POTENTIAL ROLE OF VITAMIN D RECEPTOR AGONIST IN ACETAMINOPHEN-INDUCED HEPATIC INJURY: ROLE OF CASPASE-1

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ABSTRACT:

Background *Acetaminophen (ACN) is a well-known analgesic commonly used to induce acute hepatic injury. Oxidative stress and inflammation are the main pathogenic mechanisms that contribute to ACN-induced hepatotoxicity. Vitamin D is reported to have a potential antioxidant and anti-inflammatory actions.*

Aim of the work*: The aim of the present study is to investigate the effects of pretreatment with vitamin D agonist paricalcitol in ACN induced hepatic dysfunction and to test its role in caspase-1 gene expression.*

Materials and methods*: This study was carried out on 40 male albino rats, divided equally into 4 groups. Control group received vehicle, Paricalcitol treated group (PC) received 200 ng intraperitoneally three times a week for 9 days, ACN-treated group received ACN (ACN)orally 2 g/kg body weight on day nine, and Paricalcitol-pretreated plus ACN pretreated group (PC+ACN), treated similar to PC and ACN groups.*

Results*: The PC treated normal rats did not show any significant differences from control group as regards to all the parameters measured or the histopathological changes. Histological examination showed disruption of the liver architecture in ACN group as compared to control animals. Furthermore, ACN group showed significant increase in all hepatic biomarkers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ glutamyl transferase (γGT), serum bilirubin, and a significant decrease in serum albumin. Similarly, the same group showed significant elevation of oxidative marker Malondialdehyde (MDA) and significant decrease of the antioxidant reduced glutathione (GSH) in both serum and hepatic tissue. In addition, the proinflammatory cytokines TNF-α IL-6 and IL-1β were significantly increased in both serum and hepatic tissue homogenate. Moreover, ACN-treated animals showed significant rise of mRNA of caspase-1 gene in their liver tissues. Pretreatment with paricalcitol prevented all the above parameters in the PC+ACN group. Paricalcitol pretreatment, also, down regulated the caspase-1 expression in the ACN-treated group but not in normal rats. Serum calcium and systolic blood pressure did not change significantly in all groups.*

Conclusion*: Vitamin D agonist paricalcitol has a protective effect against ACN-induced acute hepatic injury through antioxidant and anti-inflammatory actions. Moreover, it could down regulate caspase-1 gene expression in hepatic tissue with possible function as an anti-pyroptotic agent.*

Keywords: *Vitamin D agonist - Paricalcitol - ACN - Anti-inflammatory - Caspase-1 – Pyroptosis*

INTRODUCTION:

Acute hepatic failure is a severe form of liver injury that causes multiple organ failure. One of the most used pain medications worldwide, ACN (ACN), is a classical dose-dependent hepatotoxin that is responsible for almost 50% of all acute liver failure cases in the USA, the UK and many Western countries[1].

An overdose of ACN causes liver injury through a toxic metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI). The hepatic levels of reduced glutathione (GSH), required for inactivation of NAPQI, is utilized with an overdose of ACN [2]. Therefore, at overdose, the essential routes become saturated and the production of NAPQI exceeds the capacity to detoxify it. The excess NAPQI then causes liver damage associated with the generation of free radicals and oxidative stress[3]. Reactive oxygen species (ROS) in turn induce oxidative stress leading to lipid peroxidation, mitochondrial dysfunction, disruption of calcium, and nitric oxide homeostasis, and finally, cell death by apoptosis and necrosis[4].

Inflammation is closely interrelated with oxidation in the biological systems, and also plays a vital role in ACN-induced acute liver injury[5]. Powerful pro-inflammatory cyto-kines can also be released leading to a proinflammatory cascade[6].

In a process that has been termed pyroptosis, an inflammatory insult induced by ACN causes the activation of a caspase-1-dependent programmed cell death pathway that culminates in plasma membrane pore formation, cytosolic leakage and cell rupture[7]. The inflammasome is an intracellular protein complex that is a central component of pyroptosis. It regulates caspase-1 activity and acts as a gate keeper to the sensing and subsequent initiation and amplification of the inflammatory response to injury[8]. Induced-acute liver failure in animal models was associated with significantly elevated levels of cleaved caspase-1 and IL-1β and predominant pyroptotic cell death[9].

1 α, 25-dihydroxy vitamin D3 (1 α, 25(OH) 2D3 is a biologically active form of Vitamin D. Vitamin D3 is an eminent immunomodulatory that regulates adaptive immunity by processing the anti-inflammatory activity[10]. Moreover, 1,25-dihydroxyvitamin D3 improved hepatic steatosis[11] and protected mice from hepatic ischemia-reperfusion injury[12]. .Hu et al. found that liver injury caused by alcohol was augmented in Vit D deficiency (VDD) mice, which may be associated with exacerbating alcohol-induced liver oxidative stress and inflammation[13]. Supplementation with 1, 25-dihydroxyvitamin D3 was shown to reduce the expression of TNFα in the liver of mice fed ethanol[14]. Another *in vitro* study revealed that 1, 25-dihydroxyvitamin D3 protected against ethanol-induced cytotoxicity and ROS through activating an Nrf2–Aldh2 positive feedback loop[15].

Furthermore, vitamin D3 administration in animal models of acute liver injury was associated with decreased expression of apoptotic genes[16]. The role of vitamin D in pyroptotic pathway was recently proven to have a protective role in ameliorating pyropoptotic signaling pathway in acute kidney injury and in experimental autoimmune myocarditis in mice[17,18]. The role of vitamin D in the triggered pyroptotic process during ACN-induced hepatotoxicity is a rare finding in the literature.

AIM OF THE WORK:

The aim of the present study is to investigate the role of vitamin D receptor

agonist paricalcitol in ACN hepatotoxicity in rats as an animal model of acute hepatic failure. The effect of paricalcitol on the central elements of proptosis; caspase-1 and IL-1beta in the setting of ACN -induced hepatotoxicity will, also, be investigated.

MATERIALS AND METHODS:

Animals

This study was conducted in the **King Fahd Center for Medical Research**, KAU, Jeddah, Saudi Arabia from October to December 2020.The work involved 40 male albino rats 5-6 months old (200-250 gm body weight). Animals were kept under standard conditions of boarding. The rats were allowed free access to water and ad libitum feeding. The study conforms to the NIH guidelines for the care of use of laboratory animals.

Experimental design (table: 1):

Animals were initially weighed and divided into equal four groups.

Control group: treated with vehicles only.

Paricalcitol-treated group (PC): Paricalcitol was given in a dose of 200 ng intraperitoneally (IP) three times a week for 9 days. On day 9 rats received saline orally. Paricalcitol was prepared with 95% propylene glycol/5% ethyl alcohol solution and administered by intraperitoneal injection (IP). Administration of such dose was based on previous reports that proved safe administration without significant changes in blood pressure, serum calcium or phosphorus [19].

ACN-treated group (ACN): received IP 95% propylene glycol/5% ethyl alcohol solution vehicle similar to paricalcitol protocol and on day 9, received ACN), orally 2 g/kg body weight as the injury model [20, 21].

