beetroot (*Beta vulgaris* L.), locally known as Shamandar, is a vegetable plant and belongs to Amaranthaceae family [1]. Beet root contains a powerful antioxidant phenolics, Ascorbic acid, carotenoids and betalains [2]. High Pressure Liquid Chromatography analysis of this beetroot extract, confirmed the presence of bioactive polyphenols such as quercetin, sinapic acid, p-coumaric acid, syringic acid, gallic acid, coumarin, caffeic acid, chlorogenic acid and catechin [3]. Modern results suggest that *Beta vulgaris* extracts (root) have antihypertensive, hypoglycemia, antioxidant [4], anti-inflammatory, and hepatoprotective activities [5-7]. Previously, lab animals used to describe red beetroot extract as effective multi-organ tumor-suppressing agent [8].

Calcineurin inhibitors include Cyclosporine A (CsA), were used in patients after kidney, liver, heart, lung, and heart-lung transplants for graft-versus-host disease (GVHD) prophylaxis [9]. Furthermore, it is used in the majority of autoimmune disease, in dermatology such as psoriasis, autoimmune dermatitis, or chronic idiopathic urticarial [10]. Cyclosporine A have adverse side effects of acute and chronic nephrotoxicity. CsA can cause disorders in metabolic and electrolyte disorders that is weight gain, hyperglycaemia, hyperlipidaemia, hypercalcaemia, and hypomagnesaemia [11]. Experimental studies and clinical observations revealed that Cyclosporine A can lead to drug-induced liver injury (DILI). In Cyclosporine A induced liver injury, and the

change in functional and morphological nature of the liver were observed. The development of the hypermetabolic state in the liver due to the mechanism of CsA induced liver injury was investigated [12]. In Addition, inhibition of ATP-dependent transport of bilirubin and bile salts through the hepatocyte canalicular membranes as well as of bile secretion was observed in experimental animals [13].

In the experimental animals which exposed to CsA, the antioxidant used to reduce liver functional and morphological damage, which suggests the involvement of oxidative stress as one of the mechanisms of hepatotoxicity [14]. However, the hepatoprotective impact about *Beta vulgaris* extract against CsA prompted hepatotoxicity has not been scientifically investigated. So, the current study for the first time will explore the prophylactic effects for *Beta vulgaris* extract (BVE) on liver capacity, oxidative stress, inflammation, DNA damage and apoptosis prompted by CsA in rats.

Silymarin was widely used for a variety of acute and chronic liver diseases as a therapeutic agent due to its antioxidant properties [15, 16]. It has been used to protect the liver from toxic substances, treat hepatic damage, hepatitis therapy and cirrhosis for centuries [17, 18].

The aim of the present study was to evaluate the hepatotoxic effect of CsA administration on rats. The study also was extended to explore the potential impact of *Beta vulgaris* extract (BE) as a protective agent with antioxidants, anti-inflammatory and anti-apoptotic properties against the hepatotoxic effect of CsA. The study focused on the correlation between BVE and silymarin as reference drug in ameliorating the changes in the selected parameters. Histopathological studies of rat liver tissue were examined to confirm the biochemical investigations.

MATERIALS AND METHOD

Materials:

Beet Root, *Beta vulgaris* ethanolic extract (BVEE), Product (Nature's Way Product, USA). Silymarin (Silybon-70), Manufactured by Micro Labs Ltd, Cyclosporine A was supplied from Novartis Pharma AG, Sweden.Other chemicals and reagents were of analytical grade.

Methods:

Experimental Design:

This study comprised sixty male Wister rats, weighted 150-200 g, divided into six groups (ten rats /cage) were obtained from Experimental Animal Care Center of King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were housed in cages at 20-11 °C and humidity of 60% under 12 hours cycles of dark and light. The animals were housed in plastic cages. Rats were adapted to the environment for one week prior to the start of experiment. The animals were divided as follows.

Group I: Traditional animals received standard feed and water ad libitum

Group II: Hepatotoxic group, received CsA (50mg/kg weight, orally) [19] for ten consecutive days along with standard feed and water spontaneously.

Group III: Rats which was supplemented orally with BVEE (500mg/kg weight) [4]

Group IV: Treated with BVEE at the doses of 500mg/kg weight orally, for 10 days concurrently with CsA (50 mg/kg).

Group V: Animals received silymarin (100 mg/kg b.wt., orally) once daily for 10 days days [20].

Group VI: Animals received CsA as in group II and treated with silymarin as reference drug orally at a dose of 100 mg/kg b. wt for ten days.

At the end of the experiment, rats were fasted nightlong (12-14 hours). Blood specimens were gathered in tubes for clotting and serum separation. The tubes centrifuged at 2000 g for fifteen minutes and therefore the isolated serum can be stored at -20 ºC till use. The animals were then scarified underneath anesthesia and the livers were collected, washed with cold saline and maintained at -20 °C till use. Specimens of the livers were removed out, kept in 10 percent formalin and used for histopathological studies.

Biochemical analysis

Serum analysis

Serum ALT, AST, ALP, T-Bilirubin and D-Bilirubin were measured as biomarkers of liver injury utilizing an automated analyzer.

Liver tissue analysis

a) oxidative stress

Nitrite level (as an indicator of NO production) and malondialdehyde (MDA, marker of lipid peroxidation) were assayed as markers of oxidative stress. Glutathione S Transferase was estimated as an antioxidant marker, and were measured utilizing commercial kits (Nanjing, Jiancheng Co., China) using the manufacturer instruction**.**

b) DNA damage and apoptosis

8- hydroxydeoxyguanosine (8ohdg) and caspase -3 were measured using ELISA kit (Uscn Life Science Inc., Wuhan, China) according to the manufacturer's instructions.

c) Inflammation-mediated hepatotoxicity

NF-κB (Nuclear Factor-κB), and TNF-α was measured using the ELISA assay kit
(products of Thermo Scientific, Waltham, MA, USA) following the instructions supplied by the manufacturer.

Histopathological studies

Liver tissues was examined to evaluate the histomorphological changes in different experimental groups. Samples of tissues was collected and fixed in 10% formaldehyde for 24 hours. Some sections of the samples were stained with hematoxylin and eosin (H&E) for examination by ordinary optical microscope to evaluate the cytotoxic effects of CsA administration. The sections were then regarded and photographed [21].

Statistical analysis

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one-way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means ±SE and $P < 0.05$ was considered to be statistically significant.

RESULTS:

Biochemical Investigations

Figure 1 (A-E) illustrates the mean levels of serum ALT, AST, ALP, total bilirubin and direct bilirubin in control and different studied groups. Statistical analysis showed significant ($p \leq 0.05$) increase between the CsA groups and control group and significant ($p \leq 0.05$) decrease between the CsA group and medicinal plants groups. Statistical different was noticed between silymarin+CsA and Beet+CsA. No statistical different was noticed between silymarin+CsA and Beet+CsA in Bilirubin (Direct+Total).

