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Rheum Rhabarbarum L. Extract Relieved the Hepatorenal Toxicity in Pentachloronitrobenzene-Treated Rats *via*Modulating Oxidative Stress, Inflammation, and Apoptosis

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Abstract

Pentachloronitrobenzene (PCNB) is an organochlorine-fungicide that is mostly applied on soil and seeds. Many reports have proven that it is still found in soil and food product samples. This study aimed to evaluate the phytochemical profile of the Rheum rhabarbarum L. (RRL) and its protective effect against PCNB in experimental rats. The LC/MS results indicated that the RRL extract contained 18 active components identified by the LC/MS data as polyphenolic, hydroxyl stilbenes, anthraquinones, and naphthalenes compounds. The exposure to PCNB induced oxidative stress by decreasing antioxidant markers and surged MDA and NO. PCNB markedly raised the NF-κB, and caspase 3 while significantly reducing the BcL2. Moreover, liver tissues exposed to PCNB showed downregulated antioxidants-related genes (*sod1, cat, and gst*), increased pro-inflammation genes (*tnf-α, il-6, and il-1β*), and apoptosis genes. Additionally, the PCNB treatment resulted in detrimental pathological effects on the liver and kidney tissues. The simultaneous treatment of RRL and PCNB exerted hepatorenal protective effects by improving clinical symptoms in liver and renal tissues, via restoring all tested parameters, and pathological issues. Altogether, the obtained findings show that RRL extract may offer an effective strategy for ameliorating PCNB-induced hepatorenal toxicity via alleviating oxidative stress, inflammation, and apoptosis.

***Keywords*:** Hepatorenal protection, Oxidative stress, PCNB, *Rheum rhabarbarum* L.

1. introduction

Due to the uncontrolled overuse of pesticides in agriculture and the veterinary sectors, pesticide residues remain one of the most serious food/feed contaminants. Pesticide residues are still one of the core sources of food contamination. and an important worldwide contributor of health concerns for humans [1]. Pentachloronitrobenzene (PCNB), an organochlorine fungicide, is commonly applied as a seed and soil disinfectant in the agricultural and veterinary sectors. Owing to its high cumulative stability, lengthy residual activity, and inability to degrade, PCNB may easily accumulate in plants and soil, posing major threats to environmental protection and food safety, this may lead to a great potential risk to human health [2]. Although several nations began to prohibit the use of PCNB in recent years, few articles have reported the concentration of PCNB in vegetables [3], soils [4], and surface water [5]. Moreover, PCNB has been detected even in some aquatic organisms such as black trout, golden trout, and rainbow trout [6]. Furthermore, PCNB is listed as a possible carcinogen on the United States Environmental Protection Agency's Toxicity Class III chemicals list [7].

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Pesticide exposure is associated with inducing inflammation in body organs [8]. Nuclear factor kB (NF-κB) is a transcription factor involved in the inflammation process. In normal condition, NF-κB found in activation status in the cytoplasm, in stress condition NF-κB activate by release from the IκB molecules translocate to the nucleus and bind to the subunit in the DNA, and induce the proinflammatory cytokines, that are responsible for inflammation events [9].

Nowadays, herbal plants are becoming excellent sources of bioactive therapeutic components, that can exert antioxidants and anti-inflammatory activities, and exert high protect the human and animal organs against the toxic effects of various food contaminants such as pesticides [10-13], heavy metals [12, 14-16], and other food contaminants [17, 18] that can be causing oxidative stress and inflammation-related diseases.

Rheum rhabarbarum L. (RRL) belonging to the Polygonaceae family, is a known herbal medicine in traditional medication[19]. Many reports revealed that RRL contains abundant bioactive substances, including many phytochemicals groups e.g. anthraquinones, stilbenes, and flavonoids that pose several pharmacological activities including anti-inflammatory, bacteriostatic, hemostatic, lipid-lowering, and hypotensive effects [20, 21], and anticancer effects [22, 23]. This work was designed to identify the phytochemical components of RRL extract using mass chromatography and to evaluate its protective effects against the hepatorenal toxicities of PCNB-treated rats.

2. Experimental

2.1. Preparation of plant extraction

RRL rhizome was collected from an herbal store in Giza, Egypt. One hundred-gram (100 g) powder of RRL was extracted by soaking it in one liter (70% ethanol) at room temperature for forty-eight hours. Then the extract was concentrated using a rotary evaporator (Pan Chun Scientific Co.) at 30 ºC after being filtered utilizing Whatman No. 1 filter paper. The extract was freeze-dried and kept at -20 °C in hermetically glass vials. Prior to usage, the freeze-dried extract was reconstituted at a concentration of 10 mg/ml in distilled water. Weekly treatment doses of 100 and 200 mg/kg B.W. of the rat's body weight were justified.

2.2. Phytochemical analysis

The analysis of the sample was performed using liquid-chromatography electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC AC system for separation and a SCIEX Triple Quad 5500+ MS/MS system outfitted with an electrospray ionization source (ESI) for detection. The separation was performed with an Ascentis® Express 90 Å C18 Column (2.1×150 mm, 2.7 µm) was used for the separation. Two eluents A: 5 mM ammonium format pH 8; B: acetonitrile (LC grade), have been used as the mobile phases. The mobile phase gradient was conducted as follows: 5% B at 0-1 min, 5-100% at 1-20 min, 100% 20-25 min, 5% at 25.01, and 5% from 25.01-30 min. The injection volume was 5 µl, and the flow rate was 0.3 ml/min. Negative and positive ionization modes were utilized with an EMS-IDA-EPI scan from 100 to 1000 Da for MS1 for MS/MS analysis with the following parameters: curtain gas: 25 psi; IonSpray voltage: -4500; source temperature: 500°C; ion source gas 1 & 2 were 45 psi and from 50 to 1000 Da for MS2 with a decluttering potential: -80; collision energy: -35; collision energy spread: 15. Compounds' identification was performed using MS-DIAL.