Paricalcitol and ACN –treated group (PC + ACN): Paricalcitol was given in a dose of 200 ng IPi in the same schedule of paricalcitol for group 2 and on day 9 orally received ACN (2 g/kg) as the injury model in group 3. The ACN was purchased as Panadol original tablets (paracetamol 500 mg/tablet) from GlaxoSmithKline Inc. (Middlesex, UK). Paricalcitol is purchased from Sigma Aldrich Jeddah Saudi Arabia agent).

Table 1: Experimental protocol:

Group	Day 1-8	Day 9
1.Control	Vehicle injection (IP 95% propylene glycol/5% ethyl alcohol solution)	Oral saline
2. PC	Paricalcitol (200 ng IP three times a week)	Oral saline
3. ACN	Vehicle injection (IP 95% propylene glycol/5% ethyl alcohol solution)	Oral ACN (2 g/kg)
4. PC+ACN	Paricalcitol was given in a dose of 200 ng, IP three times a week for 9 days	Oral ACN (2 g/kg)

Procedures

Forty-eight hours after the last treatment, the rats were subjected to the following experimental procedures

Tail cuff systolic blood pressure and heart rate measurements:

Systolic blood pressure (SBP) was measured by tail-cuff plethysmography (BP98A, Softron, and Tokyo, Japan). The animals were kept at 25°C for 5 minutes, the first five cycles were considered as adaptation cycles. After that, another five consecutive cycles were recorded, and their average was taken as the mean systolic blood pressure.

Animals were then sacrificed; a midline abdominal incision was then made and whole blood specimen from the abdominal aorta was collected and centrifuged at 3000 rpm for 30 minutes. The sera were separated and stored at -20°C for use in measurement of biochemical parameters.

The liver was carefully dissected, rinsed in ice-cold phosphate buffer saline (pH 7.4), blotted dry on a filter paper then weighed. Part of the right lobe of the liver was cut and fixed in 10% phosphate buffered formalin (pH 7.2) and stained with conventional H&E for histopathological examination under a light microscope. The left lobe was homogenized.

Tissue homogenates were prepared using Polytron PT1200E Disperser (Kinematica AG, Luzern, Switzerland) in ice-cold phosphate-buffered saline (PBS, pH 7.2). Homogenates were centrifuged at 12,000 g for 10 min, and supernatants were kept under -80°C for further use. Both serum and the supernatants of tissue homogenates were subjected to the measurement of MDA, GSH, TNF-α and IL-1β concentrations.

Biochemical assays:

Colorimetric kits were used to determine serum calcium, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) alkaline phosphatase (ALP) activity, gamma glutamyl transferase (GGT) and serum bilirubin. The assays were done using kits supplied by (Biodiagnostic Giza Egypt) according to manufacturer instructions.

Lipid peroxidation was assessed by measuring the content of MDA, a byproduct of lipid peroxidation process. The measurement was performed using a kit supplied by Diamond Diagnostics (Cairo, Egypt), following the manufacturer's instructions. This assay depends on the reaction of MDA in samples with thiobarbituric acid (TBA) forming a red MDA-TBA complex. The complex was assessed colorimetrically at wavelength 532 nm The reduced GSH was determined colorimetrically at wavelength 405 nm by using a diagnostic kits supplied by Biodiagnostic (Giza, Egypt), we followed the manufacturer's instructions.

The levels of proinflammatory cytokines; tumor necrosis factor-α (TNF-α) and IL-*β*, were measured in the sera and liver homogenates according to the ELISA kit instructions. (R&D Systems, Inc., Minneapolis, MN, USA).

Table 2: Primers used for Real time-PCR

Caspase-1	5′-AGGAGGGAATATGTGG G-3′ 5′-AACCTTGGGCTTGTCT T-3′
GADPH	5′-GACATGCCGCCTGGA GAAAC-3′ 5′-AGCCCAGGATGCCCTT TAGT-3′

RT-PCR for identification of iNOS-mRNA in both kidney and brain tissues:

Total RNA was extracted by using TRIzol Reagent (Invitrogen, Life Technologies, USA) according to the manufacturer's instructions. Reverse transcription was carried out with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). PCR was performed with Taq DNA polymerase. The primers used for assessment of iNOs, Caspase-1 and caspase-3 genes and the GADPH gene as a control are shown in table 2.

The thermal cycle was set as follows: inactivation of reverse transcriptase at 95°C for 10 minutes, followed by 45 cycles of 95°C for 30 second, 60°C for 1 minute, and 72°C for 30 second. Dissociation curve analysis was used for confirmation of PCR results. Using ABI's SDS software, the data

were depicted as sigmoid shaped amplification plots in which the number of cycles was plotted against fluorescence linear scale. The threshold cycle serves as a tool for calculation of the starting template amount in each sample. Because the samples of the control group and also samples of the treated group were used as calibrators, the expression levels were set to one. The relative concentration of the genes were normalized against the glyceraldehyde -3-phosphate dehydrogenase GADPH and gene expression fold changes were calculated using the equation 2-ΔΔct. [(22)].

Statistical analysis:

Data were expressed as mean ± SD. The Student's t-test for unpaired data was performed to assess them as statistically significant intragroup and intergroup differences, respectively. All statistical data and statistical significance were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA), version 16. A P value less than 0.05 was considered statistically significant.

RESULTS:

Light microscopic results:

Histological observation of liver tissue in normal animals of the control group showed normal architecture with normal hepatic lobules and central veins. The hepatocytes were normally arranged in plates originating from the central vein with acidophilic cytoplasm. (Fig. 1). In the ACN-treated animals, histological findings showed distorted architecture and hepatocytes showed necrotic changes with moderate inflammatory cells (Figure 2), The histological sections of PC+ACN group pretreated with vitamin D receptor agonist Paricalcitol showed histological characteristics of the liver architecture, central vein and hepatocytes that are near to control animals (Figure 3).

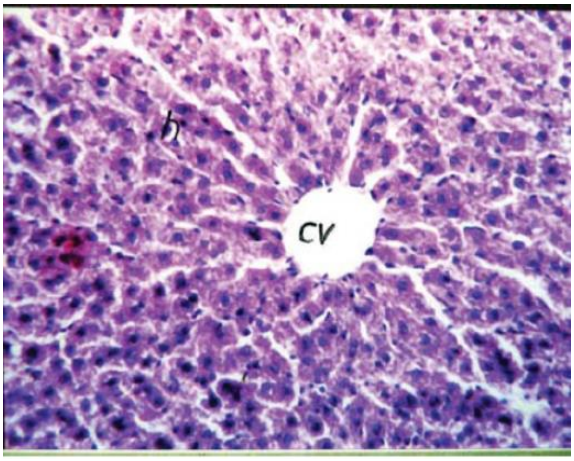

Figure 1: H and E stained hepatic section of the control rats showing the normal architecture of hepatic lobules with normal appearance of the central vein (CV) and hepatocytes (h), (H&E 64 X).