Figures 2 (F, G, H) illustrates the mean levels of nitrite, malondialdehyde and glutathione S Transferase in liver tissues of control and different studied groups. Statistical analysis showed significant ($p \leq 0.05$) increase in CsA groups in relation to control group and co-administration of medicinal plants modulated the hepatic increase in oxidative parameters. There was statistical different ($p \leq 0.05$) between the silymarin+CsA group and beet+ CsA group. Glutathione S Transferase (GST) levels were significantly ($p \leq 0.05$) decreased in CsA group, while in the medicinal plant groups was significantly ($p \leq 0.05$) increased but still lower than the normal level. Significant difference was noticed between (Silymarin+CsA) and (Beet+CsA) groups.

Figures (3, 4) illustrate the mean values of 8-hydroxydeoxyguanosine, caspase 3, NF-κB and TNF-α in liver homogenates of control and different studied groups. Statistical analysis showed significant ($p \leq 0.05$) increase between the CsA group and control group, while co- treatment with medicinal plants significantly depleted these parameters. Also, significant ($p \leq 0.05$) difference was noticed comparing the silymarin+CsA group and beet+ CsA group in all the studied parameters. No statistical difference was noticed using medicinal plants alone compared to control group which indicate the safety using of these plants.

The percentage of change was increased in CsA treated group in all parameters except in GST and modulated by using medicinal plants. The ameliorating effect of silymarin was more beneficial than beetroot extract (table 1).

Table 1: The percentage of changes of the effect of Beetroot extract and Silymarin on all studied parameters in CsA induced hepatotoxicity in rats.

Groups / Parameters	CsA	CsA+Beetrootextract	CsA+Silymarin
Serum ALT	↑ 250 %	↓ 49.63 %	↓ 75.3 %
Serum AST	↑ 114.4 %	↓ 39.52 %	↓ 56.719 %
Serum ALP	↑ 32.7 %	↓ 13.50 %	↓ 26.04 %

SerumT.bili	↑ 77.5%	↓ 39.08%	↓ 42.75%
SerumD.bili	↑ 96.9%	↓ 46.16%	↓ 46.92%
Nitrite	↑ 102.4%	↓ 35.5%	↓ 49.33 %
MDA	↑ 80.2%	↓ 33.2%	↓ 40.28%
GST	↓ 63.8%	↑ 76%	↑ 120%
8ohdg	↑ 85.1%	↓ 27.7%	↓ 43.29 %
Caspase3	↑ 38%	↓ 11.1%	↓ 21.88%
NF-kB	↑ 95.05%	↓ 10.95%	↓ 31.8 %
TNF- α	↑ 135.06%	↓ 15.46%	↓ 39.22%

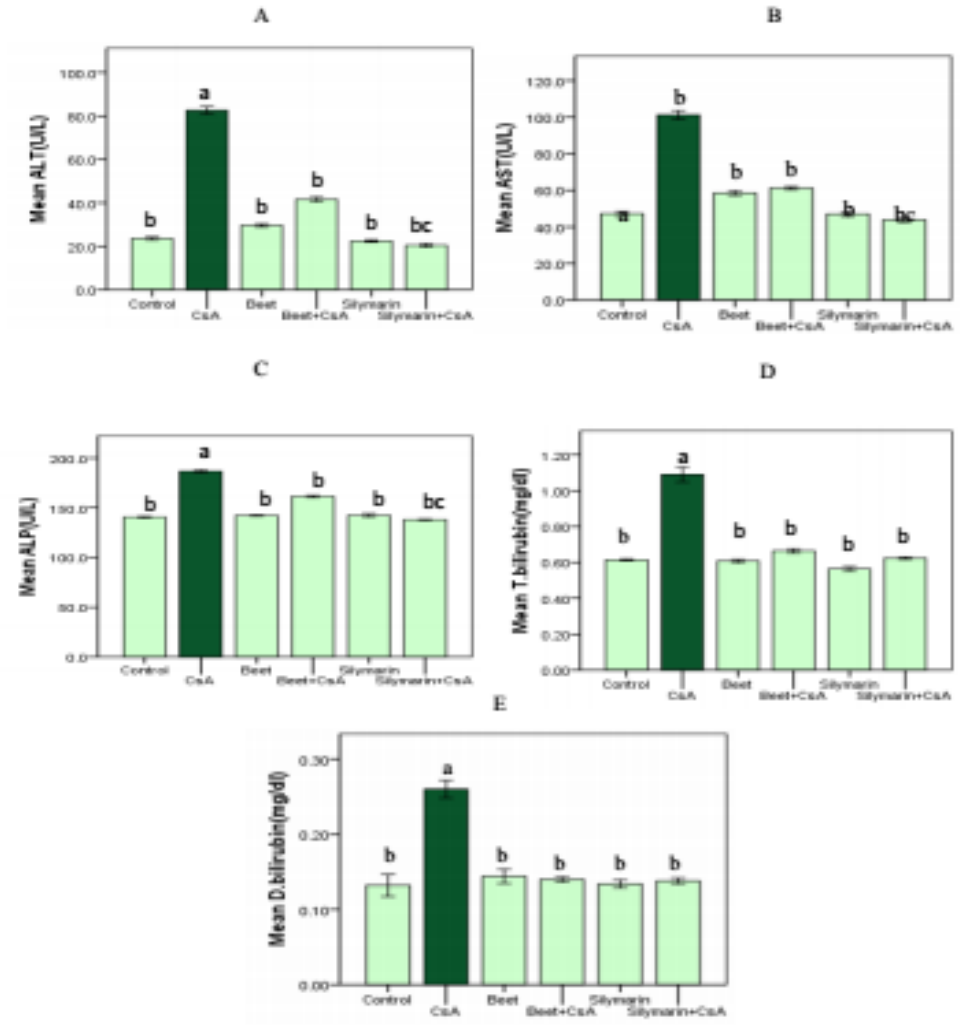


Figure 1. Effect of Beetroot extract and Silymarin on serum ALT (A), AST (B), ALP (C), total Bilirubin (D) and direct Bilirubin (E) Levels in CsA induced hepatotoxicity in rats. Data are presented as mean ± S.E. of 10 rats

a: indicates the significant difference between the control group and CsA group.

b: indicates the significant difference between the CsA group and the other groups.

c: indicates the significant difference between the silymarin+CsA group and the Beet+CsA group.

