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Mistletoe (*Viscum album* L.) extract attenuates ıtraconazole-ınduced acute oxidative stress and hepatocellular ınjury in rats

Esin Sakallı Çetin[1], Hamdi Sozen[2], Ozgur Ilhan Celik[3], Ibrahim Hakkı Cigerci[4] and Nigar Yılmaz[5]

[1]Department of Medical Biology, Faculty of Medicine, Mugla Sitki Kocman University, Mugla, Turkey,
[2]Department of Infectious Disease, Faculty of Medicine, Mugla Sitki Kocman University, Mugla, Turkey
[3]Department of Pathology, Faculty of Medicine, Mugla Sitki Kocman University, Mugla, Turkey
[4]Department of Molecular Biology and Genetics, Faculty of Science, Afyon Kocatepe University, Afyon, Turkey,
[5]Department of Medical Biochemistry, Faculty of Medicine, Mugla Sitki Kocman University, Mugla, Turkey

Abstract: In the current study, the protective effect of a mistletoe extract (Helixor®, HLX) on Itraconazole (ITZ)-induced hepatocellular injury and acute oxidative stress in rats was aimed to be investigated by histological, biochemical and comet assay methods. Four groups a control group, an HLX group (5mg/kg/14days/intraperitoneally (ip)), an ITZ group (100mg/kg/14days/oral) and an HLX plus ITZ group (5mg/kg/14days/ip+100mg/kg/14days/oral) were all created from 32 female Wistar albino rats. At the end of the experiment, AST and ALT liver enzymes, total oxidant status (TOS) levels and total antioxidant status (TAS) levels, histopathological analysis and comet assay were carried out. Highest genotoxicity, higher levels of plasma AST and ALT, higher TOS, more degeneration of liver histopathology including hepatocyte degeneration, hepatocyte apoptosis and necrosis, portal/periportal inflammation, bile ductus hyperplasia and multinuclear giant cell formation were observed in ITZ group ($p<0.05$). As opposed to that, administration of HLX plus ITZ improved histopathological changes and DNA damage and showed a dramatic decrease in AST, ALT and TOS levels ($p<0.05$) and an increase in TAS level ($p<0.001$) when compared to ITZ group. This study showed that the antioxidant properties of HLX administration significantly decreased acute oxidative stress and hepatocellular damage in rats given ITZ.

Keywords: Itraconazole, helixor, oxidative stress, antioxidant activity, comet assay

INTRODUCTION

Itraconazole (ITZ) is a synthetic triazole with broad-spectrum antifungal activity. The key component of fungal cell membranes and the underlying molecular mechanism of ITZ is the suppression of the fungal cytochrome P450 (CYP) oxidase-mediated production of ergosterol. ITZ also induces cellular oxidative stress in reaction to infections, known as an oxidative stress drug, which adds to the death of microorganisms (Li *et al*.,2022; Lin *et al*., 2019; Somchit *et al*., 2004; Zuckerman JM and Tunkel AR, 1994). ITZ is used systemically and it causes dose-dependent adverse effects such as vomiting and nausea at low doses and hypokalemia, gastrointestinal disturbances, dizziness, pruritus, diarrhea and skin rash at high doses (>400 mg/day). Hepatotoxicity is also rare but considered a potentially serious adverse effect associated with ITZ therapy (Greenblatt HK and Greenblatt DJ, 2014; Kyriakidis *et al*., 2017; Lavrijsen, 1992, 1993; Somchit, 2004, 2006; Tucker *et al*., 1990). Among ITZ users, the rate of aberrant results of liver function tests has been estimated to be 3%. After termination of ITZ treatment and administration of supportive therapy, these results recover spontaneously. In long-term ITZ therapy, hepatic dysfunction in patients has been reported to be fatal, so the liver functions of patients should be closely monitored (Rodriquez and Acosta, 1997a, 1997b; Rodriquez *et al*., 1999). The liver is the main organ of detoxification of ITZ to remove the toxic metabolites (Lou *et al*., 2011). However, the mechanism of liver damage in patients undergoing ITZ therapy remains obscure, which could be due to metabolic idiosyncrasy (Rodriquez and Acosta, 1996, 1997; Rodriquez *et al*., 1999). Recent studies have shown that the drug-metabolizing enzyme CYP3A but not 1A or 2E is associated with the pathogenesis of ITZ-induced hepatotoxicity (Penzak *et al*. 1999).