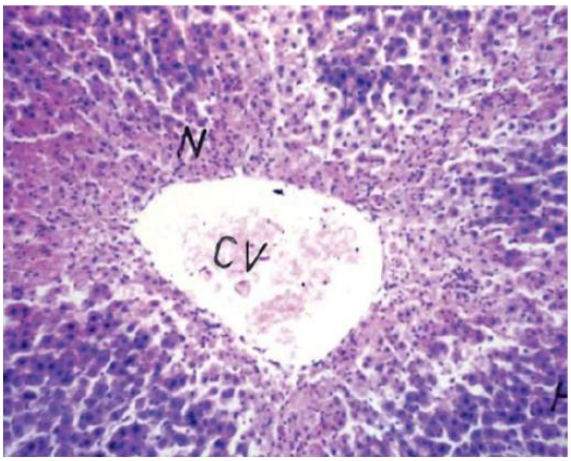

Figure 2: H and E stained hepatic section of the ACN-treated animal (ACN group) showing congestion and dilation of the central vein (CV), central lobular necrosis (N), and small dark nuclei of the hepatocytes with vacuolation are shown. (H&E 40 X).

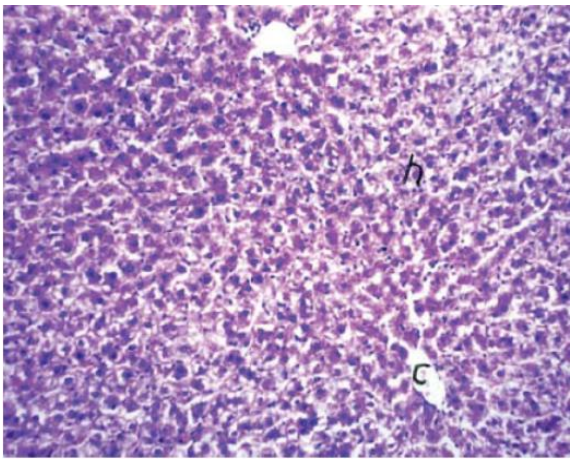

Figure 3.: H and E stained hepatic section of the ACN-injected animal pretreated with paricalcitol (ACN +PC group) showing near to normal histologic architecture with the features of central vein (CV) and hepatocytes (h) comparable to normal control. H&E,

Systolic blood pressure, serum calcium and biomarkers for liver function (table 3):

There were no significant differences in the systolic blood pressure, serum calcium and all parameters of liver functions between the control group and the PC group. On the other hand, the ACN group showed significant increase in serum ALT, AST. ALP, bilirubin and GGT and significant decrease in serum albumin when compared with the control group. The systolic blood pressure and serum calcium of the ACN group did not change significantly as compared to the control group. Pretreatment of ACN group with paricalcitol (PC +ACN) was associated with significant reduction of the hepatic markers, ALT, AST, ALP, bilirubin and GGT with significant elevation of serum albumin when compared with ACN animals not subjected to paricalcitol treatment. The PC+ACN group showed no significant differences in their systolic blood pressure or serum calcium levels as compared to ACN group.

Table 3: The means ± SD of the systolic blood pressure, serum levels of calcium (Ca^{++}), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (BIL), albumin (ALB) and gamma glutamyl transferase γ GT) in the studied groups.

	SBP	Ca^{++} mg/dl	ALT U/L	AST U/L	ALP U/L	BIL mg/dl	AlB g/dl	γ GT U/L
Control	130 ± 8.16	9.25 ±0.95	49.5 ±8.96	101.5 ±11.80	279.5 ±20.88	0.12 ±0.02	2.41 ±0.34	3.71 ±0.55
PC	134 ± 13.08	10.20 ±0.78	53.00 ±7.89	103.5 ±13.55	273 ±25.95	0.13 ±0.02	2.23 ±0.31	4.06 ±0.64
ACN	136 ± 8.43	9.95 ±1.40	162.3* ±12.41	299* ±19.55	565* ±93.48	0.18* ± 0.02	1.66* ±0.34	16.15* ±1.45
PC+ACN	136 ±9.56	11.05 ±1.82	72.10** ±15.34	143.1** ± 20.63	395** ±60.23	0.14** ± .015	1.99** +0.15	12.95** ±1.67

*P<0.05 significant from control group. **P<0.05 significant from ACN group

Oxidant, antioxidant and anti-inflammatory parameters:

As shown in table 4, there were no significant differences in the hepatic tissue level of MDA, GSH, TNF-α and IL-1 β between the control group and the PC group.

On the contrary, hepatic tissue ACN group showed significant increase in MDA, TNF-α and IL-1β and significant decrease in GSH as compared to control group.

Pretreatment of ACN animals with PC was associated with significant reduction of MDA, TNF-α and IL-1β and significant increase of GSH in the hepatic tissue of these animals in comparison to ACN animals not subjected to PC pretreatment.

Table 4: The means ± SD of the hepatic tissue content of MDA, reduced GSH, TNF-α and interleukin-1 IL-1β in the studied groups.

	MDA (nmol/gm)	GSH (µmol/gm)	TNF-α (pg/ml)	IL-1β (pg/ml)
Control	4.61±0.08	6.73±1.31	142.7±16.97	60.3±7.72
PC	4.49±0.74	6.83 ± 1.19	140.8±16.81	56.7± 6.31
ACN	16.80± 3.12*	3.96 ±0.70*	407 ±72.27*	95.70 ±13.24*
PC + ACN	7.73±1.92**	6.72±1.39**	127.7±44.3**	69.78±10.1**

*P<0.05 significant from control group. **P<0.05 significant from ACN group

As shown in figures (4-7): the PC group showed serum levels of MDA, GSH, and TNF-α and IL-1β that are not significantly different from control group. ACN group, however, exhibited significant elevation of serum MDA, TNF-α and IL-1β and significant lowering of serum GSH in comparison to normal controls.

Pretreatment of ACN group with paricalcitol was concomitant with significant drop of the serum levels of MDA, TNF- and IL-1β and significant rise of serum GSH as compared to non-treated ACN animals.

Figure 8 shows the mRNA content of hepatic homogenates in the different groups. PC group shows no significant changes in caspase-1 mRNA content of the liver as compared to control group. On the contrary, ACN group showed significant elevation of caspase 1 mRNA as compared to the control group. This content, however, was significantly decreased in the ACN group pretreated with PC when compared with ACN group non-treated with PC.