2.3. Animal and Experimental Design

Forty Sprague-Dawley rats (11-12 weeks old, weighing 160-180 g) were housed in the Animal House Colony, National Research Centre, Egypt. Prior to treatment, the rats spent a week to acclimatize to the environment. All of the groups' animals received basic feed and unlimited access to water. The rats were then divided at random into six experimental groups (eight for each) and treated for three weeks. According to Kuai, Gao, Yang, Luo, Xu, Liu, Yu, Wang, Zhang and Ma [24], the dosage of PCNB (200 mg/kg B.W.) has been chosen. At the conclusion of the experiment, the body, liver, and kidney weights were all recorded. The clinical symptoms were observed daily. The change in body weight is calculated using the following equation:

Weight change (%) = [(Initial body weight - Final body weight) / Initial body weight] x 100 (1)

2.4. Sampling

On the last day of the treatment, blood samples were taken for biochemical analysis, and all rats were then put to death by cervical dislocation. During necropsies, liver, and kidney tissues were instantly dissected, and weighed to evaluate the liver and kidneys index. For histopathological examination, other sections of liver and kidney tissues were immediately immersed in formalin solution (10%).

2.5. Histopathological examination

After the head was served, the liver and kidney tissues were then taken out and put through histopathological analysis protocol. Sections that were 5 µm thick were cut using a rotary microtome and then placed on clean glass slides. The slides were stained with hematoxylin and eosin before to examined under an Olympus light microscope [25].

2.6. Kits

The aminotransferase enzymes (ALT and AST), alkaline phosphatase, superoxide dismutase, catalase, and malondialdehyde, kits were purchased from BIO-DIAGNOSTICS Co. (Cairo, Egypt). Triglycerides (TriG) and lactate dehydrogenase (LDH) were determined using commercial kits obtained by Cusabio, Wuhan, China.

2.7. Evaluation of the Oxidative Stress

The isolated serum was used to detect the malondialdehyde (MDA) as an indicator for lipid peroxidation based on the techniques of Ohkawa, Ohishi and Yagi [26]. The approach of Sun, Oberley and Li [27] was applied to determine SOD activity. According to Sun, Oberley and Li [28] technique, the activity of CAT was detected by the decay of hydrogen peroxide. While the content of nitric oxide (NO) was evaluated using the Griess reagent.

2.8. Assessment of the DNA fragmentation in liver tissues

2.9. DNA gel electrophoresis laddering procedure

Based on the methods of Majtnerová and Roušar [29], apoptotic DNA fragmentation was analyzed qualitatively by detecting the nuclear DNA fragment bands. Briefly, liver tissues were homogenized, washed in PBS, and then lysed in 0.5 mL of DNA extraction buffer overnight at 37 °C. The lysate was next treated with 100 µg/mL DNase-free RNase for 2h at 37 °C, followed by three extractions of an equal volume of phenol/chloroform (1:1 v/v) and finally re-extraction with chloroform by centrifuging at 15000 rpm for 5 min at 4 °C. The extracted DNA was precipitated in two volumes of ice-cold 100% ethanol with a tenth volume of 3M sodium acetate, pH 5.2 for one hour at −20 °C, and then centrifuged at 15,000 rpm for 15 min at 4 °C. Following a wash with 70% ethanol, the DNA pellet was air-dried and dissolved in Tri/ EDTA, pH 8.0. The DNA was then electrophoresed on 1.5% agarose gel and stained with ethidium bromide in Tris/acetate/EDTA (TAE) buffer. DNA fragments were spotted on gels using ultraviolet transillumination, and a 50-bp DNA ladder (Invitrogen, USA) was used as a molecular size marker.

2.10. Diphenylamine Reaction Procedure

To investigate the quantitative profile of DNA fragmentation of the lover tissues, liver samples were collected immediately after sacrificing the animals. The tissues were centrifuged at 10,000 rpm for 20 min at 4°C after being lysed in 0.5 mL of lysis buffer. The pellets were re-suspended in 0.5 mL of lysis buffer. Half mL of 25% tri-chloroacetic acid (TCA) was added to the pellets (P) and the supernatants (S), and they were then incubated at 4°C for 24 h. Following centrifugation for 20 min at 10,000 rpm at 4°C, the pellets were suspended in 80 mL of 5% TCA, followed by a 20 min incubation period at 83°C. Then, 160 mL of Diphenyl Amine (DPA) solution was added to each sample and it was left to sit for one day at room temperature [30].

The proportion of calculated from an absorbance measurement at a wavelength of 600 nm and the percentage of fragmented DNA was determined utilizing the formula:

Fragmented DNA (%) = [OD(S)/ [OD(S) + OD (P)] X 100(OD: optical density, S: supernatants, P: pellets).

