Plagiarism report

by Rabia Azmat

Submission date: 18-Jun-2022 01:55PM (UTC+0500)
Submission ID: 1858949390
File name: Rabia_noor_Plagiarism_file.docx (598.33K)
Word count: 2506
Character count: 14248

Abstract

Nonylphenol (NP) is a serious environmental pollutant and is recognized for its hazardous effects on humans and animals. NP induces oxidative stress that leads to organ toxicities i.e., hepatic toxicity. However, diosmetin (DIOS) is a naturally occurring bioflavonoid, present in leaves of legume, spermin and citrus fruits and possesses several biological properties such as antioxidant, anti-inflammatory and hepatoprotectivity. The present research was designed for the evaluation of curative effects of DIOS against nonylphenol prompted hepatotoxicity in rats. 32 male albino rats were randomly categorized in 4 groups as control, NP (50 mg/kg), NP+DIOS (50 mg/kg+100 Mg/kg) and DIOS (100 mg/kg) group. Our results revealed that NP instigated substantial reduction in catalase (CAT), glutathione, peroxidase (GPx) superoxide dismutase (SOD), glutathione S-transferases (GST), glutathione reductase (GSR) and glutathione (GSH) activities, were detected. Moreover, NP administrationescalated the levels ofmalondialdehyde (MDA) with a concomitant escalation in reactive oxygen species (ROS) along with raised nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6) levels and cyclooxygenase-2 (COX-2) activity. Whereas, NP increased impairment and histopathological damage in liver. While, DIOS considerably reversed all the toxicological disorders induced by NP. The current study revealed that the DIOS have the aptitude to ameliorate hepatotoxicity induced by the NP in rats.

1. INTRODUCTION

Due to the growing population, urbanization and uncontrolled industrialization, the environmental pollution has become a serious issue in recent years (Liu et al., 2020). The nonylphenol (NP) is among the most frequent encountered persistent organic pollutants (POPs) present in the environment (Guo et al., 2021). NP is extensively utilized in the formulation of oil additives for lubrication, plastic items, cleansing agents, dyes, surfactants, cosmetic items, and resin (Jubendradass et al., 2012).Domestic and industrial wastewater discharge or agricultural and urban runoff, can introduce NP into aquatic environments (Sengul and Cevdet, 2017). In several countries, it has been reported in soil, river water, sediments and terrestrial and aquatic biota (Soares et al., 2008).

Oral ingestion, inhalation, and skin contact are the most common ways for humans to be affected by NP (Chokwe et al., 2017). NP can induce organ toxicities such as, brain (Aydogan et al., 2008), pancreas (Li et al., 2017), testis (Ijaz et al., 2021) and in liver (Kazemi et al., 2016). The NP administration prompts oxidative stress in rat's liver (Ke et al., 2021). Reactive oxygen species (ROS) production led to oxidative stress (OS) for instance, hydroxyl radicals, hydrogen peroxide and superoxide (McMillian et al., 2004)that causes an imbalance between anti-oxidants and pro-oxidants that leads to hepatotoxicity. NP exposure can cause disruption in liver parenchyma (Kazemi et al., 2016), ballooning (Kourouma et al., 2015), areas with congestion, vacuolar degeneration (Shirdel et al., 2020) and steatosis in the liver.

Flavonoids are known for their pharmacological and biological properties (Cruz et al., 2017). Flavonoids have been reported as an essential component in many nutraceutical, pharmaceutical, medicinal, and cosmetic items because they exhibit wide range of health-promoting properties (Panche et al., 2016). Diosmetin (DIOS), a bioflavonoid known as 3, 5, 7-trihydroxy-4-methoxy flavone, is abundantly present in spermin, citrus fruits, legume leavesand certain medicinal herbs, including *Rosa agrestis, Origanumvulgare, Lespedeza davurica, Chrysanthemum morifolium*and *Robiniapseudoacacia* (Patel et al., 2013). Diosmetin is reported to have the anti-inflammatory (Lee et al., 2020), antibacterial (Huang et al., 1992), antioxidant (Liao et al., 2014), anti-mutagenic (Wang et al., 2014), and anti-apoptotic (Yang et al., 2010) properties.

Considering the curative effect of diosmetin, the current research was carried out for the investigationof the modulating aptitude of DIOS against NP-prompted hepatotoxicity in rat's.

2. Materials and Methods

2.1 Experiment Animals

Male albino rats (n=32) albino rats were procured from the Animal house of University of Agriculture, Faisalabad's Pharmacology Department.Animals were accommodated at a standard temperature of 25±1°C under twelve hrs.light/dark cycles in an animal care facility. During experimentation, animals had openaccessibility to standard food and tap water ad-libitum. For acclimatization ratswere kept in laboratory environment for about one week before treatment. The Department ethical board of University of Agriculture, Faisalabad, approved all the protocols.

