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**ORIGINAL ARTICLE**

# Notch γ-Secretase Inhibitor Dibenzazepine Attenuates Cisplatin-induced Spleen Toxicity in Rats: Role of Notch Signalling Pathway

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## ABSTRACT

**Background:** Cisplatin is one of the most widely used anticancer drugs. As with most of the chemotherapeutic agents, it doesn't only target cancerous cells but is also distributed to normal cells as well causing many organs toxicity. Being the most important organ in the immune system; the spleen affection by cisplatin treatment must be carefully prevented or reversed. This current study aimed to assess the protective effect of a Notch inhibitor dibenzazepine (DBZ) against cisplatin-induced splenic toxicity as well as explore the proposed mechanism shedding light on the role of the Notch pathway in its effect.

**Methods:** Rats were treated with DBZ (2mg/kg) for 12 days with a single dose of cisplatin (7mg/kg) injected on the 8$^{th}$ day of treatment. Rats were divided into four groups: Control, DBZ, Cisplatin, and DBZ+ Cisplatin group.

**Results:** Cisplatin injection upregulated the oxidative stress markers MDA and iNOS along with reduced antioxidant enzymes glutathione and catalase. The inflammatory markers (TNFα, IL1β, and NK$\kappa$B) were also upregulated. Furthermore, the Notch-1 and Hes-1 expressions were also significantly elevated. Cisplatin-induced splenic tissue damage was further assured by light and electron microscopic histopathological examination. DBZ pre-treatment significantly restored the upregulated oxidative stress, and inflammatory as well as Notch signaling pathways toward normal levels. Additionally, the histopathological architecture impairment was improved by Dibenzazepine.

**Conclusions:** The study elucidated that DBZ protects against cisplatin-induced toxicity in rats via antioxidative and anti-inflammatory effects. Moreover, downregulating the Notch signaling pathway was proved to play a role in DBZ's protective effect against cisplatin-induced splenic toxicity.

**Keywords**: Cisplatin; Dibenzazepine; Notch; Rats; Spleen.

## INTRODUCTION

Cisplatin is a widely used and one of the standard antitumor drugs due to its high rate of success in the treatment of many solid tumors including metastatic ones. Despite its success as an anticancer chemotherapeutic agent severe organ toxicity restrict its dose and use [1]. Cisplatin employs its toxic action by linking to DNA eventually leading to impaired DNA formation, cell cycle captures thus cancer cell death [2]. Separated from cisplatin's well-established neurotoxicity, nephrotoxicity, and hemolytic uremic disorder, small consideration has been paid to cisplatin treatment dangers on the spleen which is the foremost imperative organ of the resistant framework of the immune system [2,3].

Remarkably, Oxidative stress and inflammatory pathways are actively involved in cisplatin-induced organ pathophysiology [3,4]. Hence, many drugs with antioxidant and anti-inflammatory properties evidenced to have positive results in protecting the spleen from cisplatin-induce toxicity [5].

Really, there is always need for finding the pathways incorporated in the pathogenesis of cisplatin hazardous effects on the spleen. This will be beneficial in finding out new hopeful protective drugs against these hazardous effects.

Notch signaling path was proved to have a fundamental role in controlling proliferation and metastasis of many cancers and thus believed to be a hopeful target for future cancer therapies [6,7]. There are four Notch receptors (Notch 1-4) and principally, Notch-1 receptor is the one most probably included in the upregulation of cancer stem cells (CSCs) proliferation in many cancers [8]. Hence, targeting Notch-1 by many chemotherapeutic drugs eventually result in decreasing the number of CSCs[9] Various genes are targeted by Notch-1 pathway by regulating their expression, the most important one in controlling the fate of CSCs is the Hes-1 (Hairy enhancer of split) gene [9]. Definitely, this pathway is tangled in the spread, and recurrence of CSCs leading to chemo- and radio-resistance [8].

When the ligands interact with their specific Notch receptor the specific pathway become activated [10]. Next, cleavage by two enzymes occurs these enzymes are α- and γ- secretases as shown in figure (i).

Although this pathway was incorporated in the organ toxicity evolved by many chemotherapeutic agents, we propose that the incorporation of this Notch pathway in splenic toxicity afforded by cisplatin wasn't previously cleared [10].

A γ-secretase blocker dibenzazepine (DBZ) inhibits this final enzymatic breakdown thus restricts the upregulated Notch signaling pathway by stopping the stimulation of all types of Notch receptors [11] Notably, various γ-secretase inhibitors were proved to have anti-inflammatory and anti-proliferative effects **[12,13]**. Remarkably, DBZ was proved to have anti-tumor activity on several types of cancerous cells **[14],** Consequently, we propose that DBZ will be a hopeful drug in ameliorating splenic toxicity evoked by cisplatin.

Therefore, our objectives in the present work were to explore, the probable protective consequence of DBZ use in contrast to splenic toxicity caused by cisplatin administration in rats. Additionally, the involved mechanisms in these protective effects will be explored regarding their anti-inflammatory, and anti-oxidative effects besides its promising effect on the Notch pathway.

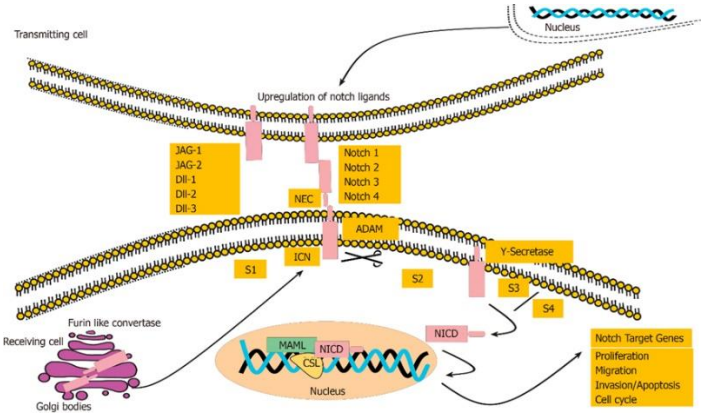


**Fig i** The activation pathway of Notch signaling receptors and their downstream signaling molecules **(**The canonical Notch signaling pathway). The interaction between Notch and its ligands from neighboring cells results in two successive cleavage events: cleavage at site S2 by ADAM protease and subsequent intramembranous cleavage at S3 by γ-secretase complex. This ligand-dependent activation process generates Notch intracellular domain (NICD). In the nucleus, NICD forms the transcription activation complex with transcription factor CSL and transcriptional coactivator such as MAML1, initiating the transcription of target genes.

## METHODS

### *Drugs and chemicals*

Cisplatin was a gift from (Merk Ltd., Cairo, Egypt) provided as a fluid (1 mg/ml) injected as a singular intraperitoneal dosage of (7 mg/kg) **[10]**. Dibenzazepine (DBZ) (Sigma-Aldrich Chemical Co, St Louis, MO, USA) was prepared in DMSO and corn oil mixture in a ratio of 1:9 **[10]** and was injected intraperitoneally (i.p.) via a 23-gauge

needle. All other used substances and solvents were commercially accessible.