Viscum album L. (VA) preparations brand names such as Iscador, Helixor (HLX), Isorel, Eurixor, Iscucin, Plenosol, etc., are anthroposophical/homeopathically produced and standardized extracts (Freuding *et al*., 2019). VA is an evergreen semi-parasitic plant of the Loranthaceae family that grows all over the world on various deciduous trees and obtaining its nutrients and water from its host. So based on the species of the host tree, harvesting time and method of extraction, VA preparations widely differ with regard to their chemical compositions. HLX is composed of lectin mainly and viscotoxins and other biologically active components like flavonoids, polysaccharides, biogenic amine, alkaloids, terpenoids, saponins, vitamins, phytosterols, hydrocarbons, long-chain fatty acids (Deliorman *et al*., 2001a, 2001b; Lorch 1993; Orru *et al*., 1997; Peumans *et al*., 1996; Radenkovic *et al*., 2006;

*Corresponding author: e-mail: esincetin@mu.edu.tr

Stirpe *et al*., 1992; Szurpnicka *et al*., 2020; Wollenweber *et al*., 2000). VA preparations have attracted attention as a potential anticancer agent because of their immunomodulatory and cytotoxic features and used in the protocols for adjuvant cancer therapies alongside standard chemotherapies or radiotherapies in Europe since the 1970s. Additionally, by stimulating the immune system, these combination therapies help to enhance the cancer patients' quality of life as a whole. (Maier and Fiebig, 2002). Besides anti-tumor, immune-modulator and anti-inflamatuar effects, VA could also enhance DNA stability and decreases mutagenicity of reactive oxygen species produced during chemotherapy and/or radiotherapy (Kovacs *et al*., 1991; Kovacs 2002; Büssing *et al*. 1995).

Our prior research demonstrated that ITZ damaged the liver, produced ROS and caused DNA damage. (Sözen *et al*., 2015). In several studies, antioxidant agents have been used to prevent oxidative stress caused by various drugs and have achieved significant success. HLX was selected as an antioxidant agent due to its powerful antioxidant capacity and for acting as a potent scavenger of free radicals, previously evaluated to prevent oxidative damage (Çetin *et al*., 2017; Kim *et al*., 2010; Oluvaseun and Ganiyu 2008; Onay Ucar *et al*., 2006, 2012; Onunogbo *et al*., 2012; Orhan *et al*., 2005). To date, no report has evaluated the defenses of HLX treatment against rats' ITZ-induced liver damage. The purpose of this work was to identify HLX's hepatoprotective and antioxidant properties against acute oxidative stress and rat hepatocellular damage caused by ITZ.

MATERIALS AND METHODS

Chemicals

Itraconazole and Helixor A (lot 4113302) were purchased from Nobel Pharmaceutical Industry and Trade Co. Ltd. and Helixor Heilmittel GmbH & CoKG, Rosenfeld, German, respectively. The *Viscum album* L. plant extract total in the Helixor A ampoules was 50mg in 1 ml of water.

Animals

A total of 200-220g 32 female Wistar albino rats were purchased from Suleyman Demirel University. Rats were kept at room temperature with food and water in a 12-hour light/12-hour dark cycle. The Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Suleyman Demirel University, Isparta, Turkey were both followed when conducting all research.

Experimental protocol

The experimental rats were therefore separated into four subgroups of eight rats each.

Group I, the control group, received oral administration of isotonic saline (0.9 % NaCl) solution.

Group II, the HLX group. received i.p. injections of Helixor A (5mg/kg bw/14 days)

Group III, ITZ group, received oral administration of ITZ (100mg/kg bw/14 day).

Group IV, HLX+ ITZ group, received i.p. injections of Helixor A (5mg/kg bw/14 day) and oral administration of ITZ (100mg/kg bw/14 day).

Specimen collection

After the experiment was finished, all of the rats were anesthetized using intramuscular injections of ketamine hydrochloride, an arylcyclohexylamine derivative (Ketalar; 50mg/kg; Eczacibasi, Istanbul, Turkey). Blood was drawn intracardially and placed in heparinized containers for AST, ALT and comet test analyses. The rats' livers were swiftly removed and they were split in half. The second half of the liver was rinsed with saline solution and stored at -80°C until further investigation, with the first half of the liver being preserved in formaldehyde solution for further histological evaluation.

Biochemical analysis

AST and ALT levels in serum were measured spectrophotometrically by autoanalyzer using Beckman Coulter kits (Unicel DxC 800 Synchron, Brea, California, USA). Erel's approach was used to test total oxidant status (TOS) and total antioxidant status (TAS) levels spectrophotometrically utilizing kits (Rel Assay, Gaziantep, Turkey). The findings of TAS and TOS were represented as mmol Trolox Equivalence per gram of tissue and μmol H_2O_2 Equivalence per gram of tissue, respectively.

Histopathological analysis

Liver tissues were preserved in 10% formaldehyde for fixation before being sliced into 5mm sections and stained with haematoxylin and eosin. On 10 slides with a 100x-400x magnification, each rat's hepatic damage was graded based on the following criteria: 0=none, 1=mild, 2=moderate, 3=severe for each criterion, by using a semiquantitative scale as follows: 1-Degeneration of centrilobular hepatocytes, 2-Ductular degeneration (bile duct hyperplasia), 3-Bile plugs, 4-Periportal inflammation, 5-Eosinophilic infiltration in the portal area and parenchyma, 6- Formation of several nuclear giant cells, 7-The presence of Xanthoma cells in the portal area, 8-The presence of apoptotic cells, 9-Necrosis.