2.2. Experimental design

Thirty-two rats wereestrangedin four groups (n = 8/group) and housedin separate steel mesh cages. Tap water and feed were provided to group I (control group) rats throughout the trial. Group II was treated with NP dissolved in distilled water from the start of the experiment. Group III received the doses of NP along with DIOS dissolved in distilled water. Finally, group IV was treated with DIOS dose orally till the end of the trial. After 30-days of treatment, all rats were anesthetized and sacrificed. The samples of blood were taken from heart using heparinized syringes and at 3000 rpm centrifuged for almost 15 minutes for plasma removal, which was then stored at -20^{0}C for further analysis. Livers of all rats were separated, blotted, and stored at -80^{0}C for histopathological analysis to study the restorative role of diosmetin on inflammatory serum markersand oxidative stress induced by nonylphenol in liver.

2.3. Biochemical analysis

Chance and Maehly (1995) procedure with slight modifications was used to assess the CAT activity.With the method given by kakkae et al. (1984) the activity of SOD was determined. Carlberg and Mannervik (1975) approach was used to assess GSR activity. The spectrophotometric methodology published by Jollow et al. (1974) was used to evaluate the glutathione peroxidase (GPx) activity in liver tissue homogenate. The reactive oxygen species were estimated in homogenate by the process of Hayashi et al. (2007). The level of malondialdehyde (MDA) in liver tissues was determined using the procedure developed by ohkawa et al. (1978).

2.4. Estimation of inflammatory serum hepatic markers

The ELISAkits were utilized for the evaluation of level of IL-1β, IL-6, TNFα, NF-κB and activity ofCOX-2 in serum according to the guidelines of the manufacturer (Bosterbio, China).

2.5. Estimation of apoptotic markers

Cusabio ELISA kits were used to investigate pro-apoptotic and anti-apoptotic indicators such as Bcl-2, Bax, caspase-9, and caspase-3.

2.7. Histopathological analysis

Histopathological examination were performed to assess NP-induced damage by the method of Fukuzawa et al. (1996). Initially, samples of liver tissue were bathed gradually in chilled saline of 0.9 percent, fixed in a 10 percent formalin solution for twenty-four hours, dehydrated in alcohol and embedded in paraffin wax. Paraffin embedded tissue's thin segments were partitioned though a microtome stained with Eosin and Hematoxylin stains and lastly studied under a light microscope at 40X (Nikon Labophot, Japan).

3. Results

3.1. Ameliorative effect of DIOS on antioxidant markers

Antioxidant potential of DIOS on NP-induced reductions in antioxidant enzymes e.g. CAT, GSH, SOD, GST, GSR and GPx, activities are illustrated in Table 1. Furthermore, NP administration indicated substantial ($p < 0.05$) attenuation in SOD, GSH, CAT, GPx, GST and GSR activities compared with control. Treatment with DIOS along with NP remarkably ($p < 0.05$) increased the activities of antioxidant serum markers in liver when compared to NP intoxicated group. There was no significance difference in the antioxidant enzymes activities in DIOS administered group and control group.

3.2. Ameliorative effect of DIOS on ROS and MDA in serum

The level of MDA, was increased considerably ($p < 0.05$) in NP group when contrasting with control group (Table 2). The ROS also exhibited a similar trend, significant ($p < 0.05$) increase in NP administered group in contrast to control group, was noticed. In comparison to the NP-treated group, co-treatment with DIOSmarkedly ($p < 0.05$) decreased ROS and MDA level in the liver

homogenate. And, no significant difference in ROS and MDA levels have been seen in the DIOS-treated group compared with control.

3.3. Ameliorativeeffect of DIOS on inflammatory markers

The anti-inflammatory effect of DIOS on NP prompted toxicity increased inflammatory hepatic serum markers levels such as NF-κB, IL-1β, TNFα, IL-6 and COX-2 activity are demonstrated in (Table 3). Moreover, NP treatment indicated a substantial ($p < 0.05$) escalation in inflammatory serum markers activities compared with control. In NP + DIOS, co-treatment group a considerable ($p < 0.05$) decrease in these inflammatory mediator's levels was seen than the NP administered rats, however, their concentrations were still significantly greater ($p < 0.05$) than the control group. While, in only DIOS treated group the mean values were similar to control group.