2.11. ELISA Analysis

The levels of nuclear factor kappa B (NF-κB), Caspase 3, (Cas 3), and B-cell lymphoma 2 (BcL2) in extracted sera were examined using ELISA kits gotten from Wuhan Cusabio company, China consistent with the manufacturer's method.

Table 1

The primer sequences of the target genes

Gene description	Gene	Accession No.	Sequences (5'—3')	Amplicon size (bp)
Cu/Zn Superoxide dismutase	*SOD1*	NM_017050.1	**F:** CATTCCATCATTGGCCGTACT R: CCACCTTTGCCCAAGTCATC	62
Catalase	*CAT*	NM_012520.2	**F**: GTACAGGCCGGCTCTCACA **R**: ACCCGTGCTTTACAGGTTAGCT	57
Glutathione	*GSH*	NM_053906.1	**F:** GGAAGTCAACGGGAAGAAGTTCACTG **R**: CAATGTAACCGGCACCCACAATAAC	64
Nuclear factor-kappa β	*NF-κB*	*NM_01276711.1*	**F**: AATTGCCCCGGCAT **R**: TCCCGTAACCGCGTA	130
Tumor Necrosis Factor α	*TNF-α*	*NM_012675.3*	**F**: ACACACGAGACGCTGAAGTA **R**: GGAACAGTCTGGGAAGCTCT	
Interleukin-6	*IL-6*	*NM_012589.2*	**F**: AAGCCAGAGTCATTCAGAGCAA **R**: GGTCCTTAGCCACTCCTTCT	149
Interleukin-1β	*IL-1B*	NM_031512.2	**F**: AAATGCCTCGTGCTGTCTGA **R**: CAAGGCCACAGGGATTTTGTC	135
Caspase-3	*CAS-3*	NM_012922.2	**F**: GTGGAACTGACGATGATATGGC **R**: CGCAAAGTGACTGGATGAACC	135
B-cell lymphoma 2	*BCL-2*	NM_016993.2	**F**: GGGATGCCTTTGTGGAACTA **R**: CTCACTTGTGGCCCAGGTAT	138
Glyceraldehyde3-phosphate dehydrogenase	*GAPDH*	*NM_001394060.1*	**F**: CCACCAACTGCTTAGCCCCC **R**: GCAGTGATGGCATGGACTGTGG	91

2.12. RNA Extraction and q-PCR Application

Trizol reagent for (Invitrogen, USA) was used with liver tissue that had been processed to extract total RNA (approximately 20 mg). A Nanodrop spectrophotometer from Thermo Fisher Scientific Inc., Wilmington, DE, USA, was employed to measure the RNA's concentration. The solidity of RNA was tested by 1% agarose gel electrophoresis based on the consistency of 18S and 28S rRNA bands. The total RNA samples were processed with RNase-free DNase I (Promega, Madison, WI, USA) before being reversed to complementary DNA (cDNA) using cDNA Kit (Invitrogen, Waltham, MA, USA) according to the manufacturer's instruction. Table 1 incorporates the primer sequences for the genes that were examined. The amplification process consisted of 40 cycles of 10 min at 95 °C, 15 s at 95 °C, and 60 s at 60 °C. The relative mRNA levels were determined using the cycle threshold approach and normalized to geometric means of GAPDH as a housekeeper gene [31]. The relative mRNA level was determined using The $2^{-\Delta\Delta CT}$ approach [32].

2.13. Serum liver and kidney functions

Serum was isolated at once from blood samples by chilling centrifuging to determine liver functions, and the serum was then tested for the biochemical parameters. According to Reitman [33], Aminotransferase enzymes (ALT, and AST) were measured calorimetrically, and alkaline phosphatase (ALP) was quantified per Goldberg and Ellis [34]. Furthermore, the supernatant of liver samples was used to determine the activity of LDH based on the approach of an LDH kit (Jian Cheng Bioengineering Institute, Nanjing, China). To minimize the number of all results divided by 1000. The separated serum was used to determine the urea and creatinine as a bio-indicator of kidney functions according to the method of Hwang and Wang [35].

3. Results

3.1. Phytochemical Analysis

The identification of metabolites based on the ESI-HPLC-MS/MS was performed in positive and negative ESI mode in Rheum rhabarbarum L ethanolic extract and the data summarized in (Figure 1; Table 2,) showed that there are eighteen metabolites have been identified in the RRL extract as polyphenolic compounds, hydroxy stilbenes, anthraquinones, and naphthalenes.

Table 2
Chemical composition of the ethanolic extract from *Rheum rhabarbarum* L.according to LC-MS analysis