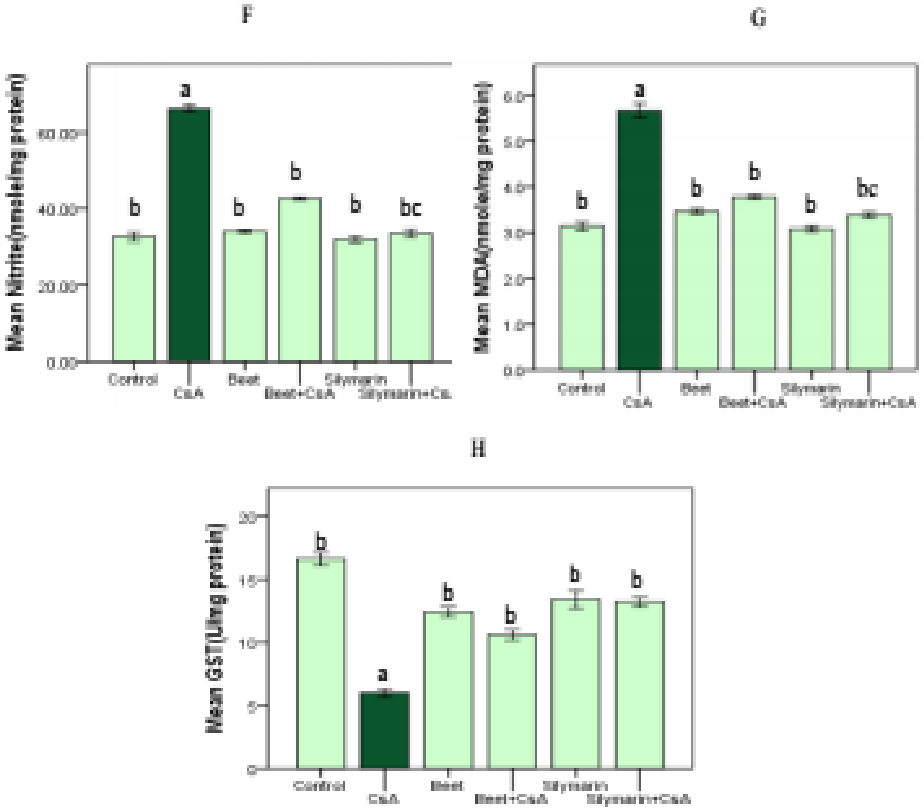


Figure 2. Effect of Beetroot extract and Silymarin on Nitrite (F) Malondialdehyde (MDA) (G), Glutathione S Transferase (GST) (H) Levels in CsA induced hepatotoxicity in rats. Data are presented as mean ± S.E. of 10 rats

a: indicates the significant difference between the control group and CsA group.

b: indicates the significant difference between the CsA group and the other groups.

c: indicates the significant difference between the silymarin+CsA group and the the Beet+CsA group.

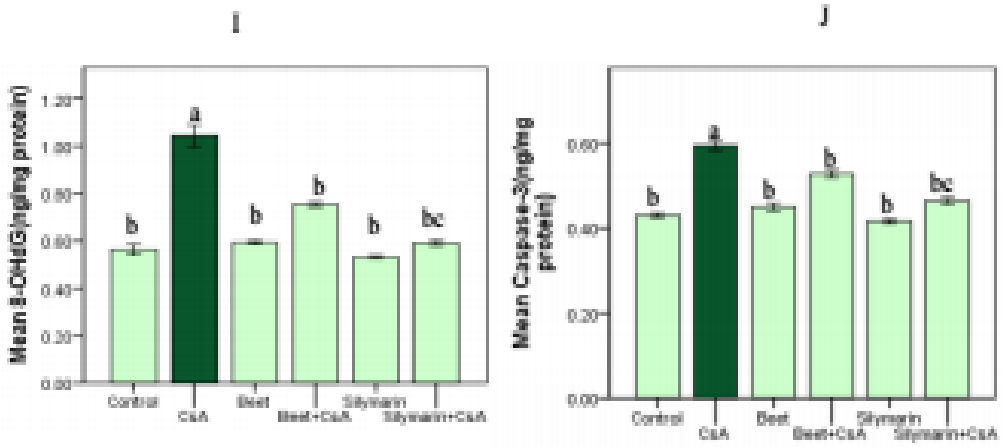


Figure 3. Effect of Beetroot extract and Silymarin on 8-hydroxydeoxyguanosine (8ohdg) (I), Caspase-3 (J) Levels in CsA induced hepatotoxicity in rats**.** Data are presented as mean ± S.E. of 10 rats

a: indicates the significant difference between the control group and CsA group.

b: indicates the significant difference between CsA group and the other groups.

c: indicates the significant difference between the silymarin+CsA group and the Beet+CsA group.

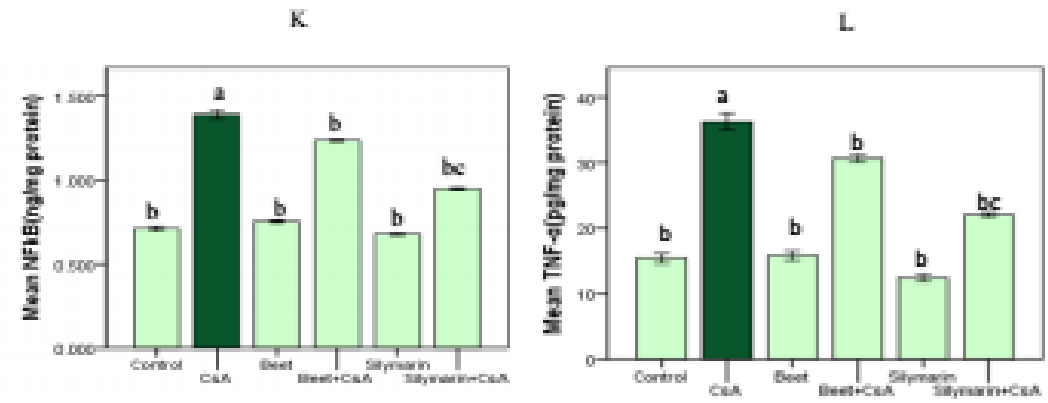
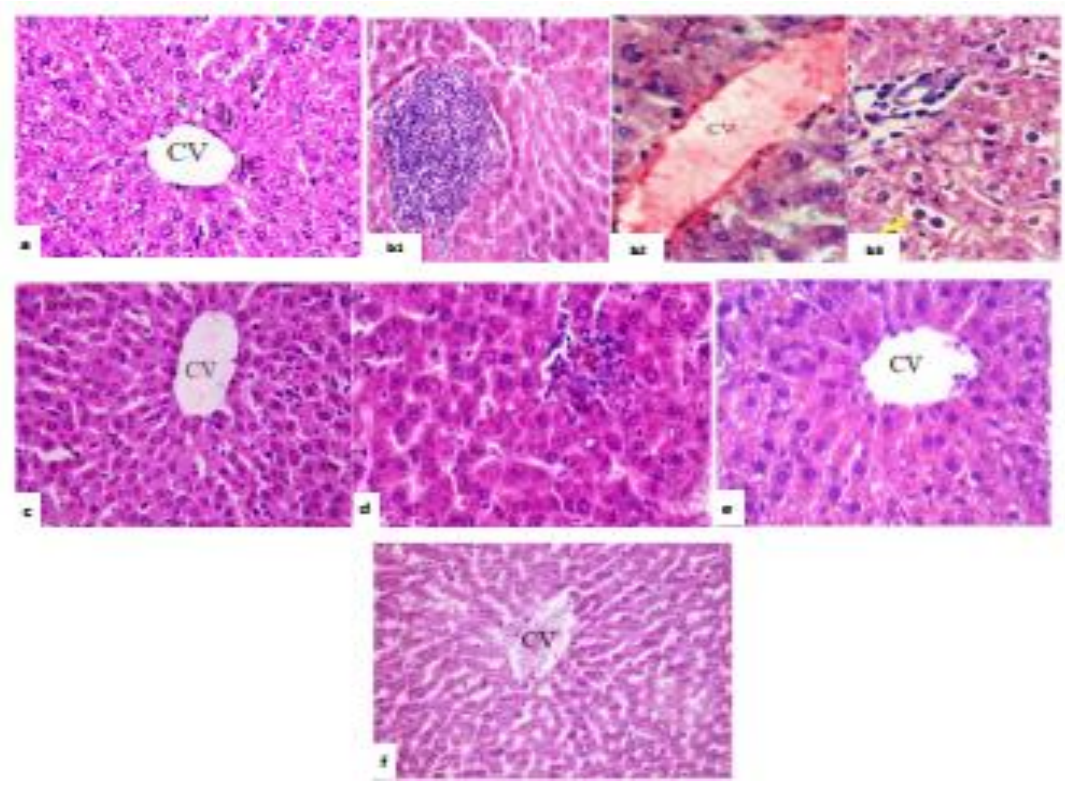