***The Animals***

Forty Sprague-Dawley male albino rats (weight from 150–200 g) were gained and kept at the animal house of faculty of medicine Zagazig university, Egypt. Rats had unrestricted admission to food and water. We kept them in suitable environment with temperature adjusted at 21-25°C. room humidity about 40–60% along with 12h cycle of light and dark. We left rats in the experiment place for about one week to adapt the surrounding environment before starting experiment.

All animal experiments comply with the ARRIVE guidelines and in harmony with *Guide for the Care and Use of Laboratory Animals (US NIH Publication NO.85-23, revised 1996)* and were revised and approved by the *Zagazig University Institutional Animal Care Unit Committee, Zagazig University, Zagazig, Egypt, with agreement number (ZU-IACUC/3/F/159/2022).*

***Experimental Design***

Animals received drug treatment for 12 days and were randomly divided into four groups (ten / per group) as follows: **Control** group, rats received i.p. mixture of DMSO and corn oil in ratio of 1:9, (as DBZ vehicle); **DBZ** group, DBZ (dose of 2 mg/kg) was administered i.p. one dose/day for 12 continuous days; **Cisplatin** group, these rats were given the vehicle i.p., once daily for 12 days and a sole dose cisplatin (dose of 7 mg/kg i.p.) on the 8th day was administered i.p. to induce spleen toxicity; **DBZ+Cisplatin** group, received DBZ (dose of 2 mg/kg; i.p.)**[10,15]** single dose/day for 12 days with a sole i.p., cisplatin injection (dose of 7 mg/kg) administered on the 8th experimental day.

Animals were sacrificed by cervical dislocation at the end of the experimental period, then spleen from all rats were dissected out and washed with phosphate buffer saline (PBS) saline and divided into 3 pieces. The first pieces of splenic tissues from the different groups were exposed to homogenization and then the homogenates were kept at −80°C till the estimates of the oxidative burden and inflammatory cytokines in addition to the Notch signaling pathway and qRT-PCR assessment. Regarding the second pieces of splenic tissues from all groups we fixe them in 10% formol saline for the immunohistochemical study and histopathological inspection by light microscope. The third pieces from the different groups cut into minor pieces and we fix them in 2.5% glutaraldehyde buffer for preparation of the ultrathin sections (inspected by electron microscope).

***Oxidative stress markers assessment in splenic tissue homogenates***

GSH, MDA levels and catalase (CAT) activity were measured in the splenic tissue homogenates of the different experimental groups. The GSH assessment method was according to that described by 16 **(16)**. Also, the MDA lipid peroxidation marker was quantified by assessing the amount of thiobarbituric acid reactive substances (TBARS) affording the method of 17 **[17]**. CAT activity was measured using a catalase measuring kit (Biodiagnostic, Cairo, Egypt) in agreement with the producer's commands.

***Inflammatory markers assessment in splenic tissue homogenates***

The inflammation markers IL-1β and TNF-α, expression in splenic tissue was judged by measurement in splenic homogenate of all groups via rats quantitative ELISA kits (R&D Systems Inc., USA). We precisely follow the manufacturer's directives.

***Immunohistochemical assessment of NF-κB and inducible nitric oxide synthase (iNOS) proteins***

We utilized the spleen blocks for the immunohistochemical valuation of NF-κB and iNOS proteins. The slides were then blocked with 5% bovine serum albumin in tris-buffered saline for two h. The section was then immunostained with the primary NF-κB antibody (rabbit polyclonal IgG to rat NF-κB p65) and iNOS antibody (rat monoclonal antibody,1:500 dilution, Transduction Laboratories, San Diego, California, USA) at a concentration of 1 μg/ml holding 5% bovine serum albumin in tris buffered saline. Afterward, these sections were incubated overnight at 4 °C. We then washed the slides with tris buffered saline, after that, the section was incubated with goat anti-rabbit secondary antibody. We then washed the section with tris buffered saline and incubated it for 5–10 min in a solution of 0.02% diaminobenzidine containing 0.01% $H_2O_2$. Counterstaining was finished using hematoxylin, and the slide was visualized and carefully inspected under a light microscope **[18]**

***The Assessment of Notch signaling Pathway by means of quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)***

Notch-1 and Hes-1 expression levels in the spleen were sensed using qRT-PCR as previously described **(19).** For total RNA extraction, we consume about 50-100 mg of splenic tissue

precisely following the Bio Easy SYBR Green I Real-Time PCR Kit (Bo Ri Technology Co, Ltd, China). Table 1 show the primer sequences for specific gene amplification. Fold variations in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method. The gene expression is measured as a relative fold change to (*GADPH) as* an internal control reference gene.

**Table 1:** The primers base sequences intended for a real-time quantitative polymerase chain reaction

| Primer name | Sequence |
|---|---|
| Notch1 | Forward CACTGTGGGCGGGTCC Reverse GTTGTATTGGTTCGGCACCAT |
| Hes1 | Forward AGCCAACTGAAAACACCTGATT Reverse GGAGTTTATGATTAGCAGTGG |
| GAPDH | Forward GGCATCCTGGGCTACACT Reverse CCACCACCCTGTTGCTGT |

***Histopathological examination by light microscopy***

Autopsy samples were taken from the spleen of all examined groups to be inspected under light microscopy. The fresh spleen parts were fixed in 10% buffered formalin at room temperature for 24 h and then immersed in paraffin. Six µm thin tissue sections were then stained with hematoxylin and eosin and pathophysiological changes were observed under a bright field and the light microscope (Leica Microsystems, Schweiz, AG, Heerbrugg, CH-9435, Switzerland) in the Department of Anatomy, Faculty of Medicine, Zagazig University, Egypt.

***Ultrastructure examination by Transmission electron microscopic (TEM)***

The slices (1 mm3 thickness) of splenic tissues were fixed in a mixture of 2.5% glutaraldehyde and 2.5% paraformaldehyde, located in phosphate buffer for 24 hours, post-fixed in 1% osmium tetra-oxide, dehydrated and embedded in resin. The slices were clipped, followed by sectioning into semi-thin and ultrathin sections. The ultrathin sections were transferred to copper grids for staining with lead citrate and uranyl acetate. The sections were inspected by a transmission electron microscope at the electron microscope unit in Mansoura University using a Zeiss EM 100 S transmission electron microscope at 60 KV to detect the ultrastructure changes.

***Histo-morphometrical analysis***

Morphometric analysis was carried out on six rats from each group. Next to the immunostaining with the anti-NF-κB and anti iNOS antibodies, perspective fields from the images taken by the light microscope at 400× magnification were selected to measure the area % of NF-κB and iNOS positive reaction in the splenic tissues from 6 animals/group. Image J analysis software (Fiji image j; 1.51 n, NIH, USA) was utilized at Anatomy and Embryology Department, Faculty of medicine, Zagazig University

**Statistical analysis.**

All gathered data were verified for normality and then analyzed using GraphPad Prism 9 software (GraphPad Software, San Diego, CA). Data were stated as mean ± standard error of mean (SEM). Different groups mean were compared using one-way analysis of variance (ANOVA) afterward Tukey post hoc test for multiple comparisons was performed. The level of significance was taken at $P < 0.05$.

