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Silymarin Attenuates NDEA-Induced Renal Oxidative Damage via Downregulation of MDA and LDH and Modulation of NF-κB/IL-6 Pathway

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ABSTRACT
N - nitrosodiethylamine (NDEA) is widely recognized as an environmental toxin capable of triggering marked oxidative imbalance and inflammatory disturbances, which over time can impair kidney function. This work explores how silymarin attenuates NDEA-induced renal oxidative damage via downregulation of MDA and LDH and modulation of NF-κB/IL-6 pathway. After a two-week acclimation period, forty-two male Wistar rats were randomly assigned to six experimental groups: an NDEA-exposed group, a corn-oil vehicle group, silymarin-only, sorafenib-NDEA (conventional drug) group, and two additional groups that received NDEA in combination with different concentration of silymarin. Following completion of the treatment protocols, blood and renal tissue samples were collected for biochemical analysis and assessment of inflammatory signaling. Rats exposed to NDEA showed substantial rises in malondialdehyde (MDA) and lactate dehydrogenase (LDH), reflecting heightened lipid peroxidation and cellular disruption. NDEA also promoted activation of NF-κB and increased IL-6, accompanied by a reduction in the anti-inflammatory cytokine IL-10. Administration of silymarin counteracted these changes by lowering MDA, LDH, NF-κB, and IL-6, indicating notable antioxidant and anti-inflammatory effects. Sugg esting a strengthened immunoregulatory response. Overall, the data indicate that silymarin, when administered, can limit the renal toxicity produced by NDEA by dampening oxidative stress and inflammatory signaling, underscoring their potential usefulness in mitigating chemically induced kidney injury.

Keywords: Silymarin, NDEA, oxidative stress, inflammation, renal toxicity.

I. INTRODUCTION

Environmental exposure to nitrosamines remains an ongoing public-health challenge due to their occurrence in processed foods, cigarette smoke, and various industrial by-products, as well as their well-established carcinogenic potential. Among the nitrosamines, N-nitrosodiethylamine (NDEA) is particularly noteworthy because its metabolic conversion generates reactive intermediates that can produce excessive reactive oxygen species (ROS) [1]. The resulting ROS burden can exceed the capacity of cellular antioxidant systems, leading to lipid and protein oxidation, genomic damage, and the activation of multiple inflammatory pathways, which collectively facilitate organ dysfunction and tumor development. Contemporary reviews underscore these mechanisms and emphasize the need to evaluate therapeutic agents with combined antioxidant and anti-inflammatory activities [2].

The kidney is especially susceptible to chemical injury owing to its substantial blood flow, ability to concentrate xenobiotics, and active metabolic machinery[3]. In experimental models, NDEA administration increases malondialdehyde (MDA), a marker of lipid peroxidation, and elevates lactate dehydrogenase (LDH), which reflects compromised membrane integrity and cytotoxicity. NDEA also stimulates inflammatory signaling, particularly through activation of nuclear factor-kappa B (NF-κB). Once activated, NF-κB enhances the production of pro-inflammatory mediators such as interleukin-6 (IL-6), while anti-inflammatory cytokines like interleukin-10 (IL-10) tend to decline, weakening the regulatory balance that normally restrains inflammation [2]. Together, these biochemical alterations promote a renal environment conducive to tissue injury and, potentially, carcinogenic progression.

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Given these mechanistic insights, interest has grown in natural or pharmacological compounds that can buffer oxidative stress while simultaneously modulating inflammatory pathways. Silymarin, a flavonolignan mixture derived from Silybum marianum, has long been recognized for its hepatoprotective actions and is now increasingly viewed as a broader antioxidant and immune-regulating agent [4]. Current experimental findings show that silymarin enhances endogenous antioxidant capacity, reduces MDA formation, stabilizes cellular membranes as reflected by lower LDH leakage, and suppresses NF-κB activation. These actions lead to reduced IL-6 levels and improved IL-10 modulation. Recent systematic reviews provide additional support for its efficacy in improving liver and kidney functional indices [5]

Sorafenib, approved for the treatment of hepatocellular and renal cell carcinoma, is primarily known as a multi-kinase inhibitor but has also been shown to influence inflammatory and fibrotic processes in renal disease models [2]. Experimental studies demonstrate that sorafenib can inhibit major inflammatory pathways, including MAPK and NF-κB signaling, while lowering pro-inflammatory cytokine expression and mitigating oxidative stress driven fibrotic responses in obstructive or toxin induced kidney injury models [6]. These findings suggest that, beyond its anticancer roles, sorafenib may possess significant renoprotective activity mediated through suppression of oxidative and inflammatory signaling.

