Hepatoprotective Effect of Niclosamide on Paracetamol-Induced Liver Toxicity in Rats

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Abstract: Liver diseases is considered one of the leading causes of death and an important barrier to increasing life expectancy. From among, liver fibrosis which is a result of a chronic damage to the liver leading to liver cirrhosis. Niclosamide is a potent anti-helminthic drug which has been used in treating the tapeworm infections. It also showed a hepatoprotective effect in induced liver toxicity models. The present study was conducted to identify the hepatoprotective effect of niclosamide in a paracetamol-induced liver toxicity. Rats were divided into five groups (6 rats per group): control group, disease group, 5 mg niclosamide group, 10 mg niclosamide group and 15 mg niclosamide group. Three doses of niclosamide (5 mg/kg, 10 mg/kg and 15 mg/kg) were injected intraperitoneal (i.p) for 4 weeks. Assessments included hepatic enzymes (AST, ALT, ALP and GGT), oxidative stress (MDA, GSH and SOD) and inflammatory markers (IL-6, TNF- α and NF-kB). The three doses of niclosamide (5 mg/kg, 10 mg/kg and 15 mg/kg) had significantly reduced the hepatic enzymes with the prominent effect at dose 15 mg/kg. There was also a significant decrease in the MDA activity, while significant increase in the GSH and SOD activity. Moreover, there was significant reduction in the levels of IL-6, TNF- α and NF-kB with the use of niclosamide (5 mg/kg, 10 mg/kg and 15 mg/kg). Therefore, in the current study, it presents the niclosamide as a promising hepatoprotective agent.

1 INTRODUCTION

Liver diseases have become a major health concern globally ranks as one of the leading causes of death and an important barrier to increasing life expectancy. From among, liver fibrosis which is a result of a chronic damage to the liver leading to the accumulation of the extracellular matrix (ECM) proteins (Friedman, 2003). The accumulation of ECM proteins will cause the formation of fibrous scar, changes in the hepatic architecture and eventually leading to liver cirrhosis (Ginès, 2004). Viral infection, autoimmune disease, alcohol intake, drug-induced and metabolic disorder are the most common etiologies of chronic liver disease, consequently, leads to liver fibrosis and cirrhosis (Friedman, 2004). Drug-induced liver toxicity manifestations can be ranging from increase in the hepatic enzymes to liver cirrhosis. An overdose of paracetamol can cause sever liver toxicity in accordance to pervious study (Vermeulen, 1992). Such toxicity arises from the generation of the toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine), which is produced from the metabolism of the paracetamol by the liver due to the oxidation through the cytochrome P450 (Cyp450) (Vermeulen, 1992;

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Cohen, 1997). Although, the toxic effect of NAPQI is neutralized by glutathione (GSH) under normal conditions, the overproduction of it will eventually deplete the GSH stores and accumulate reactive oxygen species (ROS) causing liver damage due to oxidative stress (Guo, 2016; Wang, 2017; Salem, 2018). Paracetamol induced oxidative stress also stimuli the production of inflammatory markers interleukin-6 (IL6), tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa B (NF-kB) (Liao, 2016).

Niclosamide is a potent anti-helminthic drug which has been used for long period in treating the tapeworm infections (Al-Hadiya, 2005). Niclosamide mechanism of action can be illustrated by its ability to uncouple oxidative phosphorylation in mitochondria, such mechanism protects the mitochondria by reducing ROS production (Alasadi, 2018; Al-Gareeb, 2017). In a previous study, niclosamide showed a hepatoprotective effect against methotrexate-induced liver toxicity (Zeki, 2021). Therefore, in the present study, we investigate the hepatoprotective effect of niclosamide against paracetamol-induced liver toxicity using in vivo model (albino Wistar rats).

2 MATERIALS AND METHODS

2.1 Drugs and Chemicals

Niclosamide was obtained from Sigma-Aldrich. The selection of niclosamide doses was according to a previous study (Boyapally, 2019). Dimethyl sulfoxide (DMSO) and chlorpromazine were purchased from Thermo Fisher Scientific. Normal saline solution, Polyethylene glycol (PEG) and ketamine were purchased from Sigma-Aldrich. The vehicle used for injection of niclosamide was DMSO and PEG 200 with ratio 1:4 in normal saline. The concentration of DMSO in normal saline was 1%. The chemicals useful for these studies were almost of analytical rank.