No.	RT (min)	Name	Molecular formula	Precursor mass(m/z)	MS/MS (m/z) fragments	Adduct
1	1.352	Gallic acid	$C_7H_6O_5$	331.0	270.98, 211, 169 125	$[M-H]^-$
2	2.922	Catechin	$C_{15}H_{14}O_6$	289.0	289, 245, 221, 203, 151, 137	$[M-H]^-$
3	5.400	Resveratrol glucoside	$C_{20}H_{22}O_8$	389.0	269, 227, 185	$[M-H]^-$
4	6.957	Resveratrol O-galloylglucosid	$C_{27}H_{26}O_{12}$	541	313, 169, 227, 379, 241, 389	$[M-H]^-$
5	7.181	Rhapontigenin O-glucoside	$C_{21}H_{24}O_9$	419	257, 241, 281, 299, 401, 225	$[M-H]^-$
6	7.412	Gentisin glucoside	$C_{13}H_{16}O_9$	418.9	257, 241, 224	$[M-H]^-$
7	7.671	Apigenin-8-C-glucoside	$C_{21}H_{20}O_{10}$	431.2	430.96, 311, 268.97	$[M-H]^-$
8	7.731	Rhein	$C_{15}H_8O_6$	283.0	239, 211, 183	$[M-H]^-$
9	7.789	Rhapontigenin O-galloylglucoside	$C_{28}H_{27}O_{13}$	571.0	313, 556, 169. 257,327,409, 419	$[M-H]^-$
10	8.570	Quercetin	$C_{15}H_{10}O_7$	300.96	301, 273, 179, 151, 121	$[M-H]^-$
11	9.348	Torachrysone O-glucoside	$C_{20}H_{24}O_9$	407	245, 230, 215	$[M-H]^-$
12	9.587	Deoxyrhapontigenin O-galloylglucoside	$C_{28}H_{27}O_{12}$	555	241, 265, 226, 161, 283, 253	$[M-H]^-$
13	9.971	Resveratrol dimer 1	$C_{28}H_{21}O_6$	453	359, 347, 435, 369, 411, 333, 289	$[M-H]^-$
14	10.449	Physcion	$C_{16}H_{12}O_5$	283.1	283, 268, 239.9, 212	$[M-H]^-$
15	10.798	Resveratrol dimer 2	$C_{28}H_{21}O_6$	435	435 ,369, 411, 409, 333, 347	$[M-H]^-$
16	18.518	Emodin	$C_{15}H_9O_5$	269.0	269, 241, 225, 197	$[M-H]^-$
17	22.684	Chrysophanol	$C_{15}H_{10}O_4$	253.2	253, 225, 197, 181	$[M-H]^-$
18	22.686	Chrysophanol O-glucoside	$C_{21}H_{20}O_9$	415	253, 225, 295, 277, 175, 267	$[M-H]^-$

Rt= retention time

3.2. Histopathological Findings

Figure 2 represents the photomicrographs of the liver of rats treated with RRL and PCNB. In the control rat, no visible histopathologic change was observed (A). Moreover, the liver sections of the RRL-treated groups at both high and low doses (100 and 200 mg/kg bw) showed normal unaffected architecture (B and C). Otherwise, the liver section of PCNB-treated rats showed mild inflammation, hepatocytes cytoplasmic vacuolar degeneration with pyknotic nuclei (arrow), and increased Kupffer cells (D). Foci of cellular necrosis, strongly acidophilic cytoplasm with frequent apoptotic cells (E). Whereas the liver section of PCNB plus RRL-treated rats at a low dose (F) revealed slight improvement, and the liver section of PCNB plus RRL-treated rats at a high dose (G) restored the tissue to nearly normal. (H&E ×300).

Additionally, figure 3 represented photomicrographs of the kidney of rats treated with RRL and PCNB. In the control rat, no visible histopathologic change was observed (A), renal cortex of control rats had well-developed glomerulus with normal tubular cells (inset), In the same regard, the renal cortex of rats treated with a low dose of RRL (B) revealed nearly normal tubules and some injured glomeruli. The renal cortex of rats treated with a high dose of RRL (C) disclosed most of the tissue structures are nearly normal with foci of affected tubules. The renal cortex of rats treated with PCNB (D) showed mild interstitial fibrosis and different degrees of tubular changes vascular degeneration and pyknosis in their epithelial cells (arrow), Moreover, the kidney section of PCNB plus

RRL-treated rats at the two tested doses (F and G) PCNB-exposed rats treated with a low or high dose of RRLshowing minimum histological changes in renal tubules or corpuscles. (H. & E; X 300).

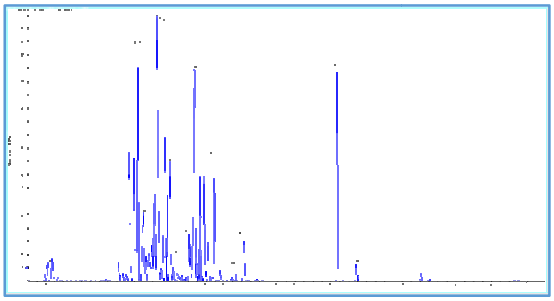

Figure1: Base Peak Chromatogram of *Rheum rhabarbarum* L. root ethanolic extracts.

3.3. RRL extract improves the body weight in PCNB-treated rats

No mortality happens between the experimental rats during the treatment period. Rats treated with PCNB showed some clinical signs such as weakness, slight hair loss, and a lessening in appetite. While rats in groups treated with RRL extract either alone or together with PCNB showed normal behavior and good health. The data in Figure 4 exemplify the effects of PCNB, and RRL on the percent of body weight change. The body weight of animals treated with PCNB reduced significantly compared to it in control rats. In the meantime, the supplementation of RRL extracts at two doses led to momentous improvements in body weight gain compared with the weight gain of rats treated with PCNB ($p \leq 0.05$). Interestingly, the treatment with the low dose was more effective in improving body weight gain either alone or in combination with PCNB.

3.4. RRL Extract Diminished Lipid Peroxidation and Improved Antioxidant Enzymes

Data in Figure 5 showed that PCNB treatment significantly heightened the serum MDA level compared to its level in the control group. Otherwise, the concurrent treatment of PCNB with the RRL extract at two tested doses meaningfully lessened the level of serum MDA compared to its level in PCNB-treated rats. Indeed, the RRL extract did not cause any significant change in MDA level compared to the control group.