Although evidence continues to grow for both silymarin and sorafenib in regulating oxidative stress and inflammatory responses, few studies have directly compared their individual mechanistic effects in an NDEA-induced renal injury setting, particularly in relation to combined assessment of MDA, LDH, NF-κB, IL-6 and IL-10, changes. Addressing this gap is important for identifying potential mechanism-driven interventions for chemically induced nephrotoxicity and early renal injury associated with carcinogenesis. Therefore, this study investigates how silymarin influences oxidative and inflammatory markers, in NDEA-treated rats, to clarify their renoprotective profiles and underlying mechanisms.

II. MATERIAL AND METHODS

Chemicals

N-nitroso diethylamine with catalog number N525465 and Silymarin were supplied by Carbanio, a chemical firm (India). LDH kit from Agappe Diagnostics (Switzerland). Sorafenib was purchased from Cipla Pharmaceutical Company (India). Kits for the enzyme-linked immunosorbent test (ELISA) of NF-kB, IL-6, and IL-10 with the catalog numbers MB-1731A, MB-1736A, and MB-7455A, respectively. They were purchased from Mornmed Medical Equipment (China). Analytical quality chemicals and reagents were procured from Sigma Aldrich, located in St. Louis, Germany.

Experimental design

Forty-two (42) male Wistar rats were acclimatized for two weeks before being divided into six groups (n=7). Group 1 served as a positive control and received a single intraperitoneal injection (i.p.) of 200 mg/kg of NDEA. Group 2 received an oral dose of 1 ml of corn oil. Group 3 was given an oral dose of 50 mg/kg/bodyweight of silymarin. Group 4 had intraperitoneal (i.p.) NDEA and then 10 mg/kg/bw sorafenib (conventional drug). Groups 5 and 6 were administered NDEA 200 mg/kg/bw and followed by silymarin 50 mg/kg and 75 mg/kg, respectively. After 3 weeks of administration of N - N-nitrosodiethylamine, animals were administered orally with daily doses of 50mg/kg, 75mg/kg silymarin, and 10mg/kg sorafenib for 14 days, according to the method of [7].

Blood and Liver Tissue Collection

After a 12-hour fast,blood samples were obtained via the ocular route after the two-week treatment period. For hematological assessments, 1 ml of blood from each animal was transferred into EDTA-coated tubes and centrifuged at 2500 rpm for 15 minutes at 4°C to separate the serum, which was subsequently held at 4°C for 30 minutes in plain tubes.

Immediately after sacrifice, the kidneys were excised, blotted dry, weighed, and rinsed thoroughly with ice-cold normal saline. A 0.2 g portion of each kidney was homogenized in 1.8 ml of phosphate buffer (10 mM, pH 7.4). The resulting homogenate was centrifuged at 4000 rpm for 15 minutes on ice, and the supernatant was carefully transferred into 2 ml Eppendorf tubes and stored at −20°C pending biochemical analysis.

Determination of Lactate Dehydrogenase (LDH) Activity

The diagnostic Agape kit was used to determine lactate dehydrogenase (LDH) by the techniques of [8]; [9]; and[10]. In the process, 10 µL of the sample and 1000 µL of the working reagent were combined, and the mixture was then incubated at 37°C for 1 minute. The change in absorbance per minute (OD/min) was then measured over 3 minutes.
LDH Activity (U/L) = (Δ OD/minutes) x 1603

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$$\text{LDH activity (U/mg protein)} = \frac{\text{LDH activity (U/L)}}{\text{Total protein concentration (mg/L)}}$$

Determination of Malondialdehyde (MDA)

The formation of TBARS (thiobarbituric acid reactive substances) was measured using the [11] technique to evaluate the peroxidation of lipids. A portion of the test sample, equal to 0.4 milliliters, was combined with 0.5 milliliters of 30% TCA and 1.6 milliliters of Tris-KCl buffer.

After that, 0.5 ml of 0.75% TBA was added to the sample and heated to 80^0C for 45 minutes in a water bath. After being chilled in ice to ambient temperature, it underwent a 10-minute, 3000 rpm centrifugation, using distilled water as a reference blank; the absorbance of the obtained clear supernatant was measured at 532nM.

nMole MDA / mg protein =

$$\frac{\text{Absorbance x Vol of mixture}}{\sum_{532} \text{x Vol of sample Xmg protein}}$$

Measurement of NF-kB, IL-6 and IL-10

This assay makes use of the quantitative sandwich enzyme immunoassay technique. 50µl of the standard was put into a standard well. 10µl of the testing sample and 40µl of the sample diluent were added to the well. 100µl of the HRP-conjugate reagent was added to each well, which was then sealed with an adhesive strip and left to incubate for 60 minutes at 37°C. Following aspiration and five rounds of washing, 400µl of wash solution was applied to each well. Each of the two chromogen solutions was applied in 50µl increments to each well. The micro-ELISA strip plate was placed out of direct sunlight, shaken gently, and incubated for 15 minutes at 37 °C .Fifty microliters of stop solution were added to each well. It became yellow after originally being blue. It changed the hue from blue to yellow. The optical density at 450 nm was measured using a microtiter plate reader in around 15 minutes. The quantity of unknown samples was ascertained using the standard curve. The standard curve was created by plotting the average optical density (450 nm) for each of the six standard concentrations.