## RESULTS

***The oxidative stress markers***

Cisplatin-injected rats showed a significant decrease in the reduced glutathione and catalase levels accompanied by a significant increase in the lipid peroxidation marker MDA in comparison with Control rats. Notably, DBZ pretreatment significantly upregulated the GSH and catalase levels while decreasing MDA level in comparison to the Cisplatin group. Furthermore, GSH, catalase and MDA levels still unchanged in animals injected with DBZ **(Figure 1a, b and c).**

iNOS expression in the rats' splenic tissues detected by immunohistochemical staining was illustrated in **Figure 1 (d:i-iv).** Splenic tissues of Control group showed minimal expression of iNOS in the red pulp with negatively stained white pulp (Fig.1d, i).

Cisplatin injection evidently upregulated the iNOS expression that revealed abundant positive cytoplasmic immuno-stained cells in the red pulp and within sinuses lining the endothelial cells with few immuno-stained cells in white pulp (Fig. 1d, ii). Alternatively, DBZ+ Cisplatin group displayed a significant decrement in iNOS immunohistochemical expression compared to Cisplatin group (Fig. 1d, iii). These results were established by morphometrical and statistical assessment of the area % of the brown staining iNOS immuno-expression (Fig.1d, iv).

***The inflammatory markers***

Cisplatin injection significantly up regulated the TNF-α and IL-1β levels compared to the Control rats. DBZ pretreatment significantly reduce the TNF-α and IL-1β levels compared to Cisplatin injected rats. Notably, DBZ only treated animals exhibited no significant variations in TNF-α and IL-1β levels in comparison with the Control group **(Fig 2 a, b).**

NF-*κ*B expression in the rats' splenic tissues detected by immunohistochemical staining as illustrated in **(Fig 2c: i-iv)** showed that splenic tissues of Control group exhibited faint expression of NF-*κ*B (Fig.2c, i). Even though, cisplatin injection markedly upregulated the NF-kB expression (Fig.2c, ii). On the other hand, DBZ+Cisplatin group displayed a significant reduction in NF-*κ*B expression compared to Cisplatin group but still revealed a significant difference from the control group (Fig. 2c, ii). These results confirmed by morphometrical and statistical assessment of the area % of the brown staining NF-*κ*B immuno-expression (Fig.2c, iv).

**Effect of DBZ pretreatment on the Notch signaling pathway**

The cisplatin-treated group showed a significantly high expression of Notch-1 and Hes-1 mRNA levels compared to the Control group. However, DBZ+Cisplatin pre-treated rats showed a significant downregulation in the Notch-1 and Hes-1 expression levels compared to the Cisplatin-treated rats. Compared to the Control group, rats treated by DBZ only did not show any significant change in the Notch-1 and Hes-1 mRNA expression levels **(Fig 3 a, b)**.

***Effect of DBZ pretreatment on* spleen histo-pathology**

The histopathological changes detected by H/E staining of the splenic tissues taken from the various experimental groups showed that Control as well as DBZ pretreated groups exhibited no histopathological alteration in the splenic tissue with distinct white pulp region surrounded by red pulp and marginal zone **(Fig 4 a-c)**. While distinct histological changes were noticed in rats injected with cisplatin, where the tissue of rats administered with cisplatin clearly showed disorganization of lymphoid follicles, dilated and congested blood vessels, deposition of hemosiderin, a characteristic of spleen damage and disorganization of the margin between white pulp and red pulp that is indicative of the toxic effect of the cisplatin on the splenic architecture **(Fig.5a-c)**. DBZ pretreatment significantly reduced hemosiderin deposition in spleen and restored the architecture of the tissue as is evident in (**Fig. 6 a- c**).

***Effect of DBZ pretreatment on ultrastructure of spleen tissues***

Ultra-structural examination of the control spleen revealed multiple lymphocytes of different sizes. These lymphocytes had nuclei with chromatin that homogenously distributed. Macrophages were large and extend their process between lymphocytes. Fibroblast cell was seen peripheral to lymphocytes **(Fig. 7a)**. Regarding cisplatin group, the splenic tissue showed lymphocytes with different degrees of marginal condensed chromatin in their nuclei. Lymphoblasts, neutrophiles, eosinophils and fibroblast cells and collagen fibers were clearly seen. Most of these cells contained dilated rough endoplasmic reticulum. Another field revealed lymphocytes, macrophage and blood sinuses with their numerous contents of RBCs. In addition, some active macrophages with multiple phagosomes, multiple vesicular body and vacuolation could be noticed. lymphocytes with apoptotic nuclei and dark cytoplasm can be observed **(Figs. 7 b,c,d,e and f)**. Interestingly, administration of DBZ reverse most of the recorded histopathological injury to near the normal architecture of the splenic tissues with few RBcs in the blood sinuses but, some eosinophils and macrophages with multiple vesicular body could be seen **(Fig 7g and h)**.

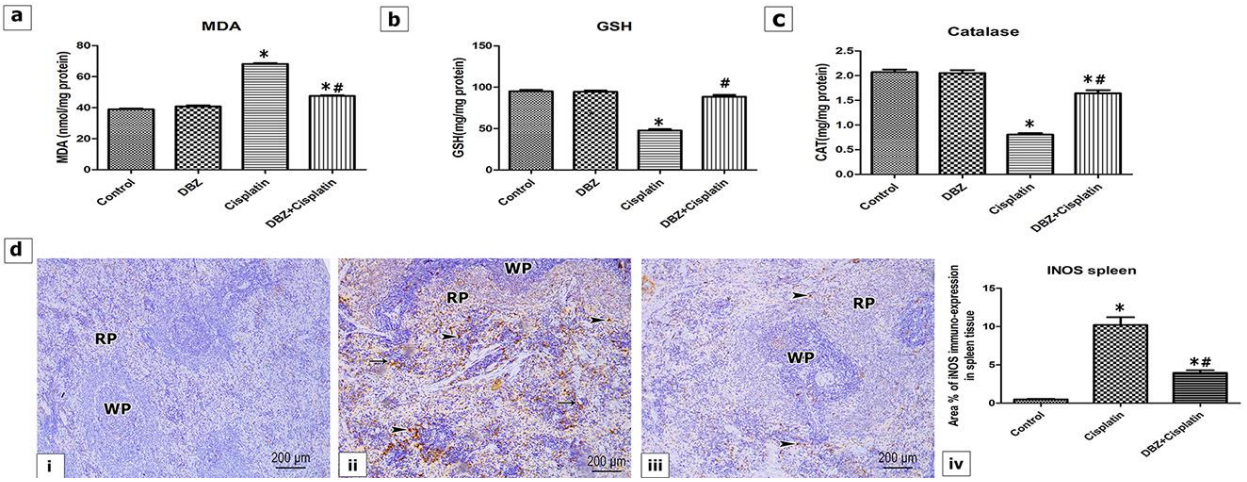


**Fig. 1** The impact of dibenzazepine treatment on the concentrations of oxidative stress markers in splenic tissues of cisplatin-treated rats. (a) malondialdehyde (MDA) (b) glutathione (GSH) and (c) catalase (CAT) in splenic tissues of cisplatin-treated rats. (d). (i: -iv) Photomicrographs of iNOS immuno-histochemical stained spleen tissues from the various studied groups. (i) Control, (ii) Cisplatin and (iii) DBZ+Cisplatin groups. Arrowhead and arrow refer to the brown color of the immuno-positive cells in the red pulp and within the sinus lining the endothelial cells respectively (RP) **Scale bar= 50 µm, x400**. (iv) Histogram displays the quantitative and statistical analysis of the area % of immuno- positive cells from the different experimental groups. * or #: Statistically significant from the control or the cisplatin group, respectively, $p < 0.05$ using ANOVA followed by Tukey-Kramer as a post-hoc test (N= 6/ group) ± S.E.M. NF-*κ*B: nuclear factor kapa B, DBZ: dibenzazepine.