Comet assay

Comet assay, the alkaline single-cell gel electrophoresis experiment, was utilized to measure the movement of DNA fragments from the nucleoid, which visually resembles a comet, in order to assess the endogenous DNA damage in lymphocytes occurring as single-strand breaks. A fluorescence microscope (Olympus-Japan) was used to examine 100 randomly selected nuclei per rat (50 cells were studied on each slide) at 400X magnification. According to the length of the comet tail, the degree of damage was assessed and was given a score between 0 and 4.

ETHICAL APPROVAL

This study was approved by the Local Ethics Committee of Suleyman Demirel University (SDU), Isparta, Turkey (Protocol Number: 27-04: Approve Date: 28.08.2011). The rats were cared in accordance with the guidelines of the Animal Care Committee of SDU.

STATISTICAL ANALYSIS

Statistical analysis was performed with the package application "SPSS version 20.0 for Windows". The Kruskal-Wallis test was used to identify significant group differences. To compare groups, the Mann-Whitney U test was used. Results were shown as mean±SD; statistical significance was determined by $p<0.05$, whereas $p<0.01$ was retained to illustrate highly statistically significant results.

RESULTS

Levels of AST, ALT, TAS and TOS

Table 1. summarizes the plasma AST and ALT concentrations. When compared to the control group, the plasma AST and ALT levels in the ITZ-treated group were considerably higher ($p<0.001$). It's interesting to note that Helixor therapy reversed the elevated AST and ALT levels in the HLX+ITZ group as compared to the ITZ group ($p<0.05$).

When compared to the control group, the ITZ-treated group had considerably higher TOS but no statistically different TAS levels. When compared to the ITZ group, the HLX+ITZ group showed a marked drop in TOS level ($p<0.05$) and an elevation in TAS level ($p<0.001$).

Histopathological results

The results of the liver histopathology for each group are listed in table 2. The liver tissues of the control and HLX groups had normal hepatic morphology during the histopathological evaluation (fig. 2A and 2B). ITZ significantly resulted in centrilobular hepatocyte degeneration ($p=0.000$) in fig. 2C, inflammation of the portal/periportal area ($p=0.000$) in fig. 2D and 2E, ductular reaction (bile duct hyperplasia) ($p=0.001$), bile duct plugs ($p=0.027$) in fig. 2E, multinuclear giant cell formation ($p=0.064$) in fig. 2F, presence of xanthomatous cells in the portal area ($p=0.144$), presence of apoptotic cells ($p=0.009$) and necrosis ($p=0.317$) in fig. 2D and 2G.

Additionally, administration of HLX plus ITZ effectively diminished both centrilobular hepatocyte degeneration ($p<0.05$) (fig. 2H) and parenchymal hepatocyte apoptosis ($p<0.05$) (fig. 2I) in the liver. Improvement was also shown in the HLX+ITZ group for the ITZ-induced ductular degeneration, the portal/periportal inflammation and the production of multinucleated giant cells, though these improvements were not determined to be statistically significant ($p=0.053$, $p=0.094$ and $p=0.064$, respectively). Infiltration of the portal/periportal region with some inflammatory cells, primarily eosinophils, was seen in the liver tissue of rats treated with the HLX and HLX+ITZ group (fig. 2F), but not in the ITZ group. These values were determined to be statistically significant between the HLX+ITZ group and the ITZ group ($p=0.027$).

Comet assay results

The comet assay findings revealed that DNA damage was significant when compared to the control group at all doses. The control group had the lowest level of genotoxic activity (10.50±1.55), whereas the ITZ group had the highest level (27.32±1.86). ITZ considerably increases DNA damage compared to the control and HLX groups while administration of HLX plus ITZ provided a great improvement effect on DNA damage. Comparing the HLX+ITZ group to the ITZ group, the reduction in DNA damage is statistically significant. DNA damage has been prevented because of HLX usage (table 3).

DISCUSSION

The current study's findings supported the effectiveness of *VA* extract HLX as a treatment for liver damage caused by ITZ. The result of the current study confirmed that *VA* extract HLX is an effective agent, offering protection against ITZ-induced liver injury. Short-term administration of ITZ to the experimental rats induced acute hepatic injury promoted by a marked elevation of the levels of diagnostic liver function enzymes in serum (AST, ALT) and tissue oxidative stress parameters (TAS, TOS), increased endogenous lymphocyte DNA damage and histopathologic alterations. Hepatotoxicity can be caused by a number of illnesses and medications. Several studies show that ITZ in an overdose can induce severe hepatotoxicity (Greenblatt HK and Greenblatt DJ, 2014; Hecht *et al*., 1997; Kyriakidis *et al*, 2017; Lavrijsen *et al*., 1992, 1993; Somchit *et al*., 2004, 2006; Tucker *et al*., 1990). One of the main mechanisms of ITZ-induced hepatotoxicity is oxidative stress. Additionally, in accordance with the results of our previous investigation, we found that using ITZ increases oxidative stress (NO, MPO), decreases the antioxidant effect (SOD, GSH-Px) and results in DNA and liver damage as consistent with the current study (Sözen *et al*., 2015).