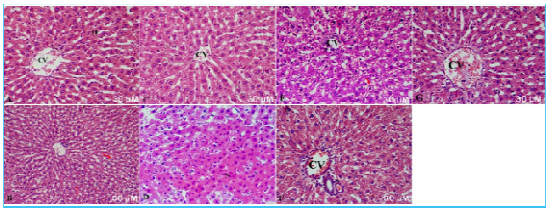

Figure 2: Histopathologic features of rat's liver treated with exposed to PCNB and/or RRL extract at two (100 and 200 mg/kg BW) Control (A), RRL extract at (100 mg/kg BW), (C) RRL extract at (200 mg/kg BW), (D and E) PCNB, (F) PCNB+ RRL extract at (100 mg/kg BW), (G) PCNB+ RRL extract at (200 mg/kg BW).

3.5. RRL extract alleviated DNA damage in liver tissues

The level of fragmented DNA in the liver tissues of rats treated with PCNB with and without RRL extract at the two low and high levels is shown in Figure 6. The rates of DNA fragmentation in the groups of rats treated with both low and high dosages of RRL were quite similar to their rates in the untreated control groups. In contrast, DNA fragmentation in the group of rats treated with PCNP was significantly increased ($P<0.01$) compared to the control group. Furthermore, the simultaneous treatment with PCNB and RRL extract at (200 mg/kg bw) decreased the DNA fragmentation considerably ($P<0.05$) compared to it in the group of rats treated with PCNB alone. The mitigation effect against PCNB-induced DNA fragmentation in PCNB+ RRL at a low dose was higher than it was in PCNB+ RRL at a high dose with no significant difference.

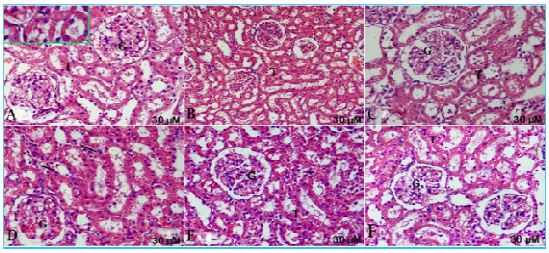

Figure 3: Histopathologic features of rat's kidney treated with PCNB and/or RRL extract at two (100 and 200 mg/kg BW) Control (A), RRL extract at (100 mg/kg BW), (C) RRL extract at (200 mg/kg BW), (D and E) PCNB, (F) PCNB+ RRL extract at (100 mg/kg BW), (G) PCNB+ RRL extract at (200 mg/kg BW).

3.6. RRL extract restored serum NF-κB, Caspase 3, and BcL2 in PCNB-treated rats

The rats exposed to PCNB alone showed significant elaboration in serum NF-κB and caspase 3, in contact led to a significant decline in the serum

Bcl-2 level compared to their control level. Otherwise, the concurrent treatment with RRL extract and PCNB significantly reduced NF-κB and caspase 3 levels and enhanced Bcl-2 compared to their levels in the PCNB-treated group (figure 7).

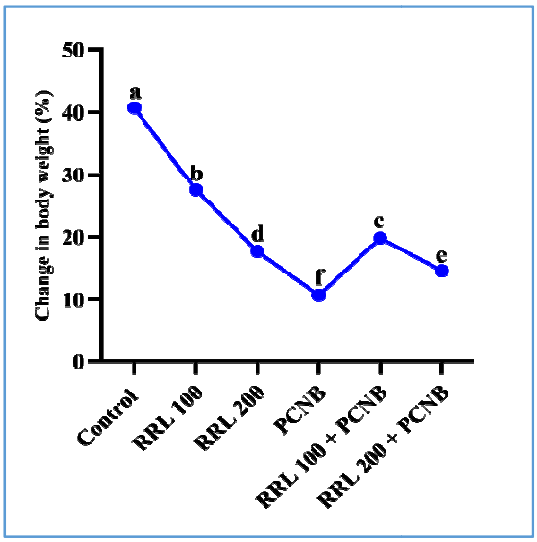

Figure 4: Change in body weight (%) of rats treated with *Rheum rhabarbarum* L (RRL) extract and Pentachloronitrobenzene (PCNB)at the end of the experiment. The different letters represent statistically significant differences ($p < 0.05$) between treatment and control.

3.7. RRL extract mitigated the expression of sod1, cat, gst, tnf-α, il-6, il-1β, casp3, and bcl-2 following to treated with PCNB exposure

The concise data in Figure 8 revealed that the oral administration of PCNB adversely affects antioxidants, inflammation, and apoptosis-related genes. Wherein the treatment of the PCNB led to a significant decline in the expression of sod1, cat, and gst, (antioxidant-related genes) compared to their expression levels in control rats. Moreover, the treatment of the PCNB caused a substantial elevated in the expression of pro-inflammation-related genes (*tnf-α, il-6, and il-1β*) assimilated to their expression level in the control rats. Additionally, the treatment of the PCNB triggered a noteworthy disruption in the expression of apoptosis-related genes, it resulted in a significant increase in the expression of the caspase 3 gene and a decline in the expression of the bcl-2 gene when compared to their expression level in the control rats. Variously, the coincidental treatment with PCNB and RRL restored the expression level of the mentioned tested genes at the tested two doses of RRL extract compared to PCNB alone. Notably, the mitigation impact of RRL extract at the high dose was pointedly higher than that was triggered by the low dose on the expression of bcl-2 genes.