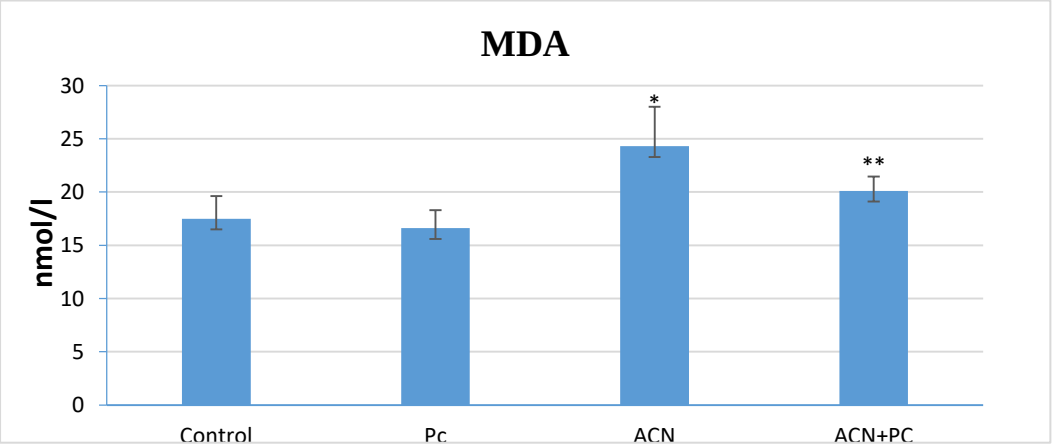

Figure 4: The serum level of MDA in the studied groups. *P<0.05 significant from control group. **P<0.05 significant from ACN group

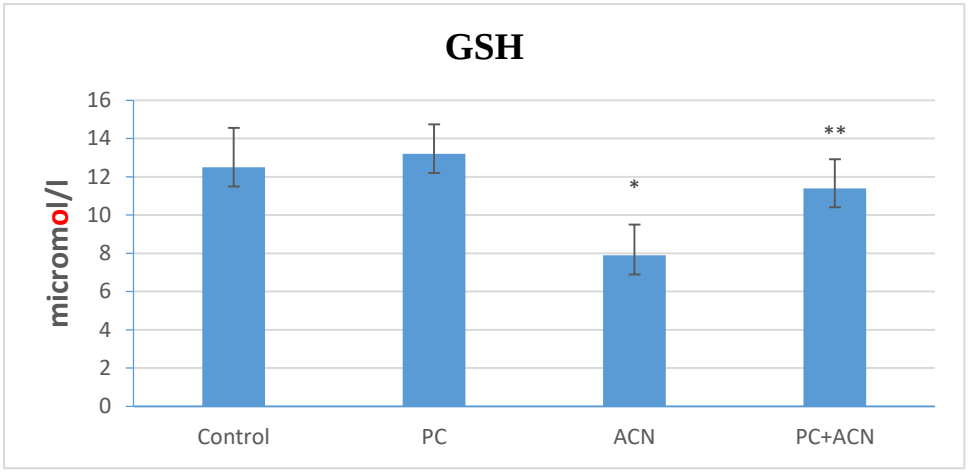

Figure 5: The serum level of GSH in the studied groups. *P<0.05 significant from control group. **P<0.05 significant from ACN group

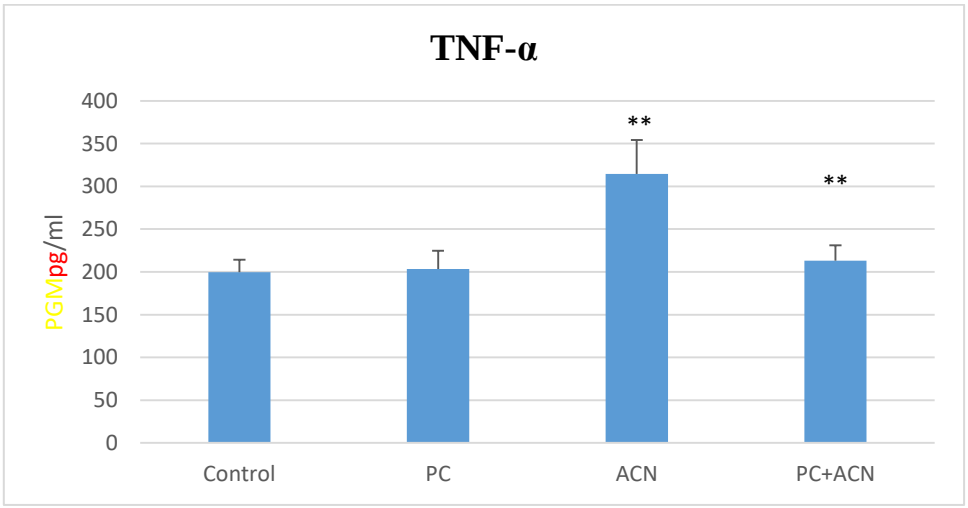

Figure 6: The serum level of TNF-α in the studied groups. *P<0.05 significant from control group. **P<0.05 significant from ACN group

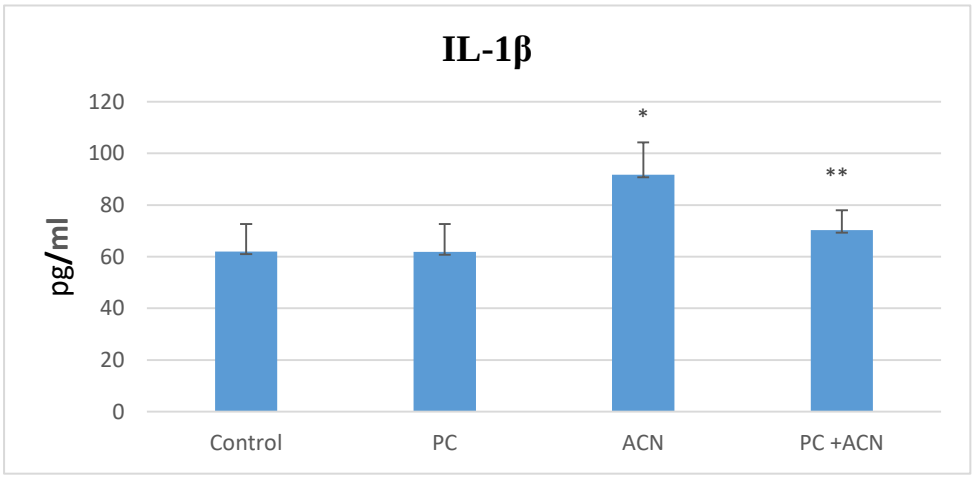

Figure 7: The serum level of IL-1β in the studied groups. *P<0.05 significant from control group. **P<0.05 significant from ACN group

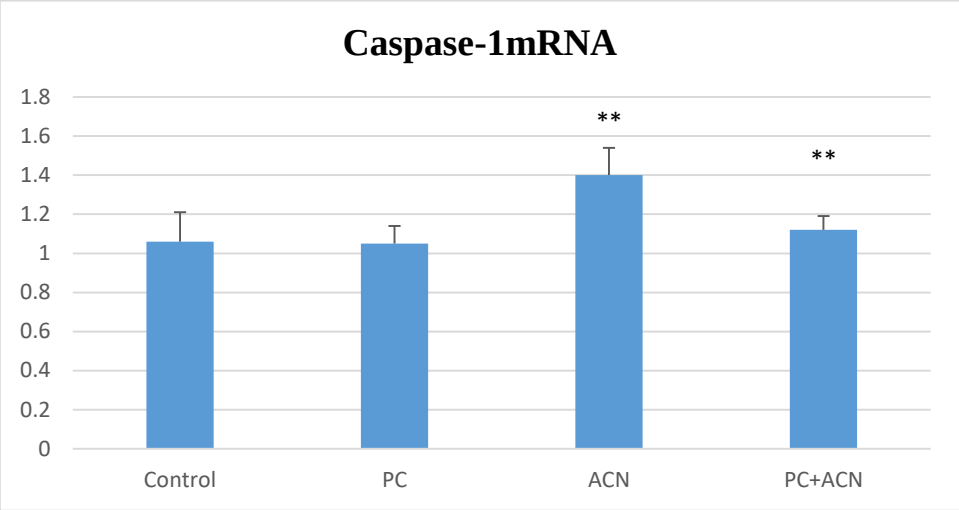

Figure 8: Caspase-1 mRNA (fold expression relative to GADPH) in hepatic tissue homogenates in the studied groups. *P<0.05 significant from control group. **P<0.05 significant from ACN group