2.2 Animals

Experiments were conducted using Male albino Wistar rats (250-300 g. n=30), procured from the Holding company for biological products and Vaccines (VACCERA, Egypt). The animals were maintained at temperature of 25 ◦C and with 12/12 h light/dark cycle. They were housed for 2 weeks prior to the pharmacological experiments to adapt the laboratory conditions. The study has been approved by recommendations approved by the Egyptian Russian University Research Ethics Committee (REC-ERU), Egypt.

2.3 Paracetamol Induced Toxicity

Paracetamol was used for the induction of acute hepatotoxicity according to the liver damage model (Sreedevi, 2009). The paracetamol was suspended in 0.5% tween-80 and the dose administered was 2 gm/kg per oral.

2.4 Experimental Design

Animals were divided into five groups with 6 rats per each group. Group 1: was the control group, rats received vehicle only. Group 2: was the disease group, rats injected with paracetamol (2 g/kg, p.o.). Group 3: was the 5 mg niclosamide group, rats were injected with paracetamol (2 g/kg, p.o.) and 5 mg/kg/day niclosamide i.p. for 4 weeks. Group 4: was the 10 mg niclosamide group, rats were injected with paracetamol (2 g/kg, p.o.) and 10 mg/kg/day niclosamide i.p. for 4 weeks. Group 5: was the 15 mg niclosamide group, rats were injected with paracetamol (2 g/kg, p.o.) and 15 mg/kg/day niclosamide i.p. for 4 weeks. The injection of niclosamide and vehicle was started at the same day of surgery.

After 4 weeks, the animals were anesthetized using ketamine (100 mg/kg) and chlorpromazine i.p. (0.75 mg/kg), then sacrificed. The blood samples were collected by puncturing the retro-orbital plexus for analysis of biochemical markers, and the liver was removed for examination of oxidative stress and inflammatory markers.

2.5 Biochemical Spectrophotometric Analysis of

2.5.1 Biomarkers for Liver Function

A colorimetric method was used to assess Serum aspartate transaminase (AST) and Alanine transaminase (ALT) according to the instructions of manufacturer's (TECO DIAG- NOSTICS, 1268 N. Lakeview Ave, Anaheim, U.S.A.). Modifications has been carried on the original methods by Doumas and Briggs, and Reitman and Frankel respectively (Doumas, 1969; Reitmen, 1957).

Colorimetric method was used to assess Gamma-Glutamyl Transferase (GGT) using kit (XpressBio 503 Gateway Dr W Thurmont, MD 21788,). It measures the level of the product p-nitroaniline

(pNA) at 405 nm, which is produced from the cleavage of the GGT substrate (©-glutamyl-p-ntiroanilide) by the enzyme. The product pNA is directly proportional to the GGT level. All the results from AST, ALT and GGT assays expressed in U/L.

2.5.2 Biomarkers for Cholestasis

Colorimetric method was used to assess Serum alkaline phosphate (ALP) according to the instructions of manufacturer (BioAssay Systems. 3191 Corporate Place, Hayward, USA). It measures the yellow-colored product at 405 nm, obtained from the hydrolysis of the p-nitrophenyl phosphate by the ALP. The yellow-colored product is directly proportional to the ALP activity. The result value was expressed as U/L.

2.5.3 Oxidative Stress Marker Malondialdehyde (MDA)

Assessment of the tissue MDA was carried according to the instructions of manufacturer (MyBioSource, sunny Southern California, San Diego, USA). Evaluation of the tissue MDA was based on the reaction with thiobarbituric acid (TBA) to produce thiobarbituric acid reactive substance (TBARS); pink chromogen; which is measured at 532-535 nm.

2.6 Enzyme- Linked Immunosorbent Assay (ELISA)

2.6.1 General Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibodies specific to the target antigens were used to pre-coat the ELISA plates. For detection antibody, Biotin conjugated antibody was used. Samples and biotin conjugated antibody were added simultaneously to the wells and washed using wash buffer. After using the wash buffer to wash the wells, avidin conjugated horseradish peroxidase (HRP) was added. To visualize the HRP reaction, tetramethylbenzidine (TMB) was used as substrate. The produced color intensity was measured at 450 nm, which is proportional to the amount of the antigen in the sample.