Figure 4. Effect of Beetroot extract and Silymarin on Nuclear Factor-κB (NF-κB) (K), Tumor Necrosis Factor alpha (TNF-α) (L) Levels in CsA induced hepatotoxicity in rats**.** Data are presented as mean ± S.E. of 10 rats.
a: indicates the significant difference between the control group and CsA group.
b: indicates the significant difference between the CsA group and the other groups.
c: indicates the significant difference between the silymarin+CsA group and the Beet+CsA group.

Liver Histopathology

Figure 5 (a - f) illustrated paraffin section of liver tissues of different studied groups. Liver sections of control group (a) exhibited normal histological features. The hepatocytes were found arranged in strands around the central vein separated from each other by large vascular spaces known as hepatic sinusoids. Hepatocytes are polyhedral with acidophilic granular cytoplasm. Animals treated with Beet or silymarin alone showed normal structure and no histopathological alterations. In contrast, liver section from rats treated with CsA (b) showed liver injury illustrated in congestion of the central vein, vacuolated cytoplasm of hepatocytes and also a large mass of inflammatory leucocytic infiltration. Liver sections from rats given CsA+ Beet showed improvement of the liver tissue with few inflammatory cells infiltrations (d). Examination of sections obtained from liver of rats treated with CsA + silymarin showed normal histopathological appearance similar to normal control liver (f).



Figures 5. A photomicrograph of hematoxylin and eosin stained sections of rat liver in control group (**a**) showing hepatocytes (h) arranged as cords radiating from the central vein (CV) and separated by blood sinusoid

(s). The hepatocytes have eosinophilic cytoplasm and round central nucleus. Note Kupffer cells (k) lining sinusoids (X100), CsA treated group (**b**) showing congested central vein (b1), vacuolated cytoplasm of some hepatocytes (yellow arrow) (b2) and inflammatory cells infiltration (b3) (X100), Beet root treated group (**c**) showing normal histopathological appearance of liver tissue structure (X100), CsA+Beet treated group (**d**) showing improvement of the liver tissue with few leucocytic infiltration (X100), silymarin treatedgroup (**e**) showing normal histopathological appearance of liver tissue structure (X100), CsA+silymarin treated group (**f**) showing near to normal liver tissue structure (X100).

DISCUSSION

The current study was carried out to determine the protective role of beetroot extract (*Beta vulgaris* L) against hepatotoxicity induced by CsA. According to the results of this study the values of serum ALT, AST, ALP and bilirubin (total and direct) in group II treated with CsA significantly increased compared to control group. The findings of our results were supported by the results of the other authors which showed that CsA initiated liver tissue and edema hypertrophy due to the release of parameters into the circulation after the cellular damage has occurred as evidence of liver toxicity [22, 23]. However, co-administration of BVEE along with cyclosporine A caused significant decrease in serum level of liver function markers ALT, AST, ALP, and bilirubin (total and direct) that suggested the protective effects of BVEE. Similar protective studies have previously been reported on various medicinal extracts against cyclosporine A [24, 25]. The results of the current study were in accordance with those of Ranju et al., (2010) [26] who reported that the treatment with the ethanolic extract of *B. vulgaris* restored the liver enzyme and bilirubin induced elevation by CCL_4. The results also were in agreement with Sadeek, (2011) [27] who reported that, beetroot treatment significantly repaired the liver enzymes activity and total bilirubin to normal levels after 28 days. Based on these findings, we suppose that beetroot has protective effect against CsA induced hepatotoxicity by suppression the infiltration of enzymes through cell membrane.

In our study, the co-administration of silymarin along with CsA repaired hepatocyte function by reducing serum levels of ALT, AST, ALP enzymes and bilirubin (total and direct). This result was in agreement with other authors who showed decreased levels of liver function enzymes when received CCl_4 along with silymarin due to its antioxidant and cell-regenerating processes. The liver protective effect of silymarin results from increased protein synthesis [28, 29]. The hepatoprotective impact of silymarin may be attributed to protection of liver cells directly through stabilizing the cell membrane by avoiding liver glutathione consumption and restricting lipid peroxidation [30]. The pharmacological characteristic of silymarin is regulating the permeability and integrity of the cell membrane, restricting leukotriene and scavenging reactive oxygen species [31].

The liver usually generates reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anions and hydrogen peroxide, but this is neutralized by an endogenous antioxidant detoxification system, including GSH, SOD and CAT, which scavenge the ROS. The imbalance between intracellular prooxidants and antioxidants contributes to DNA damage and membrane lipid peroxidation. [32]. In our result, MDA levels in liver samples of rats administrated CsA were significantly increased and this finding was similar to those observed by other studies [33, 34]. Products of oxidative stress using CsA may be caused by increase intra-mitochondrial Ca^{++} and inhibition of mitochondrial Krebs cycle, antioxidant enzymes and the production of ATP [35].

Nitric oxide (NO) is a pluripotent free gaseous radical, in particular as an important signaling molecule in almost every tissue. It is a highly responsive oxidant produced by an inducible form of NO synthase by L-arginine of liver parenchymal and nonparenchymal cells. The overproduction of NO in the liver and kidney was a major cause of endotoxin shock and various models of inflammation and damage to the liver and renal [36].

Administration of cyclosporine A in rats exhibited a significant increase in liver tissue nitrite level compared to control group. These findings were in good agreement with other results obtained by Da Costa et al., (2003) [37] who ascribed the transient increment in cytosolic calcium to CsA toxicity. The expansion in Ca^{2+} might be because of changes in cellular Ca^{2+} homeostasis in various types of cell, including influx of Ca^{2+} transmembrane and intracellular mobilization from mitochondrial and endoplasmic reticulum storage sites [38]. It is assumed that CsA may lead to alteration of the NO-forming mechanisms by affecting the NOS enzyme that mediates the formation of NO [39].