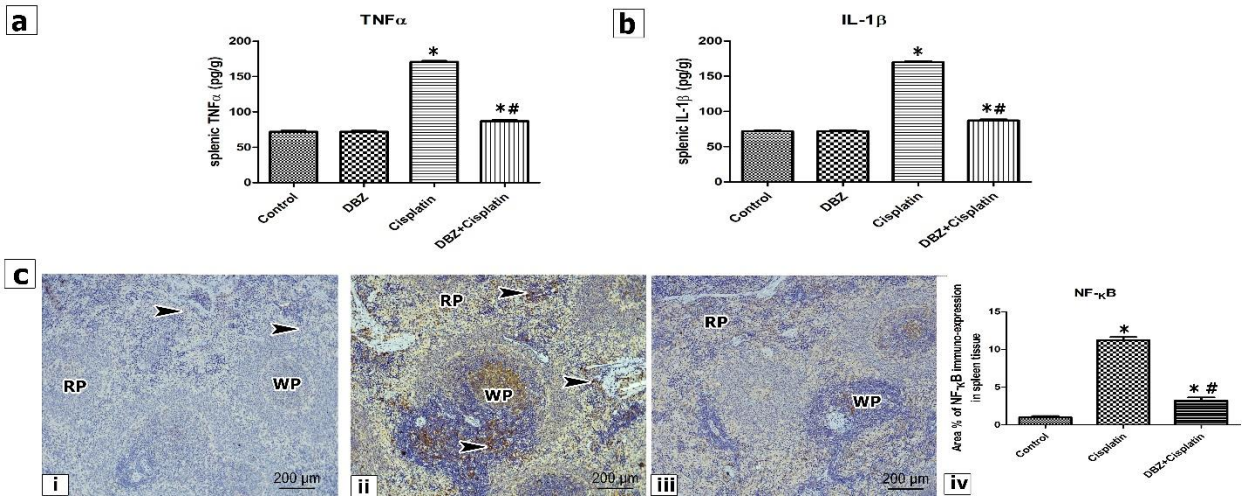


**Fig. 2:** The effect of dibenzazepine treatment on the levels of (a) tumor necrosis factor TNFα and (b) interleukin -1 beta IL-1β in splenic tissues of cisplatin-treated rats**.** (c) Photomicrographs of NF-*κ*B immuno-histochemical stained spleen tissues from the various studied groups. (i) Control, (ii) Cisplatin and (iii) DBZ+Cisplatin groups. Arrowhead refers to the brown coloration of the immuno-positive cells Scale bar= 50 µm, x400. (iv) Histogram displays the quantitative and statistical analysis of the area % of immuno- positive cells from the different experimental groups. * or #: Statistically significant from the control or the cisplatin group, respectively, $p < 0.05$ using ANOVA followed by Tukey-Kramer as a post-hoc test (N= 6/ group) ± S.E.M. NF-*κ*B: nuclear factor kapa B, DBZ: dibenzazepine.

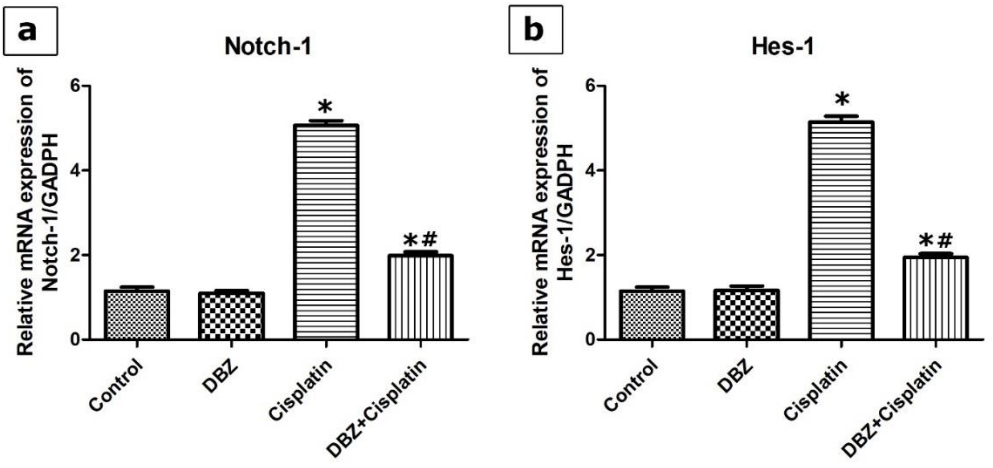


**Fig. 3:** Real-time quantitative polymerase chain reaction (qRT-PCR) shows relative mRNA expression of splenic (a) Notch-1/GADPH and (b) Hes-1/GADPH (% control) in all experimental groups (one- way ANOVA followed by Tukey post hoc test). (N= 6/ group) ± S.E.M. $^{*}p < 0.05$ vs control group, # $p < 0.05$ vs cisplatin group. DBZ: dibenzazepine

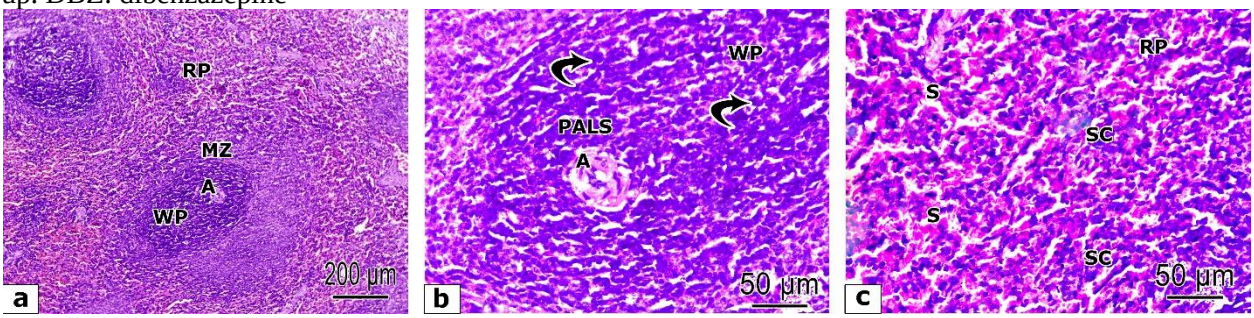


**Fig.4:** Photomicrographs of the spleen sections stained with H&E from control group shows: a) Its two main components white pulp (WP) and red pulp (RP) with distinct marginal zone (MZ) in between and the white pulp contains central arteriole (A). b) White pulp (WP) is formed of lymphocytes with dark stained nuclei (curved arrow) and contains central arteriole (A) that is surrounded by peri-arterial lymphatic sheath (PALS). c) the red pulp (RP) which contains blood sinusoids (S) and splenic cords (SC). (a) Scale bar= 200 µm, x100; (b, c) Scale bar= 50 µm, x400