Analytical Statistics

All statistical analyses were carried out using GraphPad Prism 7. a. The data were expressed using mean ± SEM, which is the standard error of the mean. One-way analysis of variance was used to determine the statistical significance, with p-values less than 0.05 (ANOVA) thought to be significant.

III. RESULTS AND DISCUSSIONS

Lactate dehydrogenase (LDH) is a definitive indirect marker of tumor hypoxia, angiogenesis, and poor prognosis in hepatocellular carcinoma (HCC). It plays a critical role in the body's energy production [2]. The results of this study clearly demonstrate a significant increase in kidney LDH levels in rats treated with N-nitrosodiethylamine (NDEA; Figure 1). In stark contrast, the groups treated with silymarin and sorafenib exhibited a remarkable recovery ($p < 0.001$), underscoring the effectiveness of silymarin in HCC treatment. Elevated LDH levels are unequivocally associated with cell damage. LDH levels in all treatment groups were statistically similar and significantly lower compared to the NDEA-induced group ($p < 0.001$). This clearly indicates that silymarin actively reduces NDEA-induced renal tissue damage. Elevated LDH serves as a clear marker of cellular injury and necrosis, as it is released from damaged cells. Moreover, this study confirms that NDEA administration significantly elevates kidney malondialdehyde (MDA) levels (Figure 2). However, treatment with 10 mg of sorafenib and varying concentrations of silymarin (50 mg and 75 mg) resulted in a significant reduction of MDA levels ($p < 0.001$).

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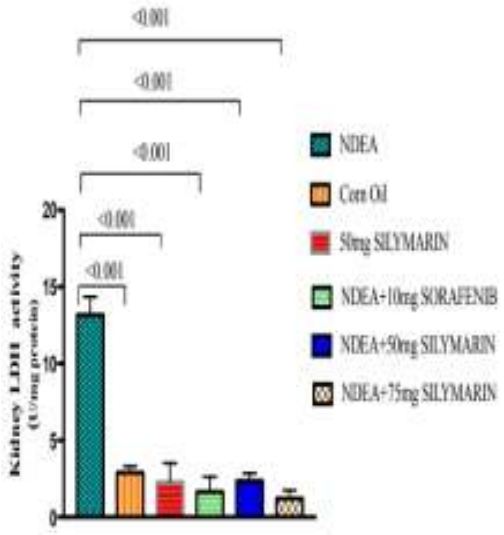

Figure 1: Effect of NDEA on kidney LDH levels and the ameliorative effect of silymarin in male Wistar rats. Values are statistically significant at $p < 0.05$.

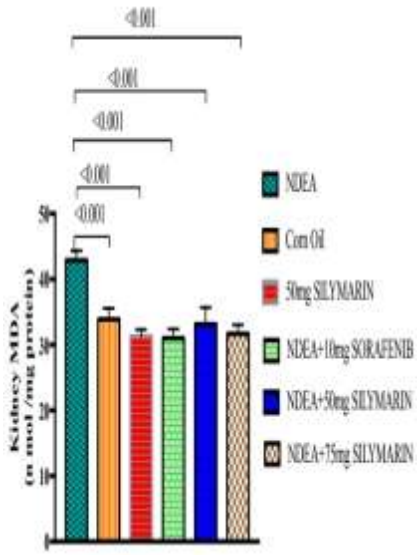

Figure 2: Effect of NDEA on kidney MDA levels and the ameliorative effect of silymarin in male Wistar rats. Values are statistically significant at $p < 0.05$.

Studies have indicated that NF-kB is a key mediator in early events that promote neoplastic lesion progression in the kidney [13]. In the current study, the carcinogen (NDEA) induced a marked elevation in the concentration of NF-κB in the damaged kidney (Figure 3), while treatment with silymarin and sorafenib administration was markedly attenuated ($p< 0.001$), the NF-κB protein concentration induced by the carcinogen. There were no significant differences in all the treated groups, including the control groups, in the cancerous kidney. This inhibition of NF-κB activity might contribute to the renoprotective effects of silymarin against carcinogen-induced renal carcinogenesis. NF-κB has been demonstrated to be a key inflammatory factor in tumorigenesis, which has been shown to be up-regulated both in human hepatocarcinoma and in experimental animals [14].