Hepatotoxicity was decreased by two weekly administration of HLX along with reduction in AST and ALT hepatocellular enzymes. Moreover, the decreased DNA damage and with improvement in liver morphology was also observed. These parameters are useful quantitative hepatocellular damage markers. While high levels of AST indicate the loss of liver functional integrity, ALT enzyme catalyzes the alanine to pyruvate and glutamate, show better parameters for diagnosing liver damage (Grespan *et al*., 2014). The measurement of increased serum AST and ALT activities indicate a

hepatocelullar damage and dysfunction with an increased damage and permeability of hepotocytes (Grespan *et al*., 2014). Our results are aggrement with earlier report by Somchit *et al*. (2006), who reported elevated ALT and AST levels induced by ITZ. The decreased serum AST and ALT activities in group HLX+ITZ relative to ITZ is obvious indication of regeneration and repair of hepatic tissue damage of rats. The same group also showed a substantial decrease in the serum indicators of liver damage, as well as an increase in TAS and a significant decrease in TOS levels in addition to this improvement (AST, ALT). The results indicate that oxidative stress plays a crucial role in ITZ-induced hepatotoxicity as well as the hepatoprotective action of mistletoe, which is mediated by its antioxidant function. In our investigation, there was no discernible difference between the TAS and TOS levels in both HLX and control groups. This may be a sign that HLX is also likely to have any effect on healthy people.

Our results corroborate the findings of Patrick-Iwuanyanwu *et al*. (2007) and Abdel-Salam *et al*. (2010) that they reported that high serum AST, ALT, ALP levels caused by CCl4 were restored by VA. Another clinical findings of HLX confirmed these investigations. Twenty-one patients were succsessfully treated with HLX preparation as monotherapy (either Iscador or Abnoba viscum) having chronic hepatitis C. During one year treatment with HLX, improved the transaminases AST and ALT levels in patients (Tusenius *et al*., 2005). However, precise mechanisms behind modulation of hepatic injury by VA are still unclear and further investigations are needed to explore this. It can be suggested that HLX may ameliorate liver damage by its phytochemical constituents, mostly flavonoids and lectins. Flavonoids are pharmaceutically active phenolic derivatives of HLX shown to be hepatoprotective in phytochemical sudies. Also, phenolic compounds and lectins are powerful antioxidants and have the capacity for acting as potent scavengers of free radicals, reducing agent and metal chelators due to their redox properties (Seewole *et al*., 1984; Wegner and Fintelmann 1999). Onunogbo *et al*. (2012) reported that the high phenolic compounds from VA extract suggests its antioxidant potential. Similarly, the antioxidant and hepatoprotective activity of *African Viscum album* in rats has been reported to be due to the presence of flovanoids (Patrick-Iwuanyanwu *et al*., 2007). In previous studies, *in vitro* antioxidant activity of methanolic extracts of VA has been already explained (Oluvaseun and Ganiyu, 2008; Onay Ucar *et al*., 2006, 2012; Orhan *et al*. 2005). Similar to this, Kim *et al*. (2010) reported on the radical scavenging activity of Korean mistletoe lectin (KML) *in vitro* as well as its anti-oxidant effects against free radicals, nitric oxide (NO), superoxide anion (O2-) and peroxynitrite-induced oxidative stress (ONOO-). Through NF-k B regulation, the protective effects of KML counteracting the oxidative stress were linked to decreased mRNA and protein expressions of COX-2 and iNOS. Animal research that examined the protective effects of HLX against oxidative stress on diabetic rats' liver, kidney, brain and heart came to similar conclusions. (Gren and Farmicki 2013; Orhan DD. *et al*., 2005; Şekeroğlu and Şekeroğlu, 2012).

According to reports, ITZ may have caused the production of ROS, which damaged genomic DNA, caused oxidative degradation of lipids and proteins and disrupted hepatocyte cell membrane permeability (Al-Dbass *et al*., 2012; Garcia-Nino *et al*., 2013). In line with these findings, we demonstrated DNA damage caused by ITZ and the VA's significant progressive effect by a significant reduction in the parameters of the comet assay. Similarly, increased DNA damage was observed in hepatocytes of pregnant rats, which were given itraconazole orally (El-Shershaby *et al*., 2015). Şekeroğlu (2012) reported the relieving effects of VA on cyclophosphamide-induced chromosomal damage in bone marrow cells of mice in addition to oxidative stress and inflamation in the heart and bladder. Another *in vitro* research that revealed similar outcomes found that VA has protective effects against oxidative nuclear and mitochondrial DNA damage brought on by H_2O_2 in HeLA cells, either directly by inhibiting ROS generation or indirectly by inducing oxidative damage repair and phase II enzymes (Onay-Ucar *et al*., 2012).