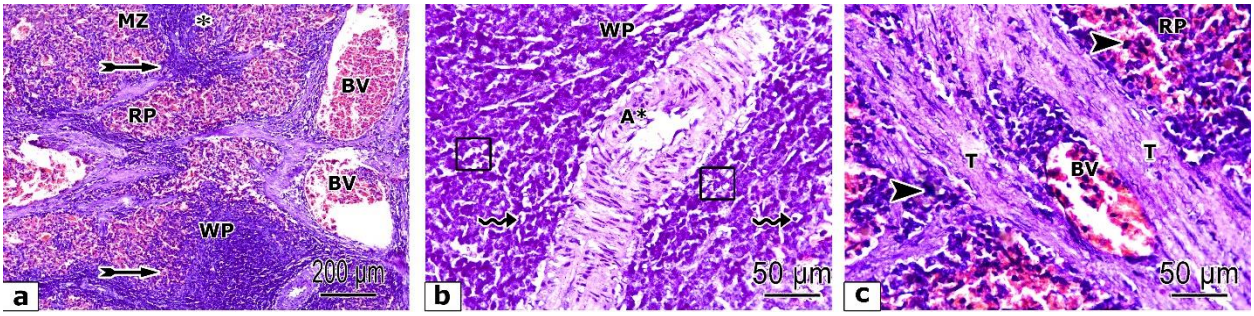


**Fig.5:** Photomicrographs of cisplatin treated group showing: a) the white pulp (WP)with disturbed lymphatic architecture (bifid tailed arrow) with appearance of germinal center (asterisk), The red pulp (RP) contains dilated congested blood vessels (BV). Note ill-defined marginal zone (MZ). b) white pulp (WP) contains several fragmented pyknotic nuclei (in sets) and vacuolated cells with fragmented pyknotic nuclei (zigzag arrows) and dilated thick wall arteriole (A*). c) Red pulp (RP) showing large dilated congested blood vessels (BV), hemosiderin laden cells (arrowhead) are also seen. Note the presence of huge splenic trabecula (T). (a) Scale bar= 200 µm, x100; (`b, c) Scale bar= 50 µm, x400.

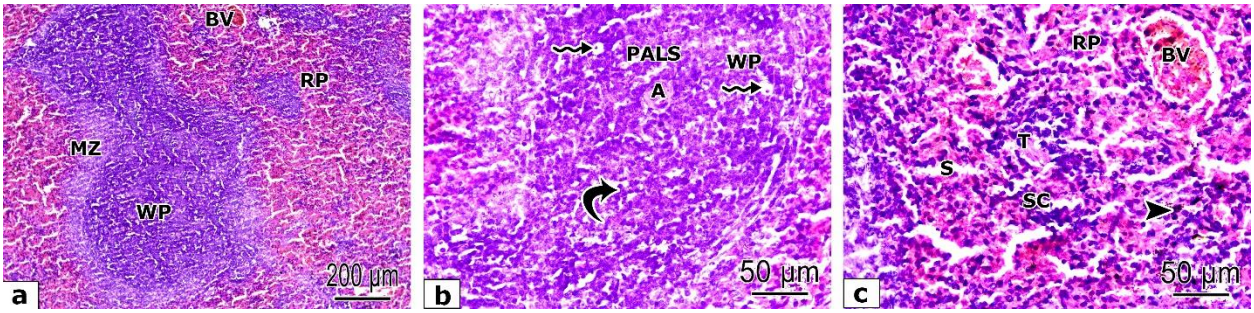


**Fig.6:** Photomicrographs of DBZ+ Cisplatin group: a) showing nearly normal splenic architecture can be seen with preservation of Its two main components white pulp (WP) and red pulp (RP) with distinct marginal zone (MZ) in between with congested blood vessel (BV). b) the white pulp (WP) is formed of lymphocytes with dark stained nuclei (curved arrow) and contains central arteriole (A) that is surrounded by peri-arterial lymphatic sheath (PALS). Pyknotic nuclei (zigzag arrow) are still detected 1I).c) The red pulp (RP) appearance is almost normal that containing blood sinusoids (S) and splenic cords (SC). While congested blood vessels (BV)and few hemosiderin-laden cells (arrowhead) are still observed. Note the presence of smaller splenic trabecula (T). (a) Scale bar= 200 µm, x100; (b, c) Scale bar= 50 µm, x400.

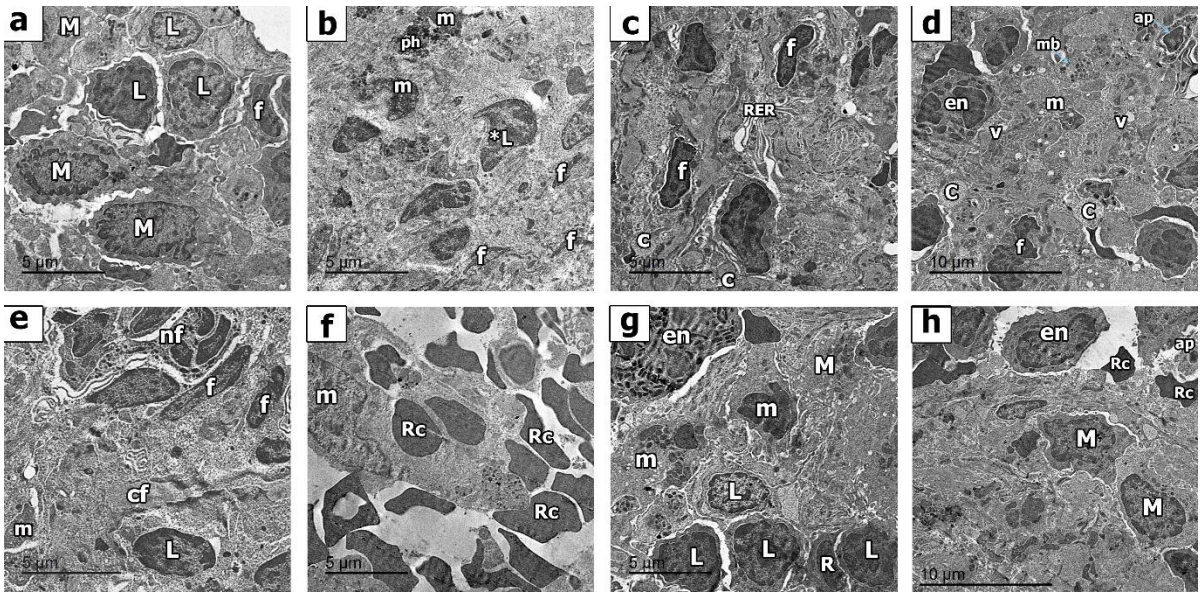


**Fig.7:** Electron photomicrographs of ultra- sections in spleen tissues from the different groups. a) control group, (b,c,d,e) Cisplatin group and (g,h) DBZ+ Cisplatin group. M: macrophage, L: lymphocytes, f: fibroblast, m: affected macrophage, ph: phagosome, RER: dilated rough endoplasmic reticulum, C: transverse collagen fibers, V: vacuolation, mb: multiple vesicular bodies, ap: apoptotic nuclei, en: eosinophile, nf: neutrophil, cf: longitudinal collagen fibers, Rc: red blood cells, R: reticular cell. (TEM, a, b, c, e , f, g: scale bar=5 µm, d &h: scale bar=10 µm).