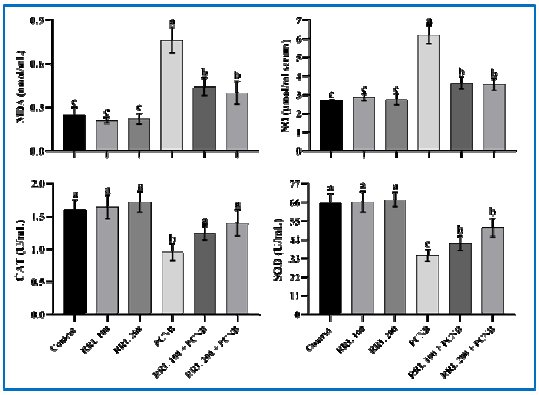

Figure 5: Biomarkers of serum oxidative stress in different experimental groups. Data are shown as mean ± SD ($n=8$). RRL= *Rheum palmatum L.* extract; PCNB= Pentachloronitrobenzene; MDA = malondialdehyde; SOD = superoxide; dismutase; CAT = catalase. The various letters indicate statistically significant differences between the treated groups and the control group ($p < 0.05$).

3.8. RRL extract improved the liver and kidney functions biomarkers

The current finding revealed that PCNB treatment resulted in a significant surge of serum liver and kidney function markers when compared to the control group. Whereas, the treatment with the PCNB plus RRL extract at once at two ominously succeeded in restoring the liver and kidney function markers toward their vales in the PCNB-treated group. While the treatment of the RRL extract alone at both of the two mentioned doses did not cause any significant difference in liver and kidney function markers when compared to the observed value in the control group (Figures 9 and 10).

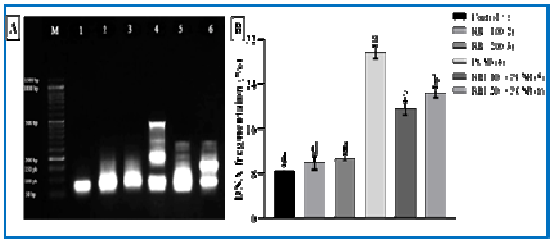

Figure 6: DNA fragmentation detected in liver tissues of rats exposed to PCNP and/or RRL extract at (100 and 200 mg/kg BW). (A) Agarose gel of DNA fragmentation extracted from liver tissues of experimental rats Line M represents DNA marker. Lines (1 to 6) represent the experimental groups. (B) DNA fragmentation detected by Diphenylamine reaction.

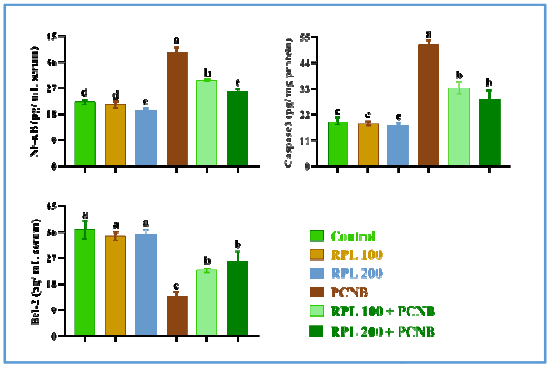

Figure 7: Effects of PCNB with or without RRL extract on the serum NF-kB, Caspase 3, and Bcl-2 protein level. Data represented as mean ± SE (*n=5*). RRL *Rheum palmatum L.* extract; PCNB= Pentachloronitrobenzene. The various letters represent statistically significant differences ($p < 0.05$) between the treated groups and the control group.

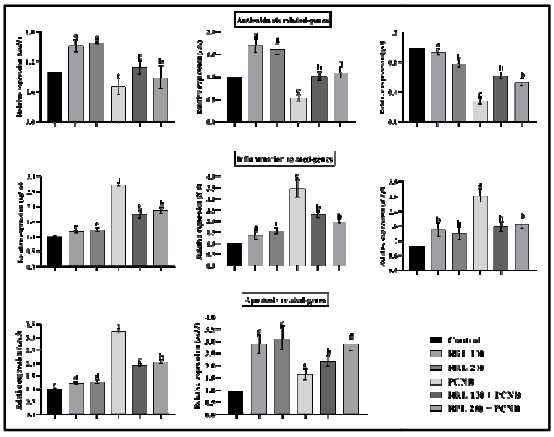

Figure 8: Relative gene expression of *sod1, cat, gst, tnf-α, il-6, il-1β, casp3, and bcl2* in different experimental groups. Data represented as mean ± SE (*n=5*). RRL *Rheum palmatum L.* extract; PCNB= Pentachloronitrobenzene. The various letters represent statistically significant differences ($p < 0.05$) between the treated groups and the control group.