ITZ-induced ROS production inhibite microsomal cytochrome P450 isoenzyme CYP3A4, which plays a key role in the detoxification of ITZ or its metabolites (Somchit *et al*., 2009). In this regard, Somchit *et al*. (2009) has revealed that at higher doses ITZ can cause an autoinhibition of its metabolism via inhibition of cytochrome P450 and may then be forced to be metabolised by an alternative route, flavin-containing monooxygenase. This metabolism may be responsible for ketoconazole-induced hepatotoxicity as suggested by Rodriguez and Acosta (1997). The HLX's inhibition and induction activity of cytochrome P450 enzymes is indicative of no interaction potential on the cytochrome P450 enzyme system (Doehmer and Eisenbraun, 2012). Clinically, these findings were confirmed by Mansky *et al*. (2005) that investigated the effect of an additional HLX therapy in the plasma concentration of the simultaneous therapy with gemcitabine and HLX therapy and found similar results.

CONCLUSİON

In conclusion, this study demonstrated that HLX were effective in alleviating the hepatotoxicity induced by ITZ by its antioxidant capacity via scavenging free radicals and reinforce the liver defence system. HLX may thus be a novel therapeutic agent for the individuals with the chronic liver disease since it seems to have considerable hepatic protective effects. In order to characterize the more likely positive benefits of HLX usage and to examine the mechanism of its hepatoprotective effects revealed in the present study, more research is needed.

Table 1: AST, ALT, TAS and TOS values in the liver of the 4 groups of the rats

	AST	ALT	TAS	TOS
Control	87.63±2.10	31.385±1.663	7.57±1.50	91,42±2.60
HLX	87.250±1.341	33.250±2.538	7.52±1.83	90.82±1.87
ITZ	211.800±4.26[a]	72.750±3.063[a]	8.55±0.90	128.24±1.85[a]
HLX+ ITZ	99.130±3.352[b]	39.380±2.576 [b]	12.15±2.07[c]	111.66±1.29 [b]

*Results are presented as mean±SD. Groups of data were compared with the Kruskal-Wallis test followed by the Mann-Whitney U test. AST; aspartate aminotransferase (u/l), ALT; alanine aminotransferase (u/l), TAS; total antioxidant status (mmol Trolox Eq/L), TOS; total oxidant status (µmol H_2O_2 Eq/L), SD=standard deviation.
[a]p<0.001 compared with control group
[b]p<0.05 compared with ITZ group
[c]p<0.001 compared with ITZ group

Table 2: Histopathological findings in the livers of the 4 groups of rats (n=8 each)

	CONTROL	HLX	ITZ	HLX + ITZ
Centrilobular hepatocytes degeneration	0	0.125	2.25[ax]	0.875[b]
Ductular degeneration (bile duct hyperplasia)	0	0	1.625[a]	0.75
Bile plugs	0	0	0.625[ax]	0.25
Periportal inflammation	0	0.5	1.625[ax]	0.875
Eosinophilic infiltration	0	0.625	0	0.875[b]
Multinuclear giant cell formation	0	0	0.5	0
Xanthomatous cells in the portal area	0	0	0.375	0
Apoptotic cells	0	0	0.625[a]	0[b]
Necrosis	0	0	0.25	0

Results are presented as the median of the scores.
[a]p<0.05 compared with control group
[b]p<0.05 compared with ITZ group
[x]p<0.001 compared with control group

Table 3: Comet assay values of the 4 groups of the rats

	Control	HLX	ITZ	HLX+ITZ
DNA damage, AU ± SD*	10.50±1.55	22.66±4.04[a]	27.32±1.86[a]	18.66±1.15[ab]

*Mean±SD. AU=arbitrary unit, SD=standard deviation.
[a]p<0.05 compared with control group
[b]p<0.05 compared with ITZ group (Duncan test).

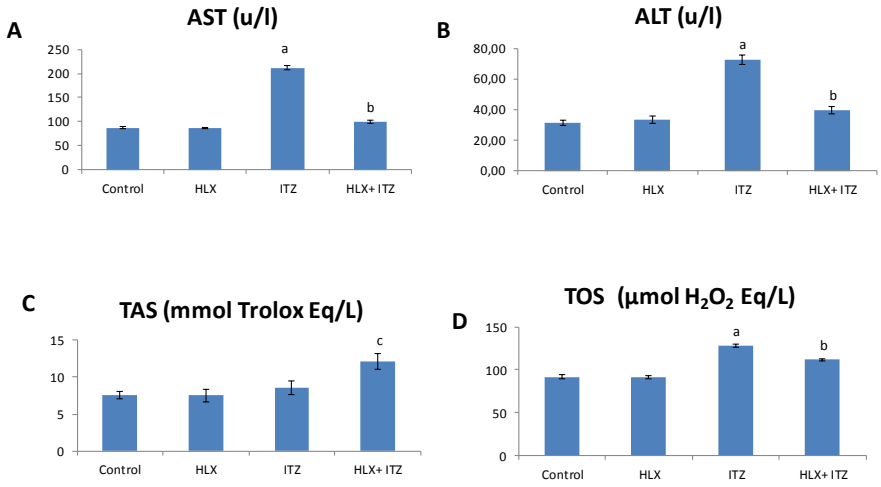

Fig. 1: Liver (A) AST, (B) ALT, (C) TAS, (D) TOS values in the control, HLX, ITZ and HLX+ITZ groups. Each group consists of 8 animals. Different characters (a, b) above the columns represent significance at p<0.05; 'c' represents significance at p<0.001.