DISCUSSION:

The present study investigated the effect of VDR agonist paricalcitol on the ACN-induced acute liver injury in rats. The ACN-treated rats showed deterioration of the liver function, which was manifested as increased activities of AST, ALT, alkaline phosphatase, serum bilirubin as well as γGT. These markers have been found to be of great value in the assessment of clinical and experimental liver damage [23]. Increase liver enzymes and other hepatic biomarkers in ACN hepatoxicity were, also, reported in previous studies [24,25].

Histopathological examination of the liver sections in the current study showed loss of hepatic integrity, and cellular necrosis, dilatation of the central veins, centrilobular necrosis, congestion and vacuolar degeneration. These findings are in agreement with previous studies[26]. We could postulate that the elevated levels of serum enzymes AST, and ALT, ALP, GGT and other biomarkers measured in this study are indicative of and attributed to the cellular leakage and the loss of functional integrity of cell membrane in the liver as previously explained [20].

Pretreatment with VDR agonist paricalcitol, was accompanied by successful protection against ACN toxicity by improving the measured hepatic biomarkers as well as the evident preservation of normal histological characteristics of the liver architecture as well as its cellular integrity. The protective role of vitamin D agonist, paricalcitol, against ACN-induced hepato-toxicity are in line with previously reported action of vitamin D3 in Tthioacetamide-induced, ACN-induced acute liver injuries[27,28].

In contradiction to the present results, it was shown that vitamin D_3 aggravated the hepatic injury induced by carbon tetrachloride administration[29]. The authors, however, attributed this effect to the elevation of serum calcium levels. Recently, however, Almaimani and his coworkers reported that the combination treatment of VD_3 and Ca^{2+} resulted in the restoration of the Ca^{2+} molecular system and alleviated lead-induced nephrotoxicity in mice[30]. It

seems that the former contradictory report depends on the animal model and the method of induction of acute liver injury. Furthermore, the vitamin D receptor agonist paricalcitol is not known to induce hypercalcemia in the dose utilized in our study [19]. Lastly, our animal model did not show significant differences in calcium levels between the different groups studied.

Our results showed significant elevation of MDA and drop of GSH in both serum and liver tissue homogenates of ACN- treated rats. Increased MDA was considered as a strong indicator for enhanced oxidative stress and excessive free radical production. [31]. GSH, a nonenzymatic antioxidant that acts as a free-radical scavenger [32]. Taken together the enhanced oxidative status and the exhausted non-enzymatic antioxidant GSH express a state of enhanced oxidative stress that was considered a main mechanism for ACN- induced liver injury [33].

The Cytochrome P450 (CYP450) enzymes are key enzymes in the metabolism of ACN to the quinine metabolite NAPQI, a reaction mainly responsible for ACN-induced injury and GSH consumption [34]. CYP450 system, especially CYP2E1 increase the formation of NAPQI with subsequent increase hepatic damage induced by ACN [35]. The NAPQI metabolite is normally efficiently detoxified by GSH, in the condition of ACN toxicity however, there is overwhelming amounts of the toxic metabolites which exceeds the antioxidant capacity. GSH deficiency occurs and the production of un-scavenged reactive oxygen species (ROS), is enhanced with consequent hepatic tissue damage [36] and cell death occurs [37].

Moreover, the enhanced ACN-related production of free radicals impairs mitochondrial respiration and results in ROS formation, leading to an overwhelming mitochondrial oxidant stress and mitochondrial dysfunction [38]. Specifically, ROS directly prompt the mitochondrial membrane permeability transition leading to mitochondrial uncoupling and severe ATP depletion which results in inner membrane permeabilization, outer membrane rupture, and cell apoptosis [39, 40].

In agreement with previous studies, [28], we showed that pretreatment with vitamin D receptor agonist paricalcitol was associated with diminished production of free radicles and lipid peroxidation with parallel preservation of the antioxidant GSH in serum and liver tissue.

Our findings demonstrated the effective suppression of oxidant production, lipid peroxidation, and inflammatory cell infiltration in liver tissues of ACN group pretreated with paricalcitol. These results clearly show that reduction of oxidative damage contributes, at least partly, to the hepatoprotective action of 1,25(OH)2D3. Previous studies demonstrated the anti-inflammatory and antioxidant actions of 1,25(OH)2D3 in various experimental settings [41]. Paricalcitol, has been demonstrated to ameliorate oxidative injury in cardiac tissue via reduction of NADPH oxidase activity [42]. The findings of another study revealed the preservation of renovascular function in hypertension by calcitriol via reduction of oxidative stress [43]. Furthermore, it was demonstrated that 1,25(OH)2 D3 alleviated the lipopolysaccharide-induced renal oxidative stress through downregulation of oxidant enzyme genes (e.g., p47phox and gp91phox) [44].

Our study in agreement with others [45], indicated that the levels of TNF-α and IL-1β were elevated in the serum and liver tissues of ACN rats. The coincident demonstration of the aggravated oxidative and inflammatory conditions is closely interrelated in the biological systems, and also plays a vital role in ACN-induced acute liver injury [5]. In addition, the key component in the inflammatory process NF-

KB p65 was excessively expressed in animal models of ACN- induced acute liver injury [45].

TNF receptor superfamily member 4(OX40), which is expressed in liver invariant natural killer T (iNKT) cells, is also involved in the development of liver failure. OX40 activates caspase-1 via TNF receptor-associated factor 6-mediated recruitment of the paracaspase MALT1, which consequently leads to massive pyroptotic death of iNKT cells and liver injury[46].

The increase expression of caspase-1 in hepatic tissue could be attributed to the inflammatory insult induced by ACN in our study with subsequent activation of a caspase-1-dependent programmed cell death pathway that culminates in plasma membrane pore formation, cytosolic leakage and cell rupture, in a process known as pyroptosis[7]. Moreover, the activation of caspase-1 could share in the elevation of other proinflammatory cytokines as it leads to cleavage of pro-IL-1β and pro-IL-18 with the production of mature, biologically active cytokines [47]. IL-1beta in turn, potentially stimulates the expression of proinflammatory factors and recruits neutrophil to the liver tissue[48].