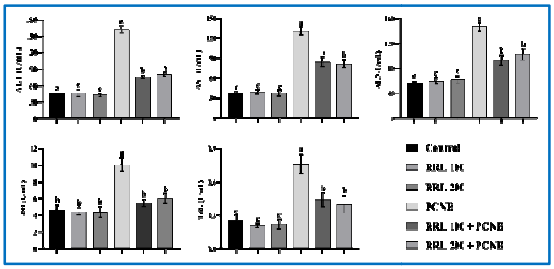

Figure 9: Biomarkers of Liver injury in various experimental groups. Data are shown as mean ± SE (*n=5*). RRL= *Rheum palmatum L.* extract; PCNB= Pentachloronitrobenzene. ALT= Alanine amino transaminase, AST= aspartate aminotransaminase, ALP alkaline phosphatase, LDH= Lactate dehydrogenase; TriG= Triglycerides. The various letters stand for the statistically significant differences ($p < 0.05$) between the treated groups and the control group.

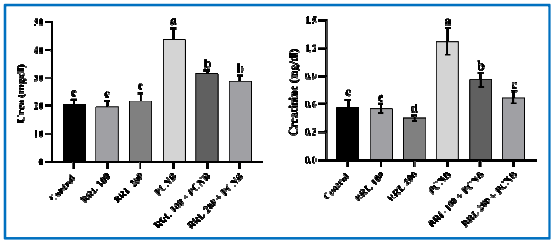

Figure 10: Kidney functions in different experimental groups. Data represented as mean ± SE (*n=5*). RRL= *Rheum palmatum L.* extract; PCNB= Pentachloronitrobenzene. The various letters represent the statistically significant differences ($p < 0.05$) between the treated groups and the control group.

4. Discussion

Although chemical pesticides are synthesized to be used to support agricultural, veterinary, and public health sectors, they negatively impact non-target living organisms and cause many toxic effects and even death [36]. In recent years, epidemiological shreds of evidence indicate that the prevalence of diseases associated with depletion of the liver and kidney functions increases exponentially particularly in developing countries or in regions in which pesticides have been excessively applied in the past [37, 38]. Nevertheless, PCNB is toxic for non-target organisms and frequently detectable around the world in surface water, vegetable, and medicinal plants, limited reports have focused on its hepatic and renal toxicity [39, 40].

Based on the current observation, it can be concluded that PCNB treatment had adversely affected body weight growth compared to the control group at the end of the experiment. The lowering in body weight due to the toxic effects of PCNB may be attributed to the oxidative stress effects of these pesticides that endanger animals, lessen appetite, and result in body weight loss [41]. These findings were in the same line as those of [42]. Many other nitrobenzene pesticides induced lowering in rats' body weight as well [43]. In contrast to rats treated with PCNB alone, the concurrent treatment with PCNB and RRL at two dosages improved the body weight. This effect was similar to the effects published by [44, 45], who stated that the extract of R. rhabarbarum considerably improved the body weight in animals when given 5, 10 and 15% of R. rhabarbarum extract combined with CCL_4 when compared to the body weight of rats treated with

CCL_4 only. Due to the generation of reactive oxygen species (ROS) during PCNB metabolism, the liver and kidneys are prone to oxidative stress-induced damage [46].

Oxidative stress caused by free radicals has been linked to hepatocyte degeneration, inflammation, and apoptosis [47]. ROS molecules are extremely active and play a crucial part in cell functioning, but they are also linked to disease [48]. The current results revealed that the PCNB treatment instigated numerous pathological events in renal and hepatic tissues. In the hepatic tissues, PCNB induced inflammation, hepatocytes cytoplasmic vacuolar degeneration with pyknotic nuclei, increased Kupffer cells, apoptotic, and necrosis. PCNB-induced caused mild interstitial fibrosis in the renal tissues, as well as varying degrees of tubular changes vascular degeneration, and pyknosis in their epithelial cells. Pathological events in liver and kidney tissues are attributed to the oxidative stress resulting from the metabolism of PCNB [49].

In contrast, the treated with RRL at two experimental doses at the same time with PCNB relived the histopathological signs in liver and kidney tissues at the end of the experiments, these results are consistent with those of [50], they found that Rheum *palmatum* L. extract reviled thickened envelopes and fibrosis of the portal area in the liver of treated rats. In addition, the Rheum ribes relieved cisplatin-induced nephrotoxicity in rats [51].

Furthermore, the current results revealed that the exposure to PCNB induced DNA damage, increased level of MDA, and decrease activities of SOD and CAT in the liver tissues of experimental rats, while its comment treatment with RRL resulted in significant mitigation of DNA damages, decreased the lipid peroxidation, and enhanced the antioxidants enzyme activities at two experimental doses. These findings corroborated those of [52-54], who found that RRL mitigated oxidative stress and alleviated the antioxidant status.

Moreover, the current findings indicated negative effects of PCNB on the antioxidants related genes (*sod1- cat, and gst*), inflammation-related genes (*tnf-α, il-6, and il-1b*), and apoptosis-related genes (*cas-3, and bcl-2*), May this effects caused by oxidative stress that induced as a result of the toxicity of PCNB. Many previous pieces of research publicized that the same effects of the BCNB have been noted in rats treated with other pesticides [55-57].

Otherwise, the simultaneous treatment of PCNB–treated rats with RRL extract led to significant improvement in the expression of all tested genes. Additionally, the present observations demonstrated that after being treated with PCNB, significant adverse effects have taken place in the biomarkers of serum liver (ALT, AST, ALP, LDH, and TriG) and kidney functions. These findings concur with those reported by Koegel, Mueller, Coulston, Korte and Chemistry [58], Tao, Yinglin, Hong and Yingjie [59]. A high level of AST, ALT, and ALP in the bloodstream indicates damage to the cell membrane of hepatic cells, which results in altered cellular permeability [60, 61], whereas a high level of serum LDH implies hepatic necrosis [62, 63].