Glutathione - S - transferase (GST) is an isoenzyme group that can detoxify different endogenous and exogenous substances by combining GSH. In this study GST was decreased in CsA treated group. Same result was obtained by Pari and Sivasankari, (2008) [40] who reported that the toxicity of cyclosporine A is known to

undergo biotransformation by hepatic glutathione S-transferase and forming aldehyde derivatives, which may produce free radical species because of oxidation by xanthine and aldehyde oxidases. Aldehydes are well known to generate free radicals that can inactivate enzyme proteins. Cyclosporine A or its active metabolites might alter the native form of antioxidant enzyme and impair their function, which support the decreased activity of these enzymes in Cyclosporine A treated rats [41]. The significant increase in MDA and Nitrite levels in group II treated with CsA accompanied by a significant decrease in GST level, suggested that oxidative stress occurs in liver.

Our result revealed that beetroot extract in combination with CsA decrease the levels of MDA, Nitrite and significantly increased GST levels which support the antioxidant properties of beetroots. These results were in agreement with Váli et al., (2007) [42] who referenced that beetroots contain essential bioactive agents (betanin and polyphenols) with a wide range of antioxidant effects. Beetroot phytoconstituents, for example terpenoids, sugars, phenolics and ascorbic acid, have potent antioxidant and liver defense effect [43]. These components are known to decrease lipid peroxidation and prevent necrosis that protected the liver tissue from oxidative harm and hepatotoxic impact brought about by CsA treatment [42]. The potential defensive role of betanin against hepatotoxicity caused by organophosphate, in which the mechanism appears to be through restricted oxygen species production and mitochondrial protection [44]. Betanin had a defensive impact against paraquat-actuated liver harm in rats. The mechanism of the protection appears to be by the inhibition of Cytochrome (CYP) 3A2 expression and protection of mitochondria. Betaine or trimethylglycine, is one of the real components of Beetroot, it restricts nitric oxide release from activated microglial cells [45]. It also improved GST in liver injury caused by ionizing radiation exposure [46].

The co-administration of silymarin along with CsA reduced NO. These results approved with other study who reported that, the silymarin reduced Nitric Oxide production, indicating that this drug has anti-inflammatory and free radicals scavenging [47]. Treatment with Silymarin diminished the seriousness of hepatic damage observed after treatment with acetaminophen (APAP) by decreasing invasion of neutrophil in hepatic tissue, which further shown its significant hepatoprotective impact. In addition, silymarin along with CsA significantly decreased liver MDA while, increased GST levels, compared to CsA group. Our results are in accordance with Nada et al., (2015) [30] Who suggested that, the silymarin has antioxidants and scavenging free radicals (ROS) to defend against oxidative stress. It can preserve the liver against oxidative damage by inhibiting lipid peroxidation and resuming reduced levels of glutathione [48]. In addition, silibinine has membrane defensive characteristics and can prevent oxidative damage to the blood constituents [49]. It was reported that, silymarin administration improved hepatocellular injury induced by diethylnitrosamine by amellurating hepatic GSH and GST levels. Silymarin posttreatment is likely to maintain GR and GST activity in the liver by restricting lipid peroxidation and maintaining GSH levels [50]. This is indicative of the potent antioxidant activity possessed by silymarin.

Apoptosis is described by chromatin condensation, organelle compaction, systematic cleavage of DNA and genetic regulation. The plasma membrane, on the other hand, is the main site of injury in necrotic cell death that causes cell swelling and extreme cell rupture [51]. In our study significant increase in caspase 3 activity was noticed in the group receiving CsA, as compared to control group. Our result agreed with the result of Wolf et al., (2000) [52] who noticed that the efficiency of caspase 3 in the hepatic cells of rats given CsA was increased compared to controls. In the situation of apoptosis, cytochrome c joins cytoplasmic scaffold (apaf-1) and procaspase 9, producing the apoptosome. Activation of procaspase 9 and other pro-apoptotic proteins activate caspase 3. Activation of caspase 3 produce programmed cell death, that is, apoptosis [53]. In nuclear and mitochondrial DNA, the most commonly discovered and studied DNA lesion is 8-hydroxydeoxyguanosine (8-OHdG), an oxidized nucleoside of DNA [54]. The administration of cyclosporine A in group II was resulted in increased level of 8- OHdG compared with control positive group. This finding was approved by the study of Josephine et al., (2008) [55] during the study of sulphated polysaccharides from SargassumWightii in Cyclosporine A- stimulated oxidative liver damage in rat. As mentioned above, in the present examination, CsA-prompted hepatotoxicity is highly confirmed by programed cell death and DNA injury. This was indicated by increasing caspase3 and 8-OHdG in the CsA administered Group.

The co-administration of beetroot has shown a decrease in caspase 3 when compared with the CsA treatment group. This result was in agreement with Abdel-Daim and Abdellatief, (2018) [56] who reported that betaine (BET) has anti-apoptotic effect by inhibiting caspase-3, caspase-8, and caspase-9 enzymes and anti-inflammatory activities. Also, in the present study it appears that beetroot along with CsA group reduced

8OHdG when compared with CsA group. This result was similar to the study of Shedid et al., (2018) [46] utilizing betaine in liver damage prompted by the exposure to ionizing radiation.

Silymarin with CsA treatment significantly reduced levels of caspase3 and 8OHdG as compared to CsA group. This result was in agreement with previous study by Al-Rasheed et al., (2015) [57] who reported that silymarin alone or in cobination with vitamin E and curcumin repaired the 8-OHdG level. This improving effect was attributed to the alteration in the redox system by scavenging free radicals and enhancing the liver's antioxidant status during hepatotoxicity by CCl_4. The data observed indicated that the silymarin reduced apoptotic cell death associated with hepatotoxicity.

NF- κB is a nuclear transcription factor specific to the cytoplasm of liver cells in which it plays a major role in the organization of inflammatory signaling pathways [58]. The most popular species of NF - κB in mammalian cells is a heterodimeric complex of p65/RelA and p50. In the cytoplasm, NF-κB is linked to its inhibitory subunit I-κB. Due to oxidative anxiety, NF-κB is activated and phosphorylated from its inhibitory subunit and translocated to the hepatic cell nucleus where it is combined to DNA. This causes the DNA transcription of numerous inflammatory genes such as cytokines, chemokines and receptors of advanced end products for glycation to be increased [59]. In the present study significant increase of NF-κB in CsA treated group compared to control was observed. This result approved by other study reported that CsA induces transcriptional activation of NF-κB in rat hepatocyte cultures [60]. Also, the result showed that administration of CsA significantly increase the level of liver tissue TNF-α. This result was in the same line as the finding of Salem et al., (2010) [61] who studied the defensive impact of trapidil and l- arginine against renal and hepatic toxicity caused by cyclosporine in rats. The data observed in the present study indicated inflammation of hepatic cells.