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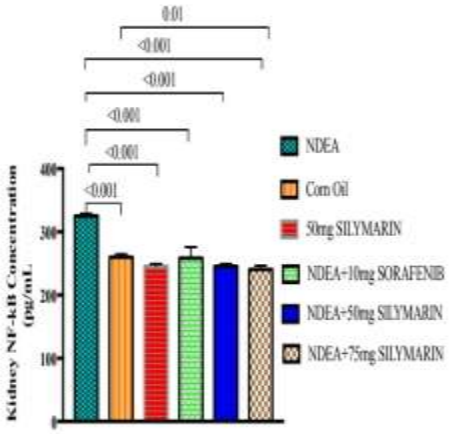

Figure 3: Effect of NDEA on kidney NF-kB concentration and the ameliorative effect of silymarin in male Wistar rats. Values are statistically significant at $p < 0.05$.

Serum cytokines serve as critical mediators in various pathological and physiological processes related to inflammation and cancer progression. In our study, the NDEA-treated groups exhibited a downregulation of interleukin 10 (IL-10), as depicted in Figure 4. In contrast, all treated groups demonstrated an upregulation of IL-10 in the kidneys ($P < 0.001$). Notably, the NDEA + 75 mg silymarin group showed the most pronounced increase in IL-10 levels when compared to other treated groups, while no significant difference was observed between the NDEA + 50 mg silymarin group and the NDEA + 10 mg sorafenib group. These findings suggest that the effects of silymarin are dose-dependent. The elevation of IL-10 may indicate a response aimed at tempering hyperinflammation and preventing tissue damage. A primary role of IL-10 during infection is to suppress the host immune response to pathogens and microbiota, thereby mitigating tissue damage and immunopathology. To achieve this, IL-10 inhibits the synthesis of pro-inflammatory cytokines. These results are consistent with prior studies [15] and [16], which also reported an upregulation of IL-10 in liver cancer. IL-10 can exert antitumor effects through mechanisms such as the activation of natural killer (NK) cells, T cells, macrophages, and the production of nitric oxide. However, high concentrations of IL-10 may indicate immune system deregulation, as elevated levels of IL-10 can allow tumors to escape immune detection [17]. Furthermore, treatment with NDEA resulted in increased IL-6 levels in the kidneys, while the administration of silymarin and sorafenib led to a reduction in IL-6 levels, as shown in Figure 5.

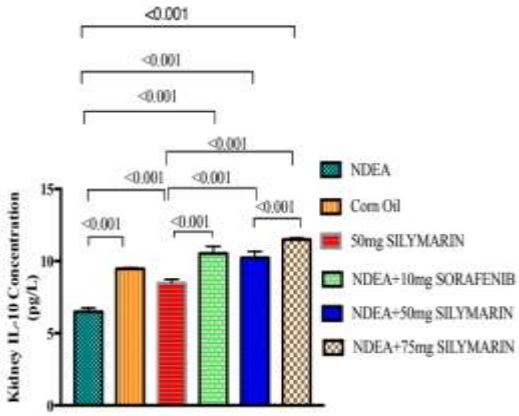

Figure 4: Effect of NDEA on kidney IL-10 concentration and the ameliorative effect of silymarin in male Wistar rats. Values are statistically significant at $p < 0.05$

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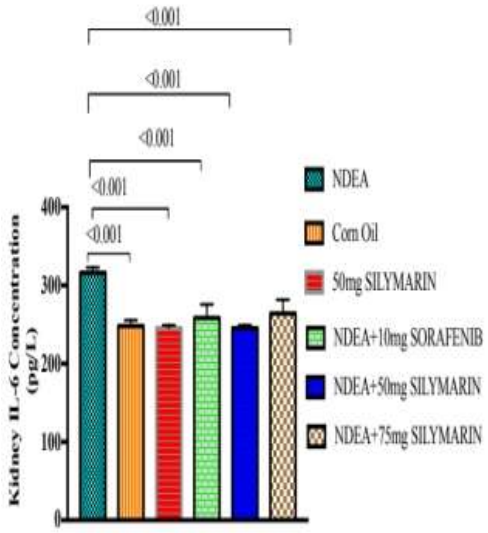

Figure 5: Effect of NDEA on kidney IL-6 concentration and the ameliorative effect of silymarin in male Wistar rats. Values are statistically significant at $p < 0.05$.

IV. CONCLUSION

This study demonstrates that NDEA induces significant renal damage characterized by elevated MDA and LDH levels, activation of NF-κB signaling, increased IL-6, and reduced IL-10, all of which contribute to oxidative and inflammatory kidney injury. Silymarin markedly counteracted these alterations by reducing lipid peroxidation, stabilizing cellular integrity, and suppressing inflammatory pathway activation. Overall, the findings validate the nephroprotective potential of silymarin against NDEA-induced renal toxicity and highlight its relevance as a promising therapeutic agent in conditions associated with oxidative stress and inflammation.

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