3.4. Ameliorative effect of DIOS on apoptotic markers

NP + DIOS effect on apoptotic and anti-apoptotic markers in liver are shown in Table 4. NP-intoxicated group had significantly ($p < 0.05$) higher level of the apoptotic markers (Bax and caspase-3) than control group, while level of anti-apoptotic marker, Bcl-2, was significantly ($p < 0.05$) reduced. DIOS co-treatment with NP, considerably ($p < 0.05$) reduced the levels of Bax, caspase-3, and caspase-9, but Bcl-2 levels were dramatically ($p < 0.05$) higher than in the NP group. In DIOS only treated group the mean values of apoptotic markers were similar to control group.

3.5. Effects of NP and DIOS on liver histopathology

For the estimation of effect of NP hepatotoxicity the hematoxylin and eosin staining was used. The histopathological analysis pointed out that the NP treatment induced disruption in the liver parenchyma, inflammatory cells infiltration, vacuolization, nucleus aggregation and necrosis as compared to control group. But, the above damages were lessened in the rats liver co-treated with DIOS and NP. In the DIOS treated rats group the histological patterns were almost normal.

4. Discussion

The present study examined the hepatoprotective effect of DIOS against NP-prompted toxicity in rats. The current research exposed that NP attenuated antioxidant enzymes together with GPx, CAT, SOD, GSR, GST and GSH activities, while elevated the ROS and MDA level in liver. Antioxidant enzymes act as the first line defense by minimizing the ROS generation and protecting the biological molecules (DNA, proteins and lipids) from the oxidative stress (Ighodaro and Akinloye, 2018). CAT and SOD are two most vital antioxidant enzymes present in human body for scavenging free radicals (Xia et al., 2015). SOD catalyzes the formation of $H_2O_2$from highly reactive superoxide anion. H_2O_2 produced inside cells is then converted into H_2O and O_2 by CAT, which inhibits H_2O_2 from being converted to a more active species like hydroxyl radical (Yang et al., 2017). Mostly in pathogenesis of various liver ailments, the oxidative stress and high LPO play a significant part (Zhao et al., 2010). The NP induced hepatic toxicity by increasing H_2O_2, which has elevated the MDA and ROS levels that resulted in decreased antioxidant index. However, Treatment with DIOS antioxidant enzyme activities were considerably recovered by attenuation of MDA and ROS levels in rat liver.

Our study also revealed that the NP prompts activation of NF-κB, along with inflammatory cytokines including TNFα, IL-1β, IL-6 and COX-2 levels. TNFα andIL-1B and are two important pro-inflammatory cytokines that are activated by inflammation and oxidative stress (Dong et al., 2014). In the inflammatory response, NF-κBis major transcription factor for the gene expression. IκB phosphorylation, activates NF-κB and ultimately NF-κB is separated from the inactive cytoplasmic complex. IκB is phosphorylated via inhibitory kappa B kinases (IKKs) causing it to be ubiquitinated and degraded by proteasomes. The IκBα degradation triggers NF-κBrelease, prompting translocation of activated NF-κBin nucleus, resulting in inflammation. NP elevated the activity of COX-2 and many cytokines levels implicated in inflammation, such as IL-6, IL-1βand TNFα, along with NF-κB activation (Subbaramaiah and Dannenberg, 2003). Our findings revealed that co-treatment with DIOS stopped the NF-κB production and alleviatedthe concentrations of IL-6, IL-1β, TNFα and COX-2.

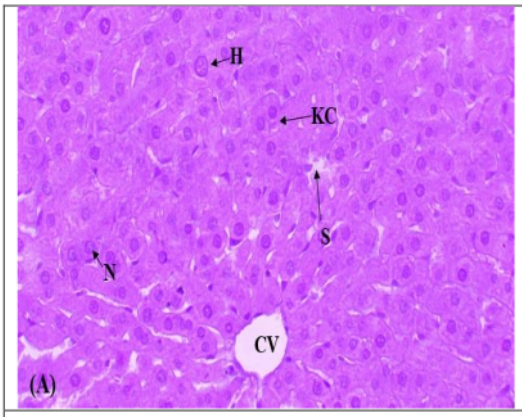
An excessive amount of ROS may trigger apoptosis and OS. As a result, apoptosis is important in hepatotoxicity (Zhang et al., 2003). Our findings revealed that NP exposure augmented the levels of caspase-3, Bax and caspase-9 and reduced the levels of Bcl-2. The present research demonstrated that NP exposure led to OS, leading to caspase-3 activation, resulting in apoptosis. Apoptosis is induced by various signals that change mitochondrial membrane permeability (particularly oxidative stress), this causes mitochondrial intermembrane proteins like cytochrome c to be released (Budihardjo et al., 1999). Apaf-1 and procaspase-9 create a complex that activates caspase-9, a pro-apoptotic enzyme released from the outer mitochondrial membrane to the cytoplasm. Apoptosis is triggered by activated caspase-9, which in turn activates caspase-3 (Arya et al., 2011). Bax is a protein with pro-apoptotic properties. Whereas, Bcl-2 is an anti-apoptosis protein (Klanova et al., 2022). Our results indicates that DIOS treatment increased the production of anti-apoptotic protein Bcl-2 while down-turning the level of apoptotic markers Bax and caspase-3. These finding suggested that DIOS had an anti-apoptotic impact against NP, which could be attributable to regulation of these apoptotic markers.