## DISCUSSION

Cisplatin is a highly used chemotherapeutic agent that's broadly described for the treatment of numerous types of tumors. Unfortunately, cisplatin has poor targeting to only cancerous cells and is distributed to other organs as well [20]. This conforms to the base for cisplatin-induced organ toxicity. Notably, the hazardous effect of this drug on the spleen is well noticed [2]. The current study sheds light for the first time to the possible protective effect of DBZ in contradiction to pathophysiology induced in the spleen by cisplatin injection in rats. Some proposed involved mechanisms were clearly elucidated in this positive protective effect, including anti-inflammatory and antioxidative mechanisms besides its effect on the Notch signaling pathway.

Cisplatin caused damage to the splenic tissue as evidenced by light and electron microscopic examination and DBZ treatment ameliorated the

extent of this histopathological damage. Cisplatin caused damage to the antioxidant defense enzymes catalase (CAT) and glutathione (GSH) along with increased expression of the lipid peroxidation marker MDA and iNOS levels. DBZ pretreatment was found to restore the GSH, CAT, MDA, and iNOS expression levels toward normal values thus reducing the oxidative damage.

Also, cisplatin-induced upregulation of the inflammatory pathways manifested by high levels of TNFα and IL-1β beside increasing activation and thus nuclear translocation of NF-κB proved by increased expression of NF-κB-p65 in immunohistochemical staining of splenic tissue. Treatment with DBZ hampered nuclear translocation of NF-κB along with a reduced transcription of the inflammatory markers TNFα and IL-1β.

To the best of our knowledge, our study is the first to shed light on the contributing role of the Notch pathway in the splenic injury provoked by cisplatin and the role of DBZ a γ secretase inhibitor in ameliorating its hazardous effects on splenic tissue of cisplatin injected rats. Our study showed that the mRNA expression levels of the Notch-1 receptor beside Hes-1 were highly upregulated in cisplatin treated rats. DBZ pretreatment restored the elevated Notch pathway molecules to near normal levels being a gamma secretase inhibitor.

In accordance with our results previous studies showed accumulation of reactive oxygen species (ROS) in the spleen following cisplatin administration led to distorted redox oxidative status homeostasis assured by reduced levels of antioxidant enzymes CAT and GSH along with upregulated oxidative stress markers MDA and iNOS levels [2, 21].

Banerjee and his colleges added that the increased TNFα level evoked by cisplatin stimulates increased expression of iNOS leading to increase NO level and generating nitrosative stress in the splenic tissue [2].

The ameliorative effect of DBZ on the oxidative stress induced by cisplatin is in the same line with preceding studies showing that antioxidant compounds such as selenium, and carnosine protects against cisplatin induced organ toxicity [2,22]. Additionally, many studies linked the inhibition of Notch-1 pathway by gamma secretase inhibitors to the down regulation noticed in the lipid peroxidation inflammatory marker MDA[23].

Added to oxidative stress; inflammation was proved to show a crucial role in the pathophysiology induced by cisplatin on rat spleen. In accordance with our results previous ones documented elevated expression of proinflammatory cytokines as TNFα and IL-1β after cisplatin injection in different animal models [3,4]. Parallel to our results Banerjee et al reported that cisplatin exposure including increases the activation and nuclear translocation of NFκB leading to increased transcription of other proinflammatory cytokines including TNFα and IL-1β [2].

The ameliorative effect of DBZ on inflammatory markers were approved by many studies on gamma secretase inhibitors as they were proved to have anti-inflammatory and antiproliferative effects on different experimental models [12,13]. Moreover, previous studies showed that dibenzazepine attenuates cisplatin-induced nephrotoxicity in rats and attributed this protective effect to its anti-inflammatory as well as antioxidant effect [10].

Pointing new pathways explaining the cisplatin induced splenic injury seems to be a hopeful solution for decreasing the cisplatin induced organ damage. The attention towards the role Notch pathway in pathogenesis, proliferation and spread of many tumors has been raised in recent years [8, 24]. And therapies targeting this harmful pathway are very promising in field of anticancer drugs (10). One of these strategies are the use of gamma secretase inhibitors including DBZ for the treatment of various cancers due to their ability to inhibit Notch receptor signaling [25,26,27]. Increasing number of studies proved that many anti cancerous drugs target the cancerous stem cells by targeting Notch-1[28,29]. Till now, the probable contribution of the Notch pathway in splenic toxicity induced by cisplatin has not been clearly elucidated, thus we found it a thought-provoking point to be discovered.

Our current results showed up regulated levels of mRNA of Notch-1 receptor and Hes-1 in the cisplatin-injected rats. These effects were ameliorated by DBZ pretreatment; which are an expected effects from such drug being a gamma-secretase inhibitor.

In addition to the attenuated expression levels of the evaluated Notch pathway components by DBZ pretreatment a cross talk between the Notch pathway and the assessed inflammatory cytokines was clearly reported by many studies. Thus, a crosstalk between TNF-α and the master inflammatory marker NF-κB and Notch pathway was proved to withstand the intrinsic inflammatory cytokines [30]. Evoked TNF-α encouraged NF-κB signaling which in teamwork with the basal Notch

signals, upregulates the expression level of the Notch targets mainly by phosphorylation of promoters of histone H3 at the Hes1. Additionally, Hes-1 proved to induce gene transcription of NF-κB, thus linking the Notch signaling pathways to the inflammatory responses induced by NF-κB upregulation [31].

These aforementioned biochemical changes evoked by cisplatin administration was correlated by histopathological damage that showed in splenic tissues by H&E staining and electron microscopic examination. In accordance with our results Milicevic al found that the volume density of red pulp, white pulp and the marginal zone of cisplatin exposed spleen was markedly reduced compared to normal ones [32,33]. Furthermore, cisplatin exposure causes injury to erythrocytes and the injured RBCs collects in the red pulp of the spleen with decreased recycling of FPN1 and ferritin protein. Thus, produces iron burden in the spleenocytes leading to hemosiderin deposition in the splenic tissue [34]. Moreover, our results in accordance with Khalaf et al who stated that the spleen sections from rats treated with cisplatin showed, noticeable histopathological changes, including disorganization of lymphoid follicles, hyperplasia in white pulp, depletion of lymphocytes in red pulp with edema. Increased hemosiderosis as well as fibrosis in the red pulp and some lymphoid follicles were also noticed along with dilated and congested blood vessels [35]. Furthermore, our results in alignment with Crăciun & Paşca who proved that Cisplatin has been found to induce ultrastructural modifications by increase the reaction of the affected tissue, revealed by the increased activity of macrophages, plasmacytes, monocytes, eosinophils and neutrophils, indicating a protective reaction of the spleen to the toxic effect of cisplatin [36].Fortunately, DBZ restored to a great extent the histopathological damage caused in the spleen by cisplatin administration a finding linked to improved oxidative stress and inflammatory status caused by cisplatin along with restoration of the disturbed Notch-1 pathway.

In conclusion, our results showed that DBZ evoked a talented protective effect in face of cisplatin-induced splenic damage in rats. These defending properties of DBZ were accomplished by tumbling oxidative stress and inflammation along with downregulating the overstimulated Notch signaling pathway a promising finding making it hopeful to attempt other Notch blockers as a splenic protective drug against chemotherapeutic induced toxicities in future studies.