In accordance with our results, significantly elevated levels of cleaved caspase-1 and IL-1β and predominant pyroptotic cell death have been observed in animal models of concanavalin A (ConA) and D-galactosamine (D-Gal)- induced liver failure [49, 50].

IL-1 receptor type 1 (IL-1R1) can amplify cell death and inflammation in hepatocytes during pyroptosis in liver failure. ALF induced by D-Gal and LPS is significantly attenuated in the liver-specific IL-1R1 knock-out mice [51].

Put all together the oxidative stress triggers a cytokine profile which stimulates the pyroptotic pathway through increasing caspase-1 levels in the hepatic tissue with subsequent activation and release of Il-1β and other cytokines that amplify the inflammatory states with subsequent hepatic cell deaths.

Our study indicated that the elevation of the levels of TNF-α, and IL-1β in ACN-induced liver injury was prevented by pretreatment with the VDR agonist paricalcitol. The anti-inflammatory action of vitamin D3 was shown *in vitro* by inhibiting the adhesion molecules, P-selectin, β1- and β2-integrin, in monocytes treated with vitamin D3[52]. In support to our findings, Boontanrart and his coinvestigators demonstrated that vitamin D3 therapy was associated with downregulated expression of the proinflammatory cytokines, IL-6 and TNF-α as well as upregulation of the anti-inflammatory cytokine, IL-10[53].

One of the central findings in this study is the downregulation of the caspase-1 gene in hepatic tissue by vitamin D receptor agonist as evident by the significant reduction of caspase-1 expression in hepatic tissues of ACN-injected animals when pretreated with this agent. The present study, thus, gives insight on the role of vitamin D as an anti-pyroptotic agent through inhibiting caspase -1 expression in ACN-induced hepatic injury. To our knowledge, the inhibitory effect of vitamin D on caspase-1 expression in ACN hepatotoxicity is a new finding. This inhibitory effect, however, was reported in acute injuries of the kidney, colon and peritoneum[17,54-55].

In conclusion, vitamin D receptor agonists improve outcomes of ACN-induced acute hepatic injury through antioxidant and anti-inflammatory pathways. On the molecular level, the current study showed the ability of this VDR agonist to suppress pyroptotic process through downregulating caspase-1 gene expression in rat hepatocytes during acute hepatic injury.

REFERENCES:

1. Lee W.M. (2012): Acute liver failure. Semin Respir Crit Care Med.; 33: 36–45.

2. Kurtovic J. and Riordan S.M. (2003): Paracetamol-induced hepatotoxicity at recommended dosage. J Inter Med; 253: 240–243.

3. Ojo O.O., Kabutu F.R., Bello M., Babayo U. (2006): Inhibition of paracetamol induced oxidative stress in rats by extracts of lemongrass (*Cymbropogon citratus*) and green tea (*Camellia sinensis*) in rats. Afr J Biotechnol; 5: 1227–1232.

4. Qinna N.A., Shubbar M.H., Matalka K.Z., Al-Jbour N., Ghattas M.A., Badwan A.A. (2015): Glucosamine enhances paracetamol bioavailability by reducing its metabolism. J Pharm Sci.; 104(1): 257–265.

5. Woolbright B.L. and Jaeschke H. (2017): Role of the inflammasome in ACN-induced liver injury and acute liver failure. J Hepatol.; Apr; 66(4): 836-848.

6. Linkermann A., Stockwell B.R., Krautwald S., Anders H.J. (2014): Regulated cell death and inflammation: an auto-amplification loop causes organ failure. Nat Rev Immunol.;14(11): 759-767

7. Vande Walle L. and Lamkanfi M. Pyroptosis. (2016): Curr Biol.; Jul 11; 26(13): R568-R572.

8. Zuurbier C.J., Abbate A., Cabrera-Fuentes H.A., Cohen M.V., Collino M., De Kleijn D.P.V., Downey J.M., Pagliaro P., Preissner K.T., Takahashi M., Davidson S.M (2019): Innate immunity as a target for acute cardioprotection. Cardiovasc Res.; Jun 1; 115(7): 1131-1142.

9. Wu J., Lin S., Wan B., Velani B., Zhu Y. (2019): Pyroptosis in Liver Disease: New Insights into Disease Mechanisms. Aging Dis.; Oct 1; 10(5): 1094-1108.

10. Gatenby P, Lucas R, Swaminathan A. (2013): Vitamin D deficiency and risk for rheumatic diseases: an update. Curr Opin Rheumatol.; Mar;25(2): 184-91.

11. Li R., Guo E., Yang J., Li A., Yang Y., Liu S., Liu A., Jiang X. (2017): 1,25$(OH)_2$ D_3 attenuates hepatic steatosis by inducing autophagy in mice. Obesity (Silver Spring); Mar; 25(3): 561-571.

12. Yang J., Chen Q., Tian S., Song S., Liu F., Wang Q., Fu Z. (2015): The role of 1,25-dyhydroxyvitamin D3 in mouse liver ischemia reperfusion injury: regulation of autophagy through activation of MEK/ERK signaling and PTEN/PI3K/Akt/mTORC1 signaling. Am J Transl Res.; Dec 15; 7(12): 2630-2645.

13. Hu C.Q., Bo Q.L., Chu L.L., Hu Y.D., Fu L., Wang G.X., Lu Y., Liu X.J., Wang H., Xu D.X. (2020): Vitamin D Deficiency Aggravates Hepatic Oxidative Stress and Inflammation during Chronic Alcohol-Induced Liver Injury in Mice. Oxid Med Cell Longev.; Feb 27: article ID:5715893: 1-14

14. Trépo E., Ouziel R., Pradat P., Momozawa Y., Quertinmont E., Gervy C., Gustot T., Degré D., Vercruysse V., Deltenre P., Verset L., Gulbis B., Franchimont D., Devière J., Lemmers A., Moreno C. (2013): Marked 25-hydroxyvitamin D deficiency is associated with poor prognosis in patients with alcoholic liver disease. J Hepatol.; Aug; 59(2): 344-50.

15. Zhang H., Xue L., Li B., Zhang Z., Tao S. (2019): Vitamin D Protects Against Alcohol-Induced Liver Cell Injury Within an NRF2-ALDH2 Feedback Loop. Mol Nutr Food Res.; Mar; 63(6): e1801014: 1-9

16. Seif AA, Abdelwahed DM. Vitamin D ameliorates hepatic ischemic/reperfusion injury in rats. J Physiol Biochem. 2014 Sep;70(3):659-66.

17. Jiang S., Zhang H., Li X., Yi B., Huang L., Hu Z., Li A., Du J., Li Y., Zhang W. (2021): Vitamin D/VDR attenuate cisplatin-induced AKI by down-regulating NLRP3/Caspase-1/GSDMD pyroptosis pathway. J Steroid Biochem Mol Biol.; Feb;206, 105789: 1-12

18. Liu N., Su H., Zhang Y., Liu Z., Kong J. (2017): Cholecalciterol cholesterol emulsion attenuates experimental autoimmune myocarditis in mice via inhibition of the pyroptosis signaling

pathway. Biochem Biophys Res Commun.; Nov 4; 493(1): 422-428.