Our histopathological findings indicatedthat NP administration disturbs balance between antioxidant enzymes and ROS which resulted inOS induction inrats liver. NP increased lipid peroxidation in liver tissues which leads to morphological defects. The evident hepatic damage documented in NP-treated group were disruption in liver parenchyma,swelling of supporting and

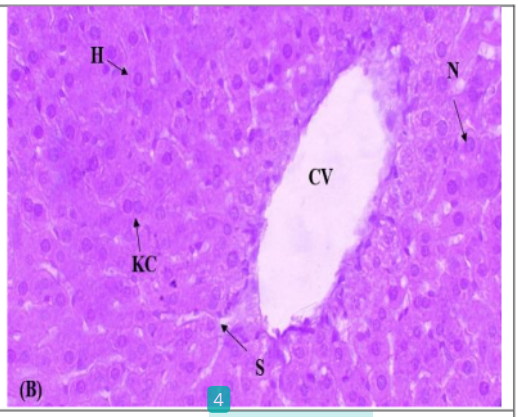
connective tissues, necrosis, nucleus aggregation, inflammatory cells infiltration and vacuolization. However, the above damages were mitigated in the rats liver co-treated with DIOS. It is possible that the antioxidant, anti-inflammatory, and anti-apoptotic characteristics of DIOS are responsible for its restorative effects.

Conclusion

In conclusion, our investigation demonstrated that DIOS exhibits strong protective efficacy against oxidative stress that is an integral part of NP induced hepatic damage. The levels of inflammatory serum markers, hepatic serum markers, activities of endogenous antioxidant enzymesand histological anomalies were all successfully restored with DIOS. Thetherapeutic potential of DIOS is linked with its hepatoprotective, antioxidant and anti-inflammatory activities.



(A) Control



(B) NP (50 mg/kg)

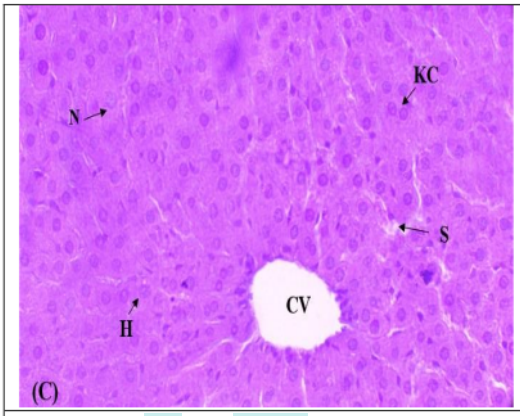
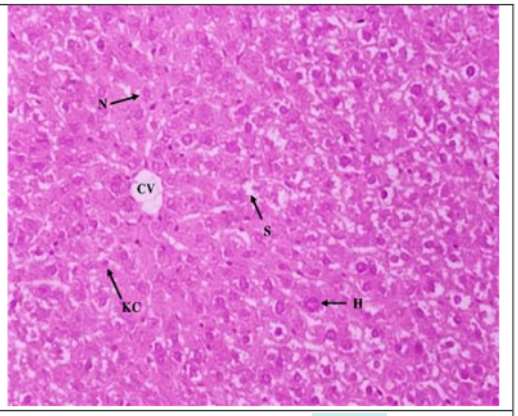
	
(C) NP (50 mg/kg) + DIOS (100 mg/kg)	**(D) DIOS (50 mg/kg)**

Table 1. Impact of NP and DIOS on antioxidant markers in rat's liver.

Table 2. Impact of NP and DIOS on MDA and ROS in rat's liver.

Table 3.Impact of NP and DIOS on inflammatory serum markers in rat's liver.

Table 4. Impact of NP and DIOS on apoptotic markers in rat's liver.