**Conflict of Interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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## REFERENCES

1. Dasari, S., & Tchounwou, P. B. Cisplatin in cancer therapy: molecular mechanisms of action. Eur. J. Pharmacol*2014; 740*,364–378. https://doi.org/10.1016/j.ejphar.2014.07.025
2. Banerjee, S., Sinha, K., Chowdhury, S., & Sil, P. C. Unfolding the mechanism of cisplatin-induced pathophysiology in spleen and its amelioration by carnosine. *Chem. Biol. Interact 2018*; *279*, 159–170. https://doi.org/10.1016/j.cbi.2017.11.019
3. Gao, H., Zhang, S., Hu, T., Qu, X., Zhai, J., Zhang, Y., Tao, L., Yin, J., & Song, Omeprazole protects against cisplatin-induced nephrotoxicity by alleviating oxidative stress, inflammation, and transporter-mediated cisplatin accumulation in rats and HK-2 cells. *Chem. Biol. Interact* 2019; 297, 130–140. https://doi.org/10.1016/j.cbi.2018.11.008
4. Soetikno, V., Sari, S., Ul Maknun, L., Sumbung, N. K., Rahmi, D., Pandhita, B., Louisa, M., & Estuningtyas, A. Pre-Treatment with Curcumin Ameliorates Cisplatin-Induced Kidney Damage by Suppressing Kidney Inflammation and Apoptosis in Rats. *Drug Res* 2019; *69*(2), 75–82. https://doi.org/10.1055/a-0641-5148
5. Noori, S., & Mahboob, T. Antioxidant effect of carnosine pretreatment on cisplatin-induced renal oxidative stress in rats. *IJCB 2010*; 25(1), 86–91. https://doi.org/10.1007/s12291-010-0018-x
6. Wang, J., Wang, C., Meng, Q., Li, S., Sun, X., Bo, Y., & Yao, W. (2012). siRNA targeting Notch-1 decreases glioma stem cell proliferation and tumor growth. *Mol. Biol. Rep* 2012; *39*(3), 2497–2503. https://doi.org/10.1007/s11033-011-1001-1
7. Yan, B., Liu, L., Zhao, Y., Xiu, L. J., Sun, D. Z., Liu, X., Lu, Y., Shi, J., Zhang, Y. C., Li, Y. J., Wang, X. W., Zhou, Y. Q., Feng, S. H., Lv, C., Wei, P. K., & Qin, Z. F. Xiaotan Sanjie decoction attenuates tumor angiogenesis by manipulating Notch-1-regulated proliferation of gastric cancer stem-like cells. *World J. Gastroenterol.* 2014; *20*(36), 13105–13118. https://doi.org/10.3748/wjg.v20.i36.13105
8. Kontomanolis, E. N., Kalagasidou, S., Pouliliou, S., Anthoulaki, X., Georgiou, N., Papamanolis, V., & Fasoulakis, Z. N. The Notch Pathway in Breast Cancer Progression. *Sci. World J 2018*; 2415489. https://doi.org/10.1155/2018/2415489
9. Wu, W. R., Shi, X. D., Zhang, F. P., Zhu, K., Zhang, R., Yu, X. H., Qin, Y. F., He, S. P., Fu, H. W., Zhang, L., Zeng, H., Zhu, M. S., Xu, L. B., Wong, P. P., & Liu, C. Activation of the Notch1-c-myc-VCAM1 signalling axis initiates liver progenitor cell-driven hepatocarcinogenesis

and pulmonary metastasis. *Oncogene 2022*; *41*(16), 2340–2356. https://doi.org/10.1038/s41388-022-02246-5

10. Abd El-Rhman, R. H., El-Naga, R. N., Gad, A. M., Tadros, M. G., & Hassaneen, S. K.. Dibenzazepine Attenuates Against Cisplatin-Induced Nephrotoxicity in Rats: Involvement of NOTCH Pathway. *Front. Pharmacol. 2020; 11*, 567852. https://doi.org/10.3389/fphar.2020.567852
11. Nowell, C. S., & Radtke, F. Notch as a tumour suppressor. *Nat. Rev. Cancer 2017; 17*(3), 145–159. https://doi.org/10.1038/nrc.2016.145
12. Zhao, C., Li, J., Yang, J., Yang, L., Chen, P., Dou, J., & Zhao, S. Inhibitor of γ-secretase alleviates middle ear inflammation by regulating Th2 response in OVA-mediated allergic OME in vivo. *Immunobiology 2019; 224*(6), 765–773. https://doi.org/10.1016/j.imbio.2019.08.010
13. Michelon, C., Michels, M., Abatti, M., Vieira, A., Borges, H., Dominguini, D., Barichello, T., & Dal-Pizzol, F. The Role of Secretase Pathway in Long-term Brain Inflammation and Cognitive Impairment in an Animal Model of Severe Sepsis. *Mol. Neurobiol. 2020; 57*(2), 1159–1169. https://doi.org/10.1007/s12035-019-01808-1
14. Al-Qawasmeh R. A., Lee Y., Cao M. Y., Gu X., Viau S., Lightfoot J., et al. 11-Phenyl-[b,e]-dibenzazepine compounds: novel antitumor agents. *Bioorg. Med. Chem. Lett* 2009; 19, 7–104. 10.1016/j.bmcl.2008.11.001
15. Ahmed, L. A., Abd El-Rhman, R. H., Gad, A. M., Hassaneen, S. K., & El-Yamany, M. F. Dibenzazepine combats acute liver injury in rats via amendments of Notch signaling and activation of autophagy. *aunyn Schmiedebergs Arch. Pharmacol. 2021; 394*(2), 337–348. https://doi.org/10.1007/s00210-020-01977-0
16. ELLMAN G. L. Tissue sulfhydryl groups. Arch. Biochem.1959; *82*(1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6
17. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica chimica acta 1978; 90*(1), 37–43. https://doi.org/10.1016/0009-8981(78)90081-5
18. Buchwalow I. B., Böcker W. *Immunohistochemistry: basics and methods*. 2002; 1st Edn Berlin, Germany: Springer.
19. Yan, B., Zhou, Y., Feng, S., Lv, C., Xiu, L., Zhang, Y., Shi, J., Li, Y., Wei, P., & Qin, Z. β -Elemene-Attenuated Tumor Angiogenesis by Targeting Notch-1 in Gastric Cancer Stem-Like Cells. *: eCAM, 2013*, 268468. https://doi.org/10.1155/2013/268468
20. Abuzinadah, M. F., & Ahmad, A. Pharmacological studies on the efficacy of a thymoquinone-containing novel polyherbal formulation against cisplatin-induced hepatorenal toxicity in rats. *J. Food Biochem 2020; 44*(2), e13131. https://doi.org/10.1111/jfbc.13131
21. Omar, H. A., Mohamed, W. R., Arafa, e., Shehata, B. A., El Sherbiny, G. A., Arab, H. H., & Elgendy, A. N. Hesperidin alleviates cisplatin-induced hepatotoxicity in rats without inhibiting its antitumor activity. *Pharmacol Rep 2016; PR*, *68*(2), 349–356. https://doi.org/10.1016/j.pharep.2015.09.007
22. Kamel K. M., Fawzy H. M., Metwally S. A., Abd El-Latif H. A., El-sayed M. E. Protective effects of onion oil and selenium against cisplatin-induced nephrotoxicity and oxidative stress in rats. *Egypt. J. Hosp. Med.2015;* 58, 18–25. 10.12816/0009356
23. Song, N. J., Yun, U. J., Yang, S., Wu, C., Seo, C. R., Gwon, A. R., Baik, S. H., Choi, Y., Choi, B. Y., Bahn, G., Kim, S., Kwon, S. M., Park, J. S., Baek, S. H., Park, T. J., Yoon, K., Kim, B. J., Mattson, M. P., Lee, S. J., Jo, D. G., … Park, K. W. (2016). Notch1 deficiency decreases hepatic lipid accumulation by induction of fatty acid oxidation. *Sci. Rep* 2016; *6*, 19377. https://doi.org/10.1038/srep19377
24. Meurette, O., & Mehlen, P. Notch Signaling in the Tumor Microenvironment. *Cancer cell 2018; ,34*(4), 536–548. https://doi.org/10.1016/j.ccell.2018.07.009
25. Aoyama, T., Takeshita, K., Kikuchi, R., Yamamoto, K., Cheng, X. W., Liao, J. K., & Murohara, T. gamma-Secretase inhibitor reduces diet-induced atherosclerosis in apolipoprotein E-deficient mice. Biochem 2009; *383*(2), 216–221. https://doi.org/10.1016/j.bbrc.2009.03.154
26. Kang, J. H., Kim, B. S., Uhm, T. G., Lee, S. H., Lee, G. R., Park, C. S., & Chung, I. Y. Gamma-secretase inhibitor reduces allergic pulmonary inflammation by modulating Th1 and Th2 responses. *Am. J. Respir 2009; 179*(10), 875–882. https://doi.org/10.1164/rccm.200806-893OC
27. Acharya, A., Hans, C. P., Koenig, S. N., Nichols, H. A., Galindo, C. L., Garner, H. R., Merrill, W. H., Hinton, R. B., & Garg, V. Inhibitory role of Notch1 in calcific aortic valve disease. *PloS one*2011; *6*(11), e27743. https://doi.org/10.1371/journal.pone.0027743
28. Zhen, Y., Zhao, S., Li, Q., Li, Y., & Kawamoto, K. Arsenic trioxide-mediated Notch pathway inhibition depletes the cancer stem-like cell population in gliomas. *Cancer letters 2010; 292*(1), 64–72. https://doi.org/10.1016/j.canlet.2009.11.005
29. Li, Y., Zhang, J., Ma, D., Zhang, L., Si, M., Yin, H., & Li, J. (2012). Curcumin inhibits proliferation and invasion of osteosarcoma cells through inactivation of Notch-1 signaling. *The FEBS journal 2012; 279*(12), 2247–2259. https://doi.org/10.1111/j.1742-4658.2012.08607.x
30. Maniati, E., Bossard, M., Cook, N., Candido, J. B., Emami-Shahri, N., Nedospasov, S. A., Balkwill, F. R., Tuveson, D. A., & Hagemann, T. Crosstalk between the canonical NF-κB and Notch signaling pathways inhibits Pparγ expression and promotes pancreatic cancer progression in mice. *J. Clin. Investig 2011; 121*(12), 4685–4699. https://doi.org/10.1172/JCI45797
31. Cao, Q., Li, P., Lu, J., Dheen, S. T., Kaur, C., & Ling, E. A. Nuclear factor-κB/p65 responds to changes in the Notch signaling pathway in murine BV-2 cells and in amoeboid microglia in postnatal rats treated with the γ-