In vitro and in vivo studies, literature survey indicated that anti - inflammatory activity of quercetin, coumarin, caffeic acid and chlorogenic acid (beetroot components) was evident [62-65]. In the present study the coadministration of beetroot extract along with cyclosporine A caused significant decrease in the level of liver tissueNF-kB and TNF-α. These results approve by the study of Afifi et al., (2016) [66] who used quercetin as a protective agent against thioacetamide induced hepatotoxicity in rats due to the reduction of biomarkers of oxidative stress, inflammatory cytokines, TNF-α , NF-κB and fragmentation of DNA. Also, it was approved by Shi et al., (2013) [67] who utilized chlorogenic acid to decrease hepatic inflammation and fibrosis by inhibiting the signaling pathway of toll-like receptor 4.

Our result showed the silymarin with CsA significantly decreased NF-kB and TNF-α. These results were supported by other study who reported that silymarin is a powerful inhibitor of NF-kB stimulation caused by a wide range of inflammatory agents. The inhibition of activation of NF - kB by silymarin correlated with suppression of IkBa phosphorylation and degradation, nuclear translocation of p65 and NF-kB dependent transcription of the reporter gene [68]. The protective effect of silymarin is due to its pharmacological properties include the regulation of permeability and integrity of the cell membrane, inhibition of leukotriene, scavenging of reactive oxygen species, elimination of NF-kB activity, depression of protein kinases and collagen production [69]. This result indicates that the silymarin is a potent anti-inflammatory and protective against hepatotoxicity, induced by CsA.

Histopathological changes in the liver induced by CsA, showing disruption of normal liver architecture; liver injury in congestion of the central vein, vacuolated cytoplasm of hepatocytes and also a large mass of inflammatory leucocytic infiltration. The histopathological changes were in agreement with Nacar, et al; (2015) [70] who reported that CsA caused damage to hepatic tissue, using histological and biochemical methods to study the defensive effect of erdosteine against cyclosporine-induced injury to rat liver. However, in our study liver sections of CsA+Beetroot (BVEE) treated group revealed improvement of the liver tissue but few inflammatory cells infiltrations still found. These changes in histopathological were supported with Olumese and Oboh, (2018) [71], who reported that the beetroot would ameliorated liver damage induced by CCL4 intoxication, by treating rats by 250mg and 500 mg of the beetroot extract and 100mg silymarin.

Liver sections of CsA+silymarin revealed complete restoration of tissue hepatocytes. These results were approved by Wang et al., (2018) [72] who revealed that triptolide (TP) made evident harm to the hepatic architecture and prompted serious pathological variations, including vacuole formation, inflammatory penetration and focal necrosis. However, the presence of disintegrated hepatic cells was significantly reduced by pretreatment with silymarin, which support that the protective role of silymarin was more beneficial than beetroot extract (*Beta vulgaris* L).

From all the above results and by calculating the percent of changes in all the parameters studied, it could be expressed that there is no critical interaction between beetroot extract and CsA when utilized in combination on

any of the previously mentioned parameters. It follows that the two treatments (Beetroot extract and CsA) can be safely combined without fear of serious responses. It was announced that the medication is usually taken orally through the digestive system similar to food and herbs. Therefore, each pharmacokinetic profile, i.e absorption, distribution, metabolism and/or excretion, can be changed whenever, mixed with herb. Some medicines affect the ability of the body to absorb herbs. Similarly, some herbs and food can lessen or increase the impact of a drug [73].

CONCLUSION

In conclusion, this study indicates that Beetroot extract (BVEE) has a hepatoprotective capacity against hepatotoxicity prompted by CsA. The hepatoprotective impact of BVEE can be attributed to the antioxidant, anti-apoptosis and anti-inflammatory properties. These finding may have important implications in the development of new therapeutic strategy aimed at manipulating BVEE as a supplement for prophylaxis from hepatotoxicity.

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REFERENCES

1. Vali, L., Et Al., Liver-Protecting Effects Of Table Beet (Beta Vulgaris Var. Rubra) During Ischemia-Reperfusion. Nutrition, 2007. 23(2): P. 172-178.
2. Clifford, T., Et Al., The Potential Benefits Of Red Beetroot Supplementation In Health And Disease. Nutrients, 2015. 7(4): P. 2801-2822.
3. Indu, R., Et Al., Antioxidant Properties Of Polyphenolic Rich HPLC Standardized Extract Of Beta Vulgaris L. Roots. Int J Res Dev Pharm L Sci, 2017. 6(3): P. 2619-24.
4. El Gamal, A.A., Et Al., Beetroot (Beta Vulgaris L.) Extract Ameliorates Gentamicin-Induced Nephrotoxicity Associated Oxidative Stress, Inflammation, And Apoptosis In Rodent Model. Mediators Of Inflammation, 2014. 2014.
5. Charde, S.Z.R.C.M., Antioxidant And Anti-Inflammatory Activity Of Ethanolic Extract Of Beta Vulgaris Linn. Roots. International Journal, 2011. 2(4).
6. Jain, S., V.K. Garg, And P.K. Sharma, Anti-Inflammatory Activity Of Aqueous Extract Of Beta Vulgaris L. Journal Of Basic And Clinical Pharmacy, 2011. 2(2): P. 83.
7. Singh, A., Et Al., Wound Healing Activity Of Ethanolic Extract Of Beta Vulgaris. 2011.
8. J Kapadia, G., Et Al., Cytotoxic Effect Of The Red Beetroot (Beta Vulgaris L.) Extract Compared To Doxorubicin (Adriamycin) In The Human Prostate (PC-3) And Breast (MCF-7) Cancer Cell Lines. Anti-Cancer Agents In Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 2011. 11(3): P. 280-284.
9. Tedesco, D. And L. Haragsim, Cyclosporine: A Review. Journal Of Transplantation, 2012. 2012: P. 7.
10. Khattri, S., Et Al., Cyclosporine In Patients With Atopic Dermatitis Modulates Activated Inflammatory Pathways And Reverses Epidermal Pathology. Journal Of Allergy And Clinical Immunology, 2014. 133(6): P. 1626-1634.
11. Korolczuk, A., Et Al., Oxidative Stress And Liver Morphology In Experimental Cyclosporine A-Induced Hepatotoxicity. Biomed Research International, 2016. 2016.
12. Zhong, Z., Et Al., Cyclosporin A Causes A Hypermetabolic State And Hypoxia In The Liver: Prevention By Dietary Glycine. Journal Of Pharmacology And Experimental Therapeutics, 2001. 299(3): P. 858-865.
13. Hulzebos, C.V., Et Al., Cyclosporin A And Enterohepatic Circulation Of Bile Salts In Rats: Decreased Cholate Synthesis But Increased Intestinal Reabsorption. Journal Of Pharmacology And Experimental Therapeutics, 2003. 304(1): P. 356-363.