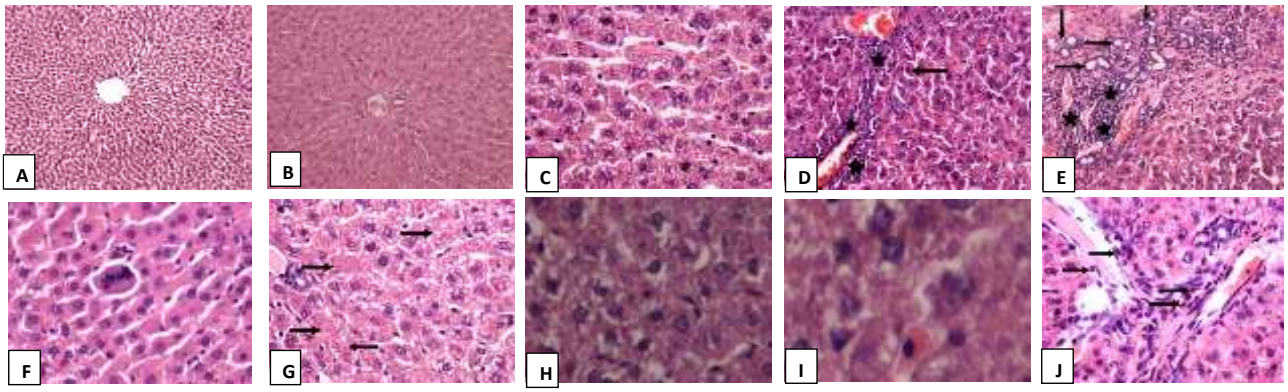

Fig. 2: (A) Control group normal liver morphology, (B) HLX group (H&E,×100): normal liver morphology-normal hepatocytes around central vein, normal portal areas, (C) ITZ group (H&E,×400): hepatocyte degeneration, (D) ITZ group (H&E,×200): hepatocyte apoptosis (arrow) and portal inflamation (stars), (E) ITZ group (H&E,×200): Bile ductus hyperplasia (arrows) and portal inflamation (stars), (F) ITZ group (H&E,×400): Multinuclear giant cell, (G) ITZ group (H&E,×400): hepatocyte necrosis (arrows), (H) ITZ+HLX group (H&E,×400): decreased hepatocyte degeneration, (I) ITZ+HLX group (H&E,×400): decreased hepatocyte apoptosis, (J) ITZ+HLX group (H&E,×400): eosinophil infiltration in portal area (arrows), H&E=hematoxylin and eosin.

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REFERENCES

Bussing A, Regnery A and Schweizer K (1995). Effects of Viscum album L. on cyclophosphamide-treated peripheral blood mononuclear cells *in vitro*: Sister chromatid exchanges and activation/proliferation marker expression. *Cancer Lett.,* **94**(2): 199-205.

Büssing A and Schietzel M (1999). Apoptosis-inducing properties of Viscum album L. extracts from different host trees, correlate with their content of toxic mistletoe lectins. *Anticancer Res.,* **19**(1A): 23-28.

Çetin ES, Tetiker H, Çelik Öİ, Yılmaz N and Ciğerci İH (2017). Methotrexate-induced nephrotoxicity in rats: protective effect of mistletoe (*Viscum album L.*) extract. *Complement Med. Res.*, 24(6): 364-370

Deliorman D, Calis I and Ergun F (2001a). A new acyclic monoterpene glucoside from *Viscum album* ssp. *Album*. *Fitoterapia,* **72**(2): 101-105.

Deliorman D, Ergun F and Sener B and Palittapongarnpim P (2001b). Evaluation of antibacterial activity of Viscum album subspecies. *Pharm. Biol.,* **39**(5): 381-385.

Doehmer J and Eisenbraun J (2012). Assessment of extracts from mistletoe (*Viscum album*) for herb-drug interaction by inhibition and induction of cytochrome P450 activities. *Phytother. Res.,* **26**(1): 11-7.

El-Shershaby A, Dakrory AI and El-Dakdoky MH and Ibrahim J (2015). Biomonitoring of the Genotoxic and Hepatotoxic Effects and Oxidative Stress Potentials of Itraconazole in Pregnant Rats. *Birth Defects Res. B. Dev. Reprod Toxicol.,* **104**(2): 55-64

Freuding M, Keinki C, Micke O, Buentzel J and Huebner J (2019). Mistletoe in oncological treatment: A systematic review: part 1: Survival and safety. *J. Cancer Res. Clin. Oncol*., **145**(3): 695-707.