The aminotransferase enzymes are recognized to play critical roles in coordinating many cellular physiological functions, promoting transamination reactions to facilitate xenobiotic detoxification, and regulating several metabolic processes [64, 65]. Triglyceride is also a biomarker for liver toxicity, which is an indicator of the alteration of fat metabolism [66]. In the same trend, the alleviation of the kidney function biomarkers in response to the treatment of PCNB was noted at the end of the experiment. The release of urea and creatinine into the blood circulation is a prominent sign of kidney toxicity [67, 68]. Overproduction of free radicals as a result of exposure to environmental and food hazards can lead to a reduction in the amounts of cellular antioxidants, disrupting redox equilibrium. Our findings indicated that PCNB therapy caused oxidative stress in the liver tissues of treated rats, as evidenced by an increase in MDA and NO activity as oxidative stress biomarkers [69].

Consequences, the PCNB induced the NF-κB, it's a transcription factor exist in the cytoplasm by binding to the protein, but under the oxidative stress conditions translocate to the nucleus and bind to subunit in DNA to induce the pro-inflammatory cytokines [70]. In the present investigation, the exposure to PCNB led to significant increase of NF-κB and release the pro-inflammatory cytokines to the blood stream. Otherwise, RRL extract treatment relieved the inflammatory via declining the NF-κB. On the same regards, the exposure to PCNB led to significant increase the pro-apoptotic protein (caspase 3) and decrease the level of anti-apoptotic protein

(Bcl-2), that may be attributed to excessive inflammation and oxidative stress conditions [71].

Altogether, the results revealed that RRL extract alleviated PCNB-induced liver and kidney injuries reflected by repairing hepatic and renal histopathology, liver DAN damages, moderated the antioxidants, inflammation, and apoptosis, and decreased the plasma kidney and liver function biomarkers. RRL treatment decreased serum MDA NO, NF-κB and caspase 3 levels and enhanced BcL2 compared to their levels in PCNB-treated group, as well as upregulated the antioxidant related genes (sod1, gpx1, and gsh), downregulated the expression of proinflammatory genes (tnf-α, il-6, and il-1β), downregulated the pro-apoptosis-related genes (casp-3 and bax) and upregulated the anti-apoptotic gen(bcl-2).

The protective effects of RRL extract are attributed to its antioxidants and anti-inflammation activities [54, 72]. The current results showed that RRL extracted contains eighteen bioactive components such as emodin, rhein, physcion, gallic acid, quercetin, catechin, and resveratrol dimmers. The phytochemical composition of RRL extract was reported to dominate polyphenolic compounds, hydroxy stilbenes [73, 74], anthraquinones, and naphthalenes [45].

Anthraquinones mainly include emodin, aloe-emodin, rhein, physcion, chrysophanol, and their derivatives [75]. Emodin belongs to anthraquinones, which are found in several plants such as Rheum spp. This component has various actions including antioxidants and anti-inflammatory [76], antibacterial [77], and anticancer activities [78]. Moreover, emodin has also been identified as having potential antiviral activity against coronaviruses [79], anti-HIV, anti-human cytomegalovirus, anti-HSV, and anti-Epstein-Barr virus activities [80]. Furthermore, rhein (4, 5-dihydroxyanthraquinone-2-carboxylic acid) has been used medicinally in China for more than 1,000 years. Rhein has many pharmacological effects, including anti-inflammatory, antioxidant, anticancer, hepatoprotective, nephroprotective, and antimicrobial activities [81, 82].

In addition, physcion belongs to anthraquinones and has a variety of pharmacological properties including anti-inflammatory, laxative, hepatoprotective, anti-microbial, and antiproliferative effects [83]. Other literature confirmed our finding; the components in the extract of RRL exerted many pharmaceutical activities like antioxidants [84-87].

5. Conclusion

In conclusion, RRL protects the liver and kidneys from PCNB- toxicity by restoring the pathological signs, decreasing lipid peroxidation and nitric oxide levels and improving the antioxidant enzymes. Additionally, the simultaneous treatment of RRL extract upregulated the antioxidants-related genes, downregulated the pro-inflammation-related genes, downregulated of caspase 3 gene, and up-regulated the anti-apoptotic gene (bcl2). Besides, the RRL extract led to a decrease in the level of DNA damage in liver tissues. As obtained results, the RRL ethanoic extract contained polyphenolic compounds, hydroxyl stilbenes, anthraquinones, and naphthalene; these components exerted antioxidants, anti-inflammation, and anti-apoptotic activities, and protected the liver and kidney in PCNB-treated rats.

6. Conflicts of interest

The authors declare no competing interests.

7. Formatting of funding sources

No funding for this work.

8. Ethics Statement

The experimental procedure and animal management were carried out through subsequent experiments approved by the Committee of Animal Ethics in the National Research Centre, Dokki, Cairo, Egypt (No:084120923).

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10. Author conurbations

MS: Investigation, Experimental carrying Data curation, software and figure creation; MS, MMA, AAE: Investigation, experimental carrying; MIMI: phytochemical analysis; WKBK, DNA fragmentation; NSH: histopathology study, AAE, Supervision, Writing -review & editing; Validation.

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