14. Akbulut, S., Et Al., Effects Of Antioxidant Agents Against Cyclosporine-Induced Hepatotoxicity. Journal Of Surgical Research, 2015. 193(2): P. 658-666.
15. Qato, D.M., Et Al., Use Of Prescription And Over-The-Counter Medications And Dietary Supplements Among Older Adults In The United States. Jama, 2008. 300(24): P. 2867-2878.
16. Anthony, K., Et Al., Antioxidant And Anti-Hepatitis C Viral Activities Of Commercial Milk Thistle Food Supplements. Antioxidants, 2013. 2(1): P. 23-36.
17. Mayer, K.E., R.P. Myers, And S.S. Lee, Silymarin Treatment Of Viral Hepatitis: A Systematic Review. Journal Of Viral Hepatitis, 2005. 12(6): P. 559-567.
18. Wellington, K. And B. Jarvis, Silymarin: A Review Of Its ClinicalProperties In The Management Of Hepatic Disorders. Biodrugs, 2001. 15(7): P. 465-489.
19. Ezejiofor, A.N., N.A. Udowelle, And O.E. Orisakwe, Nephroprotective And Antioxidant Effect Of Aqueous Leaf Extract Of Costus Afer Ker Gawl On Cyclosporin-A (Csa) Induced Nephrotoxicity. Clinical Phytoscience, 2016. 2(1): P. 11.
20. Yuvaraj, P. And A. Subramoniam, Hepatoprotective Property Of Thespesia Populnea Against Carbon Tetrachloride Induced Liver Damage In Rats. Journal Of Basic And Clinical Physiology And Pharmacology, 2009. 20(2): P. 169-178.
21. Bancroft, J.D. And M. Gamble, Theory And Practice Of Histological Techniques. 2008: Elsevier Health Sciences.
22. Erdem, Ş.R., Et Al., Cyclosporine A-Induced Acute Hepatotoxicity In Guinea Pigs Is Associated With Endothelin-Mediated Decrease In Local Hepatic Blood Flow. Life Sciences, 2011. 88(17-18): P. 753-760.
23. Ahmed, S.A., Hepatoprotective Effects Of L-Carnitine Against Cyclosporine A-Induced Liver Injury In White Albino Rats; A Newly Proposed Mechanism Of Action. International Journal Of Research In Pharmacology & Pharmacotherapeutics.(3)5. 2016
24. Kshirsagar, A., Et Al., Hepatoprotective And Antioxidant Potential Of Calotropis Gigantea In Cyclosporine-An Induced Hepatotoxicity. Research Journal Of Pharmacology And Pharmacodynamics, 2010. 2(5): P. 343-347.
25. Ezejiofor, A.N. And O.E. Orisakwe, Asessment Of The Hepatoprotective And Antioxidant Effect Of Aqueous Leaf Extract Of Costus After "Ker Gawl" On Cyclosporine A Induced Hepatotoxicity. Toxicology International, 2015. 22(3): P. 83-91.
26. Ranju, P., Et Al., Hepatoprotective Activity Of Beta Vulgaris Against Ccl4 Induced Acute Hepatotoxicity In Rats. Archives Of Applied Science Research, 2010. 2(1): P. 14-18.
27. Sadeek, E.A., Protective Effect Of Fresh Juice From Red Beetroot (Beta Vulgaris L.) And Radish (Raphanus Sativus L.) Against Carbon Tetrachloride Induced Hepatotoxicity In Rat Models. African J. Biol. Sci, 2011. 7(1): P. 69-84.
28. Pradhan, S.C. And C. Girish, Hepatoprotective Herbal Drug, Silymarin From Experimental Pharmacology To Clinical Medicine. Indian Journal Of Medical Research, 2006. 124(5): P. 491-504.
29. Huda, M.N. And M.A. Mosaddik, Hepatoprotective Activity of Sharbat Chylosin a Polyherbal Formulation Against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. World Journal of Pharmacy and Pharmaceutical Sciences, 2018. 7(9).
30. Nada, S.A., Et Al., Protective Effect Of Grape Seed Extract And/Or Silymarin Against Thioacetamide-Induced Hepatic Fibrosis In Rats. J Liver, 2015. 4(178): P. 2167-0889.1000178.
31. Shelbaya, L.A., Evaluation Of Protective And Antioxidant Activity Of Milkthistle On Paracetamol-Induced Toxicity In Rats. Journal Of American Science, 2013. 9(10): P. 272-278.
32. Czaja, M.J. Cell Signaling In Oxidative Stress-Induced Liver Injury. In Seminars In Liver Disease. 2007: Thieme Medical Publishers.
33. Kurus, M., Et Al., Oral L-Arginine Protects Against Cyclosporine-Induced Hepatotoxicity In Rats. Experimental And Toxicologic Pathology, 2008. 60(4-5): P. 411-419.
34. Akbulut, S., H. Elbe, And C. Eris, Effects Of Antioxidant Agents Against Cyclosporine-Induced Hepatotoxicity. Journal Of Surgical Research, 2015. 193: P. 658-666.
35. Serkova, N.J., U. Christians, And L.Z. Benet, Biochemical Mechanisms Of Cyclosporine Neurotoxicity. Molecular Interventions, 2004. 4(2): P. 97.
36. Clemens, M.G., Nitric Oxide In Liver Injury. Hepatology, 1999. 30(1): P. 1-5.