19. Fryer RM, Rakestraw PA, Nakane M, Dixon D, Banfor PN, Koch KA, Wu-Wong JR, Reinhart GA. (2007): Differential inhibition of renin mRNA expression by paricalcitol and calcitriol in C57/BL6 mice. Nephron Physiol.; 106(4): 76-81.
20. Kumar Bellamakondi P., Godavarthi A., Ibrahim M. (2018): *Caralluma umbellata* Haw. protects liver against paracetamol toxicity and inhibits CYP2E1. Bioimpacts.; 8(1): 23-30.
21. Abo El-Magd NF, Eraky SM. (2020): The molecular mechanism underlining the preventive effect of vitamin D against hepatic and renal acute toxicity through the NrF2/ BACH1/ HO-1 pathway. Life Sci. Mar 1: 244,117331: 1-13
22. .Livak K.J., and Schmittgen T.D. (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods; Dec; 25(4): 402-8.
23. Achliya GS, Wadodkar SG, Dorle AK. (2004): Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride induced hepatic damage in rats. J Ethnopharmacol.; 90(2): 229-32.
24. Ilavenil S., Al-Dhabi N.A., Srigopalram S., Ock Kim Y., Agastian P., Baru R., Choi K.C., Valan Arasu M. (2016): ACN Induced Hepatotoxicity in Wistar Rats--A Proteomic Approach. Molecules. Jan 28; 21(2): 161: 1-12
25. Al-Asmari A. K., Al-Said M. S., Abbasmanthiri R., Al-Buraidi A., Ibrahim K. E. and Rafatullah S. (2020): Impact of date palm pollen (Phoenixdactylifera) treatment on paracetamol-induced hepatorenal toxicity in rats. Clinical Phytoscience 6:16-27.
26. Akgun E, Boyacioglu M, Kum S. (2021): The potential protective role of folic acid against ACN-induced hepatotoxicity and nephrotoxicity in rats. Exp Anim.; Feb 6; 70(1): 54-62.
27. Özdemir-Kumral Z.N., Erkek B.E., Karakuş B., Almacı M., Fathi R., Yüksel M., Cumbul A., Alican İ. (2019): Potential Effect of 1,25 Dihydroxyvitamin D_3 on Thioacetamide-Induced Hepatotoxicity in Rats. J Surg Res.; Nov; 243: 165-172.
28. El-Boshy M., BaSalamah M.A., Ahmad J., Idris S., Mahbub A., Abdelghany A.H., Almaimani R.A., Almasmoum H., Ghaith M.M., Elzubier M., Refaat B. (2019): Vitamin D protects against oxidative stress, inflammation and hepatorenal damage induced by acute paracetamol toxicity in rat. Free Radic Biol Med.; Sep;141: 310-321.
29. Yoshioka H., Usuda H., Miura N., Fukuishi N., Nonogaki T., Onosaka S. (2017): Vitamin D3-induced hypercalcemia increases carbon tetrachloride-induced hepatotoxicity through elevated oxidative stress in mice. PLoS One. Apr 27; 12(4): e0176524: 1-15
30. Almaimani RA, Almasmoum H, Ghaith MM, El-Boshy M, Idris S, Ahmad J, Abdelghany AH, BaSalamah MA, Mahbub A, Refaat B. (2019): Enhanced remedial effects for vitamin D_3 and calcium co-supplementation against pre-existing lead nephrotoxicity in mice: The roles of renal calcium homeostatic molecules. Biochim Biophys Acta Mol Basis Dis.; Feb 1; 1865(2): 512-524.
31. Lee, J. C., and Durand, T. (2019): Lipid peroxidation: Analysis and applications in biological systems. Antioxidants; *8*: 40–41.
32. Spyropoulos, B.G.; Misiakos, E.P.; Fotiadis, C.; Stoidis, C.N. (2011): Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis. Dig. Dis. Sci.; 56: 285–294.
33. Neag M.A., Catinean A., Muntean D.M., Pop M.R., Bocsan C.I., Botan E.C., Buzoianu A.D. (2020): Probiotic Bacillus Spores Protect Against ACN Induced Acute Liver Injury in Rats. Nutrients; Feb27; 12(3): 632: 1-16
34. Chen, C., Gonzalez, F.J., Idle, J.R., (2007): LC-MS-based metabolomics in drug

metabolism. Drug Metab. Rev.; 39: 581–597.

35. Michael SL, Pumford NR. Mayeux PR. Niesman MR, Hinson JA. Pretreatment of mice with macrophage in activators decreases ACN hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatol 1999;30(1):186-95.
36. Madkour, F.F. and Abdel-Daim, M.M. (2013): Hepatoprotective and Antioxidant Activity of Dunaliella salina in Paracetamol-induced Acute Toxicity in Rats. Indian J. Pharm. Sci.; 75: 642.
37. Cha, H.; Lee, S.; Lee, J.H.; Park, J.W. (2018): Protective effects of p-coumaric acid against ACN -induced hepatotoxicity in mice. Food Chem. Toxicol.; 121: 131–139.
38. Du K., Ramachandran A., Jaeschke H. (2016): Oxidative stress during ACN hepatotoxicity: Sources, pathophysiological role and therapeutic potential. Redox Biol.; 2016 Dec; 10: 148-156.
39. Kim J.S., He L., Lemasters J.J. (2003): Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem Biophys Res Commun.; May 9;304(3): 463-70.
40. Kon, K., Kim, J.S., Jaeschke, H., Lemasters, J.J., (2004): Mitochondrial permeability transition in ACN-induced necrosis and apoptosis of cultured mouse hepatocytes. Hepatology; 40: 1170–1179.
41. Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC. (2010): Vitamin D and inflammation. Joint Bone Spine; 77: 552-557.
42. Husain K., Ferder L., Mizobuchi M., Finch J., Slatopolsky E. (2009): Combination therapy with paricalcitol and enalapril ameliorates cardiac oxidative injury in uremic rats. Am J Nephrol.; 29: 465-472.
43. Dong J., Wong S.L., Lau C.W., Lee H.K., Ng C.F., Zhang L., Yao X., Chen Z.Y., Vanhoutte P. M., Huang Y. (2012): Calcitriol protects renovascular function in hypertension by down-regulating angiotensin II type 1 receptors and reducing oxidative stress. Eur Heart J.; Dec;33(23): 2980-90.
44. Xu S., Chen Y.H., Tan Z.X., et al. (2015): Vitamin D3 pre treatment alleviates renal oxidative stress in lipopolysaccharide induced acute kidney injury. J Steroid Biochem Mol Biol.; 152: 133-141.
45. Huang W., Wang Y., Jiang X., Sun Y., Zhao Z., Li S. (2017): Protective Effect of Flavonoids from Ziziphus jujuba cv. Jinsixiaozao against ACN-Induced Liver Injury by Inhibiting Oxidative Stress and Inflammation in Mice. Molecules.; Oct 20; 22(10): 1781: 1-18
46. Lan P., Fan Y., Zhao Y., Lou X., Monsour H.P., Zhang X., et al. (2017). TNF superfamily receptor OX40 triggers invariant NKT cell pyroptosis and liver injury. *J Clin Invest*.; June 1, 127(6): 2222-2234.
47. Rathinam V.A. and Fitzgerald K.A. (2016): Inflammasome Complexes: Emerging Mechanisms and Effector Functions. Cell; 165: 792-800.
48. Tsutsui H, Cai X, Hayashi S. (2015): Interleukin-1 Family Cytokines in Liver Diseases. Mediators Inflamm.; 2015: 2015, 630265: 1-19
49. Luan J., Zhang X., Wang S., Li Y., Fan J., Chen W., et al. (2018): NOD-Like Receptor Protein 3 Inflammasome-Dependent IL-1beta Accelerated ConA-Induced Hepatitis. Front Immunol; 9, 758: 1-13
50. Wang J., Ren H., Yuan X., Ma H., Shi X., Ding Y. (2018): Interleukin-10 secreted by mesenchymal stem cells attenuates acute liver failure through inhibiting pyroptosis. Hepatol Res.; 48: 194 -202.
51. Gehrke N, Hövelmeyer N, Waisman A, Straub BK, Weinmann-Menke J, Wörns MA, Galle PR, Schattenberg JM. (2018): Hepatocyte-specific deletion of IL1-RI attenuates liver injury by blocking IL-1 driven autoinflammation. J Hepatol.; May;68(5): 986-995.
52. Riek A.E., Oh J., Sprague J.E., Timpson A., de las Fuentes L., Bernal-Mizrachi L., Schechtman K.B., Bernal-Mizrachi C.