Garcia-Nino WR, Tapia E and Zazueta C, Zatarain-Barrón ZL, Hernández-Pando R, Vega-García CC adn Pedraza-Chaverrí J (2013). Curcumin pretreatment prevents potassium dichromate-induce hepatotoxicity, oxidative stress, decreased respiratory complex i activity and membrane permeability transition pore opening. *Evid. Based Complement Altern. Med.,* **2013**: 424692.

Gardin NE (2009). Immunological response to mistletoe (Viscum album L.) in cancer patients: A four-case series. *Phytother. Res.,* **23**(3): 407-411.

Greenblatt HK and Greenblatt DJ (2014). Liver injury associated with ketoconazole: Review of the published evidence. *J. Clin. Pharmacol*., **54**(12): 1321-9.

Greń A and Formicki G (2013). Effects of iscador and vincristine and 5-fluorouracil on brain, liver and kidney element levels in alloxan-induced diabetic mice. *Biol Trace Elem Res.,* **152**(2): 219-24.

Hecht FM, Wheat J, Korzun AH,Hafner R, Skahan KJ, Larsen R, Limjoco MT, Simpson M, Schneider D, Keefer MC, Clark R, Lai KK, Jacobson JM, Squires K, Bartlett JA and Powderly W (1997). Itraconazole maintenance treatment for histoplasmosis in AIDS: A prospective, multicenter trial. *J. AIDS.,* **16**(2): 100-107.

Kim BK, Choi MJ and Park KY *et al*. (2010). Protective effects of Korean mistletoe lectin on radical-induced oxidative stress. *Biol. Pharm Bull.,* **33**(7): 1152-8.

Kovacs E (2002). The *in vitro* effect of Viscum album (VA) extract on DNA repair of peripheral blood mononuclear cells (PBMC) in cancer patients. *Phytother. Res.,* **16**(2): 143-147.

Kovacs E, Hajto T and Hostanska K (1991). Improvement of DNA repair in lymphocytes of breast cancer patients treated with Viscum album extract (Iscador). *Eur. J. Cancer,* **27**(12): 1672-1676.

Kyriakidis I, Tragiannidis A, Munchen S and Groll AH (2017). Clinical hepatotoxicity associated with antifungal agents. *Expert Opin. Drug Saf.*, **16**(2): 149-165.

Lavrijsen AP, Balmus KJ and Nugteren-Huying WM *et al*. (1993). Liver damage during administration of itraconazole. *Ned Tijdschr Geneeskd*, **137**(1): 38-41.

Lavrijsen APM, Balmus KJ and Nugteren-Huying WM *et al*. (1992). Hepatic injury associated with itraconazole. *Lancet*, **340**(8813): 251 -52.

Li CL, Fang ZX, Wu Z, Hou YY, Wu HT and Liu J (2022). Repurposed itraconazole for use in the treatment of malignancies as a promising therapeutic strategy. *Biomed. Pharmacother.*, **154**: 113616

Lin WC, Chen LH, Hsieh YC, Yang PW, Lai LC, Chuang EY, Lee JM and Tsai MH (2019). miR-338-5p inhibits cell proliferation, colony formation, migration and cisplatin resistance in esophageal squamous cancer cells by targeting FERMT2. *Carcinogenesis.*, **40**(7): 883-892

Lorch E (1993). Neue Untersuchungen uber Flavonoide in Viscum album ssp. abietis, album und austriacum. *Zeitschrift für Naturforschung C*, **48c**: 105-107.

Lou HY, Fang CL and Fang SU *et al*. (2011). Hepatic failure related to itraconazole use successfully treated by corticosteroids. *Hepat. Mon.*, **11**(10): 843-6.

Maier G and Fiebig HH (2002). Absence of tumor growth stimulation in a panel of 16 human tumor cell lines by mistletoe extracts *in vitro*. *Anti-Cancer Drug,s* **13**(4): 373-379.

Mansky PJ, Wallerstedt DB and Monahan BP (2005). Mistletoe extract/Gemcitabine combination treatment: An interim report from the NCCAM/NCI phase I study in patients with advanced solid tumors. *J. Clin.*, **23**(16S): 3098

Oluwaseun AA and Ganiyu O (2008). Antioxidant properties of methanolic extracts of mistletoes (Viscum album) from cocoa and cashew trees in Nigeria. *Afr. J. Biotechnol.*, **7**(17): 3138-3142.

Onunogbo C, Ohaeri OC and Eleazu CO *et al*. (2012). Chemical composition of mistletoe extract (Loranthus micranthus) and its effect on the protein, lipid metabolism and the antioxidant status of alloxan induced diabetic rats. *J. Med. Res.*, **1**(4): 057-062.

Orhan DD, Aslan M and Sendogdu N *et al*. (2005). Evaluation of the hypoglycemic effect and antioxidant activity of three Viscum album subspecies (European mistletoe) in streptozotocin-diabetic rats. *J. Ethnopharmacol.*, **98**(1-2): 95-102.