37. Da Costa, M.C., Et Al., Cyclosporin A Tubular Effects Contribute To Nephrotoxicity: Role For Ca2+ And Mg2+ Ions. Nephrology Dialysis Transplantation, 2003. 18(11): P. 2262-2268.
38. Rao, M.R., Et Al., Cyclosporin A-Induced Changes In Nitric Oxide Synthase Activity In The Rat Kidney: In Vitro And In Vivo Assay. Indian J Physiol Pharmacol, 1996. 40(2): P. 2.
39. Gallego, M.J., Et Al., Blockade Of Endothelium-Dependent Responses In Conscious Rats By Cyclosporin A: Effect Of L-Arginine. American Journal Of Physiology-Heart And Circulatory Physiology, 1993. 264(3): P. H708-H714.
40. Pari, L. And R. Sivasankari, Effect Of Ellagic Acid On Cyclosporine A-Induced Oxidative Damage In The Liver Of Rats. Fundamental & Clinical Pharmacology, 2008. 22(4): P. 395-401.
41. Jimenez, R., Et Al., Glutathione Metabolism In Cyclosporine A-Treated Rats: Dose-And Time-Related ChangesIn Liver And Kidney. Clinical And Experimental Pharmacology And Physiology, 2000. 27(12): P. 991-996.
42. Váli, L., Et Al., Liver-Protecting Effects Of Table Beet (Beta Vulgaris Var. Rubra) During Ischemia-Reperfusion. Nutrition, 2007. 23(2): P. 172-178.
43. Krajka-Kuźniak, V., Et Al., Betanin, A Beetroot Component, Induces Nuclear Factor Erythroid-2-Related Factor 2-Mediated Expression Of Detoxifying/Antioxidant Enzymes In Human Liver Cell Lines. British Journal Of Nutrition, 2013. 110(12): P. 2138-2149.
44. Ahmadian, E., Et Al., Betanin Reduces Organophosphate Induced Cytotoxicity In Primary Hepatocyte Via An Anti-Oxidative And Mitochondrial Dependent Pathway. Pesticide Biochemistry And Physiology, 2018. 144: P. 71-78.
45. Amiraslani, B., Et Al., Recognition Of Betaine As An Inhibitor Of Lipopolysaccharide-Induced Nitric Oxide Production In Activated Microglial Cells. Iranian Biomedical Journal, 2012. 16(2): P. 84.
46. Shedid, S.M., N. Abdel-Magied, And H.N. Saada, Role Of Betaine In Liver Injury Induced By The Exposure To Ionizing Radiation. Environmental Toxicology, 2018.
47. Freitag, A.F., Et Al., Hepatoprotective Effect Of Silymarin (Silybum Marianum) On Hepatotoxicity Induced By Acetaminophen In Spontaneously Hypertensive Rats. Evidence-Based Complementary And Alternative Medicine, 2015. 2015.
48. He, L., Et Al., Antioxidants Maintain Cellular Redox Homeostasis By Elimination Of Reactive Oxygen Species. Cellular Physiology And Biochemistry, 2017. 44(2): P. 532-553.
49. Kshirsagar, A., Et Al., Silymarin: A Comprehensive Review. Pharmacognosy Reviews, 2009. 3(5): P. 126.
50. Pradeep, K., Et Al., Silymarin Modulates The Oxidant-Antioxidant Imbalance During Diethylnitrosamine Induced Oxidative Stress In Rats. European Journal Of Pharmacology, 2007. 560(2-3): P. 110-116.
51. Telford, W.G., L.E. King, And P.J. Fraker, Rapid Quantitation Of Apoptosis In Pure And Heterogeneous Cell Populations Using Flow Cytometry. Journal Of Immunological Methods, 1994. 172(1): P. 1-16.
52. Wolf, A., Et Al. In Vitro Induction Of Apoptosis In Rat Hepatocytes By Cyclosporine A. In Anales De La Real Academia De Farmacia. 2000.
53. Circu, M.L. And T.Y. Aw, Reactive Oxygen Species, Cellular Redox Systems, And Apoptosis. Free Radical Biology And Medicine, 2010. 48(6): P. 749-762.
54. Wu, L.L., Et Al., Urinary 8-Ohdg: A Marker Of Oxidative Stress To DNA And A Risk Factor For Cancer, Atherosclerosis And Diabetics. Clinica Chimica Acta, 2004. 339(1-2): P. 1-9.
55. Josephine, A., Et Al., Role Of Sulphated Polysaccharides From Sargassum Wightii In Cyclosporine A-Induced Oxidative Liver Injury In Rats. BMC Pharmacology, 2008. 8(1): P. 4.
56. Abdel-Daim, M.M. And S.A. Abdellatief, Attenuating Effects Of Caffeic Acid Phenethyl Ester And Betaine On Abamectin-Induced Hepatotoxicity AndNephrotoxicity. Environmental Science And Pollution Research, 2018: P. 1-9.
57. Al-Rasheed, N., Et Al., Assessment Of The Potential Role Of Silymarin Alone Or In Combination With Vitamin E And/Or Curcumin On The Carbon Tetrachloride Induced Liver Injury In Rat. Brazilian Archives Of Biology And Technology, 2015. 58(6): P. 833-842.
58. Luedde, T. And R.F. Schwabe, NF-Kb In The Liver-Linking Injury, Fibrosis And Hepatocellular Carcinoma. Nature Reviews Gastroenterology & Hepatology, 2011. 8(2): P. 108.

59. Zhu, W. And P.C.W. Fung, The Roles Played By Crucial Free Radicals Like Lipid Free Radicals, Nitric Oxide, And Enzymes NOS And NADPH In Ccl4-Induced Acute Liver Injury Of Mice. Free Radical Biology And Medicine, 2000. 29(9): P. 870-880.
60. Andrés, D., EtAl., Relationship Between The Activation Of Heat Shock Factor And The Suppression Of Nuclear Factor-Kb Activity In Rat Hepatocyte Cultures Treated With Cyclosporine A. Biochemical Pharmacology, 2002. 64(2): P. 247-256.
61. Salem, N.A., Et Al., ProtectiveEffect Of Trapidil And L-Arginine Against Renal And Hepatic Toxicity Induced By Cyclosporine In Rats. Renal Failure, 2010. 32(8): P. 959-968.
62. Li, Y., Et Al., Quercetin, Inflammation And Immunity. Nutrients, 2016. 8(3): P. 167.
63. Sandhiutami, N.M.D., Et Al., In Vitro Assesment Of Anti-Inflammatory Activities Of Coumarin And Indonesian Cassia Extract In RAW264. 7 Murine Macrophage Cell Line. Iranian Journal Of Basic Medical Sciences, 2017. 20(1): P. 99.
64. Armutcu, F., Et Al., Therapeutic PotentialOf Caffeic Acid Phenethyl Ester And Its Anti-Inflammatory And Immunomodulatory Effects. Experimental And Therapeutic Medicine, 2015. 9(5): P. 1582-1588.
65. Chen, W.-P. And L.-D. Wu, Chlorogenic Acid Suppresses Interleukin-1β-Induced Inflammatory Mediators In Human Chondrocytes. International Journal Of Clinical And Experimental Pathology, 2014. 7(12): P. 8797.
66. Afifi, N.A., Et Al., Quercetin Protects Against Thioacetamide Induced Hepatotoxicity In Rats Through Decreased Oxidative Stress Biomarkers, The Inflammatory Cytokines;(TNF-A),(NF-K B) And DNA Fragmentation. Der Pharma Chem, 2016. 8(9): P. 48-55.
67. Shi, H., Et Al., Chlorogenic Acid Reduces Liver Inflammation And Fibrosis Through Inhibition Of Toll-Like Receptor 4 Signaling Pathway. Toxicology, 2013. 303: P. 107-114.
68. Manna, S.K., Et Al., Silymarin Suppresses TNF-Induced Activation Of NF-Kb, C-Jun N-Terminal Kinase, And Apoptosis. The Journal Of Immunology, 1999. 163(12): P. 6800-6809.
69. Saller, R., R. Meier, And R. Brignoli, The Use Of Silymarin In The Treatment Of Liver Diseases. Drugs, 2001. 61(14): P. 2035-2063.
70. Nacar, A., Et Al., Investigation Of The Protective Effect Of Erdosteine Against Cyclosporine-Induced Injury In Rat Liver With Histological And Biochemical Methods. TurkishJournal Of Medical Sciences, 2015. 45(6): P. 1390-1395.
71. Olumese, F.E. And H.A. Oboh, Hepatoprotective Effect Of Beetroot Juice On Liver Injury In Male Sprague-Dawley Rats. Annals Of Tropical Pathology, 2018. 9(1): P. 83.
72. Wang, L., Et Al., Protective Effects Of Silymarin On Triptolide-Induced Acute Hepatotoxicity In Rats. Molecular Medicine Reports, 2018. 17(1): P. 789-800.
73. Soliman, H.A., N.A. Eltablawy, And M.S. Hamed, The Ameliorative Effect Of Petroselinum Crispum (Parsley) On Some DiabetesComplications. J Medicinal Plants Studies, 2015. 3: P. 92-100.