(2012): Vitamin D suppression of endoplasmic reticulum stress promotes an antiatherogenic monocyte/macrophage phenotype in type 2 diabetic patients. J Biol Chem.; Nov 9; 287(46): 38482-94.

53. Boontanrart M, Hall SD, Spanier JA, Hayes CE, Olson JK. (2016): Vitamin D3 alters microglia immune activation by an IL-10 dependent SOCS3 mechanism. J Neuroimmunol.; Mar 15;292: 126-36.

54. Xiong Y., Lou Y., Su H., Fu Y., Kong J. (2016): Cholecalciterol cholesterol emulsion ameliorates experimental colitis via down-regulating the pyroptosis signaling pathway. Exp Mol Pathol.; Jun; 100(3): 386-92.

55. Ko J., Kang H.J., Kim .DA., Ryu E.S., Yu M., Lee H., Lee H.K., Ryu H.M., Park S.H., Kim Y.L., Kang D.H. (2019): Paricalcitol attenuates TGF-β1-induced phenotype transition of human peritoneal mesothelial cells (HPMCs) via modulation of oxidative stress and NLRP3 inflammasome. FASEB J.; Feb; 33(2): 3035-3050.

الدور المحتمل لمنبه مستقبلات فيتامين د فى الاصابة الكبدية الناجمة عن عقار الاسيتامينوفين ودور الكاسبيز-١

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[١]قسمى الفسيولوجى كلية الطب جامعة عين شمس بالقاهرة جمهورية مص العربية و جامعة الملك عبدالعزيز بجده المملكة العربية السعودية [٣]قسم التشريح بكلية الطب جامعة الازهر بالقاهرة وجامعة الملك عبدالعزيز بجده

يعرف عقار الاسيتامينوفين باستخدامه الشائع كمسكن للالم كما انه يستخدم فى احداث اصابات كبدية فى حيوانات التجارب وتعتبر زيادة المواد المؤكسده والالتهابات من اهم الاليات المرضيه اللتى تتسبب فى الاصابة الكبديه الناجمه عن عقار الاسيتامينوفين. ومن هنا فان هذا البحث يهدف الى فحص تاثير المعالجه المسبقه بمنبه مستقبلات فيتامين د الباريكالسيتول على الخلل الوظيفى الناجم عن عقار الاسيتامينوفين فى الفئران. قد اجريت هذه الدراسة على عدد اربعين فأرا من الفئران البيضاء واللتى قسمت الى اربع مجموعات متساوية مجموعه ضابطه والمجموعه الثانيه تم حقنها بالباريكالسيتول وكانت الجرعة ٢٠٠ نانوجرام لكل كيلوجرام من وزن الفئران واعطيت اربع جرعات فى ٨ ايام . المجموعه الثالثه تم حقنها بالاسيتامينوفين ٢ جرام لكل كيلوجرام من وزن الفئران عن طريق الفم فى اليوم التاسع من التجربه اما المجموعه الرابعه فتم معالجتها بالباريكالسيتول والاسيتامينوفين معا بنفس طريقو معالجة المجموعه الثانيه والثالثه.

وقد اظهرت النتائج ان حقن الفئران بمادة الاسيتامينوفين بجرعه واحده ٢جرام لكل كيلوجرام من وزن الفئران احدث خللا فى انسجة الكبد ووظائفه بذياده فى انزيمات الكبد ونسبة البليروبين ونقصا حادا فى تركيز الالبومين فى الدم. كما تسبب الاسيتامينوفين فى زيادة درجة الاكسده حيث زادت ماده المالوندالدهيد ونقص مضاد الاكسده الجلوتاثيون فى دم وكبد الفئران المحقونة وكانت التغييرات ذات دلاله احصائية. كما ذادت عوامل الالتهاب والمعروفه بالسيتوكينات مثل الانترلوكين بيتا ١ والانترلوكين ٦ وكذلك عامل الاورام المميت الفا زياده ذات دلاله احصائية فى كل من الدم ونسيج الكبد فى الفئران المحقونه بالاسيتامينوفين. كما زادت نسبة التعبير الجينى للكاسبيز ١ فى انسجة الكبد لتلك الفئران زياده ذات دلالة احصائية.

كما اظهرت النتائج ان المعالجه المسبقه بمنبه مستقبل فيتامين د للفئران المحقونه بالاسيتامينوفين ادت الى منع حدوث التغييرات السابقه. فقلت الذياده فى انزيمات الكبد وقلت نسبة المالوندالدهيد وزادت مضادات الاكسده الجلوتاثيزن كما نقصت نسبة السيتوكينات فى الدم وانسجة الكبد كما قل التعبير الجينى للكاسبيز ١ وكانت التغييرات ذات دلالة احصائيه عند مقارنتها بمجموعة الاسيتامينوفين الغير معالجه بالباركالسيتول.

نستنتج من هذه الدراسه قدرة منبه مستقبلات فيتامين د الباريكالسيتول على الوقاية من الاصابة الكبدية الناجمه عن عقار الاسيتامينوفين فى الفئران وان هذه المقدره تتم من خلال عمل فيتامين د كمضاد للاكسده ومضاد للالتهابات كما اظهرت الدراسه قدرة منبهات فيتامين د على تقليل التهبير الجينى للكاسبيز ١ فى خلايا الكبد وبالتالى تقليل الالتهاب الكبدى فى هذه الحالة.