Groups	ROS (U/mg tissue)	MDA (nmol/mL)
Control	1.26 ± 0.06^{c}	0.63 ± 0.06^{c}
NP	5.66 ± 0.12^{a}	1.91 ± 0.11^{a}
NP + DIOS	1.84 ± 0.15^{b}	1.02 ± 0.08^{b}
DIOS	1.25 ± 0.06^{c}	0.63 ± 0.05^{c}

Means of Control, NP, NP + DIOS and DIOS groups that do not have similar letter are substantially different.

Groups	NF-κB	TNFα	IL-1β (ng/mL)	IL-6(ng/mL)	COX-2 (ng/L)

Control	14.5 ± 0.65[a]	6.63 ± 0.51[a]	22.93 ± 1.03[a]	6.49 ± 0.39[a]	24.55 ± 0.87[a]
NP	61.03 ± 4.32[b]	17.40 ± 0.81[b]	81.91 ± 186[b]	25.83 ± 1.19[b]	78.76 ± 3.47[b]
AUC +NP	23.90 ± 1.13[a]	10.77 ± 0.60[a]	33.17 ± 0.92[c]	13.60 ± 0.33[c]	34.88 ± 1.14[c]
AUC	14.04 ± 0.56[a]	8.64 ± 0.45[a]	22.50 ± 1.54[a]	6.43 ± 0.38[a]	24.18 ± 1.11[a]

Means of Control, NP, NP + DIOS and DIOS groups that do not have similar letter are substantially different.

Groups	**Bax (pg/mL)**	**Bcl-2 (ng/mL)**	**Caspase-3 (pg/mL)**	**Caspase-9 (pg/mL)**

Control	2.50 ± 0.26^{a}	17.74 ± 0.67^{a}	1.45 ± 0.163^{a}	3.24 ± 0.13^{a}
NP	8.9 ± 0.56^{b}	5.86 ± 0.19^{b}	13.83 ± 0.53^{b}	21.34 ± 1.38^{b}
NP + DIOS	2.99 ± 0.17^{c}	14.81 ± 0.67^{c}	3.16 ± 0.23^{c}	5.56 ± 0.22^{c}
DIOS	2.44 ± 0.18^{a}	17.78 ± 0.74^{a}	1.38 ± 0.17^{a}	3.21 ± 0.14^{a}

Means of Control, NP, NP + DIOS and DIOS groups that do not have similar letter are substantially different.

Plagiarism report

ORIGINALITY REPORT

9%	5%	6%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.science.gov Internet Source	2%
2	www.scielo.br Internet Source	1%
3	Kiran Kousar Noor, Muhammad Umar Ijaz, Nazia Ehsan, Arfa Tahir et al. "Hepatoprotective role of vitexin against cadmium-induced liver damage in male rats: A biochemical, inflammatory, apoptotic and histopathological investigation", Biomedicine & Pharmacotherapy, 2022 Publication	1%
4	Hernandez, J.P.. "Gender-specific induction of cytochrome P450s in nonylphenol-treated FVB/NJ mice", Toxicology and Applied Pharmacology, 20061015 Publication	1%
5	"Biomedical Translational Research", Springer Science and Business Media LLC, 2022 Publication	<1%
	slideheaven.com	

6	Internet Source	<1%
7	T. K. Lim. "Edible Medicinal And Non-Medicinal Plants", Springer Science and Business Media LLC, 2012 Publication	<1%
8	labanimres.biomedcentral.com Internet Source	<1%
9	Tarek S. Ibrahim, Mohamed M. Hawwas, Ehab S. Taher, Nabil A. Alhakamy et al. "Design and synthesis of novel pyrazolo[3,4-d]pyrimidin-4-one bearing quinoline scaffold as potent dual PDE5 inhibitors and apoptotic inducers for cancer therapy", Bioorganic Chemistry, 2020 Publication	<1%
10	Zhuo Li, Syed Waqas Ali Shah, Qin Zhou, Xiujie Yin, Xiaohua Teng. "The contributions of miR-25-3p, oxidative stress, and heat shock protein in a complex mechanism of autophagy caused by pollutant cadmium in common carp (Cyprinus carpio L.) hepatopancreas", Environmental Pollution, 2021 Publication	<1%
11	repository.up.ac.za Internet Source	<1%
	www.ncbi.nlm.nih.gov	

12	Internet Source	<1%
13	Chi-Ho Lee, Ah-Young Kim, Chang-Won Pyun, Michihiro Fukushima, Kyu-Ho Han. "Turmeric (Curcuma longa) whole powder reduces accumulation of visceral fat mass and increases hepatic oxidative stress in rats fed a high-fat diet", Food Science and Biotechnology, 2013 Publication	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		