secretase complex blocker DAPT. J. Neurosci 2010; *88*(12), 2701–2714. https://doi.org/10.1002/jnr.22429
32. Milićević, Z., Slepcević, V., Nikolić, D., Zivanović, V., & Milićević, N. M. Effects of cis-diamminedichloroplatinum II (cisplatin) on the splenic tissue of rats: a histoquantitative study. *Exp. Mol. Pathol 1994; 61*(2), 77–81. https://doi.org/10.1006/exmp.1994.1027
33. Wen, S. W., Everitt, S. J., Bedő, J., Chabrot, M., Ball, D. L., Solomon, B., MacManus, M., Hicks, R. J., Möller, A., & Leimgruber, A. Spleen Volume Variation in Patients with Locally Advanced Non-Small Cell Lung Cancer Receiving Platinum-Based Chemo-Radiotherapy. *PloSone*2015; *10*(11),e0142608. https://doi.org/10.1371/journal.pone.0142608
34. Wang, Y., Juan, L. V., Ma, X., Wang, D., Ma, H., Chang, Y., Nie, G., Jia, L., Duan, X., & Liang, X. J. Specific hemosiderin deposition in spleen induced by a low dose of cisplatin: altered iron metabolism and its implication as an acute hemosiderin formation model. *Curr2010;* 11 (6), 507–515. https://doi.org/10.2174/138920010791636149
35. Khalaf, Abdelazeem Ali, et al. Protective effect of Echinacea purpurea (Immulant) against cisplatin-induced immunotoxicity in rats. DARU J. Pharm. Sci. 2019; 27.1: 233-241.
36. Crăciun, C., & Pașca, C. Structural and ultrastructural data on side effects of cisplatin in spleen, kidney and liver of rats. Acta Metallomica 2014; 11(1), 9-22.

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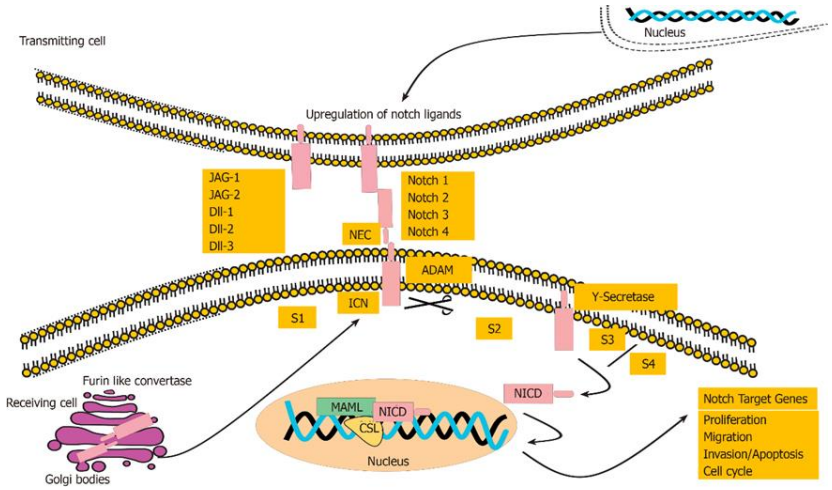


**Fig i The activation pathway of Notch signaling receptors and their downstream signaling molecules (**The canonical Notch signaling pathway). The interaction between Notch and its ligands from neighboring cells results in two successive cleavage events: cleavage at site S2 by ADAM protease and subsequent intramembranous cleavage at S3 by γ-secretase complex. This ligand-dependent activation process generates Notch intracellular domain (NICD). In the nucleus, NICD forms the transcription activation complex with transcription factor CSL and transcriptional coactivator such as MAML1, initiating the transcription of target genes.