Orru S, Scaloni A and Giannattasio M *et al*. (1997). Aminoacid sequence, S-S bridge arrangement and distribution in plant tissues of thionins from Viscum album. *Biol. Chem.*, **378**(9): 989-996.

Önay-Uçar E, Erol O and Kandemir B *et al*. (2012). Viscum album L. Extracts Protects HeLa Cells against Nuclear and Mitochondrial DNA Damage. *Evid Based Complement Alternat. Med.*, **2012**(2012): 958740.

Önay-Uçar E, Karagöz A and Arda N (2006). Antioxidant activity of Viscum album ssp. Album. *Fitoterapia*, **77**(7-8): 556-560.

Penzak SR, Gubbins PO and Gurley BJ *et al*. (1999). Grapefruit decrease the systemic availability of itraconazole capsule in healthy volunteers. *Ther. Drug Monit.*, **21**(3): 304-309.

Peumans WJ, Verhaert P and Pfuller U *et al*. (1996). Isolation and partial characterization of a small chitinbinding lectin from mistletoe (*Viscum album* L.). *FEBS Letters*, **396**(2-3): 261-265.

Radenkovic M, Ivetic V and Popovic M *et al*. (2006). Neurophysiological effects of Mistletoe (*Viscum album* L.) on isolated rat intestines. *Phytother Res*, **20**(5): 374-377

Rodriguez RJ and Acosta Jr D (1997), N-deacetyl ketoconazole induced hepatoxicity in a primary culture system of rat hepatocytes. *Toxicology*, **117**(2-3): 123-131.

Rodriquez RJ and Acosta D Jr (1996), Inhibition of mitochondrial function in isolated rate liver mitochondria by azole antifungals. *J. Biochem. Toxicol.*, **11**(3): 127-31.

Rodriquez RJ and Acosta D Jr (1997). Metabolism of ketoconazole and deacetylated ketoconazole by rat hepatic microsomesand flavin-containing monooxygenase. *Drug Metab. Dispos.*, **25**(6): 727-77.

Rodriquez RJ, Proteau PJ and Marquez BL and *et al*. (1999). Flavin-ontaining monooxygenase-525 mediated metabolism of N-deacetyl ketoconazole byrat hepatic microsomes. *Drug Metab. Dispos.*, **27**(8): 880-86.

Sekeroğlu ZA and Sekeroğlu V (2012). Effects of Viscum album L. extract and quercetin on methotrexate-induced cyto-genotoxicity in mouse bone-marrow cells. *Mutat. Res.*, **746**(1): 56-9

Somchit N, Ngee CS, Yaakob A, Ahmad Y and Zakaria ZA (2009). Effects of cytochrome p450 inhibitors on itraconazole and fluconazole induced cytotoxicity in hepatocytes. *J. Toxicol.*, **2009**(2009): 912320.

Somchit N, Norshahida AR and Hasiah AH *et al*. (2004). Hepatotoxicity induced by antifungal drugs itraconazole and fluconazole in rats: A comparative *in vivo* study. *Hum. Exp. Toxicol.*, **23**(11): 519- 525

Somchit N, Wong CW, Zuraini A, Ahmad Bustamam A, Hasiah AH, Khairi HM, Sulaiman MR and Israf DA (2006). Involvement of phenobarbital and SKF 525A in the hepatotoxicity of antifungal drugs itraconazole and fluconazole in rats. *Drug Chem. Toxicol.*, **29**(3): 237-53

Stirpe F, Barbieri L and Batteli MG, Soria M and Lappi DA (1992). Ribosome-inactivating proteins from plants: Present statusand future prospects. *Bio/ Technology*, **10**(4): 405-412.

Szurpnicka A, Kowalczuk A and Szterk A (2020). Biological activity of mistletoe: *In vitro* and *in vivo* studies and mechanisms of action. *Arch. Pharm. Res.*

43(6): 593-629.

Tucker RM, Haq Y, Denning DW and Stevens DA (1990). Adverse events associated with itraconazole in 189 patients onchronic therapy. *J. Antimicrob. Chemother.*, **26**(4): 561-66.

Wegner T and Fintelmann V (1999). Flavonoids and bioactivity. *Wien. Med. Wochenschr,* **149**(8-10): 241-7.

Wollenweber E, Wieland A and Haas K (2000). Epicuticular waxes and flavonol aglycones of the European mistletoe, Viscum album L. *Zeitschrift. Fur. Naturforschung,* **55(**5-6): 314-317.

Yesilada E, Deliorman D, Ergun F, Takaishi Y and Ono Y (1998). Effects of the Turkish subspecies of Viscum album on macrophage-derived cytokines. *J. Ethnopharmacol.,* **61**(3): 195-200.

Zuckerman JM and Tunkel AR (1994). Itraconazole: A new triazole antifungal agent. *Infec. Control Hosp. Epidemiol.,* **15**(6): 397-410.