2.6.2 Assessment of Oxidative Stress Markers Superoxide Dismutase (SOD) And Glutathione (GSH)

Assessment of the SOD and GSH tissue levels was carried according to the instructions of manufacturer (MyBioSource, sunny Southern California, San Diego, USA), (Blue gene Biotech CO., LTD, Shanghai, China), respectively. The results values were expressed as mmol/mg protein.

2.6.3 Assessment of Inflammatory Markers

Assessment of the interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa B (NF-kB) was carried according to the instructions of the manufacturer (Cat no. MBS175908, MyBioSource, sunny Southern California, San Diego, USA), (Cat no. MBS9711597, MyBioSource, sunny Southern California, San Diego, USA) and (Cat no. MBS268833, MyBioSource, sunny Southern California, San Diego, USA), respectively. The result value of NF-kB level was expressed as ng/mg protein, however, the results values for both IL-6 and TNF-α were expressed as pg/mg protein.

2.6.4 Statistical Analysis

The results were all expressed as mean ± S.D. To compare between different groups, one-way analysis of variance (ANOVA) was used followed by Tukey's test. The data considered statistically significant when the $P < 0.05$. All of the statistical analysis and graphs were done using GraphPad Prism version 5 software (ISI Software, United States).

3 RESULTS

3.1 Effect of Niclosamide on Liver Function and Cholestasis Markers

After 4 weeks, there was a significant increase in the AST, ALT, ALP and GGT values in the disease group by 5, 6, 5 and 8-fold respectively, when compared to the control group. Treatment with niclosamide in group 3 (5 mg/kg/day) cause a significant decrease in the values of AST, ALT, ALP and GGT by 40%, 45%, 50% and 53% respectively, when compared to the disease group. On the other hand, treatment with niclosamide in group 4 (10 mg/kg/day) cause a significant decrease in the values of AST, ALT, ALP and GGT by 59%, 67%, 70% and 78% respectively, when compared to the disease group. Furthermore, treatment with niclosamide in group 5 (15 mg/kg/day) cause a significant decrease in the values of AST, ALT, ALP and GGT by 63%, 70%, 73% and 80% respectively, when compared to the disease group (Table 1).

Table 1: Effect of Niclosamide (5, 10 and 15 mg/kg) on paracetamol induced changes in liver functions.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
Control	45.06 ± 5.312	32.64 ± 4.51	52.83 ± 4.13	23.95 ± 3.46
Disease	235.50 ± 5.39[a]	198.01 ± 7.66[a]	264.01 ± 5.81[a]	188.0 ± 7.37[a]
5 mg Niclosamide	138.50 ± 7.78[a, b]	108.30 ± 7.36[a, b]	132.7 ± 6.09[a, b]	89.82 ± 4.93[a, b]
10 mg Niclosamide	98.50 ± 3.85[a, b]	66.42 ± 5.60[a, b]	80.45 ± 5.08 [a, b]	42.82 ±4.21[a, b]
15 mg Niclosamide	89.02 ± 4.63[a, b]	60.76 ± 6.59[a, b]	73.99 ± 6.53[a, b]	37.27 ± 6.40[b]

Data are represented as mean ± SD of 6 rats per group. a: significant difference from control group, and b: significant difference from disease group at $p < 0.05$ using ANOVA followed by Tukey's post-hoc test.

3.2 Effect of Niclosamide on Oxidative Stress and Antioxidant Capacity

Comparing the level of the MDA in the control group to the disease group. There was a significant increase with around 6 folds in the disease group. Treatment with niclosamide in the group 3 (5 mg/kg/day), group 4 (10 mg/kg/day) and group 5 (15 mg/kg/day) caused a significant reduction by 40%, 64% and 69% respectively, when compared to the disease group (Figure 1). Moreover, GSH and SOD levels were significantly decrease in the disease group by 75% and 70% respectively, when compared to the control group. Treatment with niclosamide in group 3 (5 mg/kg/day), group 4 (10 mg/kg/day) and group 5 (15 mg/kg/day) caused a significant increase in the levels of GSH by 2 folds, 3.5 folds and 4 folds respectively, when compared to the disease group (Figure 2). While levels of SOD were increased with niclosamide treatment in group 3 (5 mg/kg/day), group 4 (10 mg/kg/day) and group 5 (15 mg/kg/day) by 1.7 folds, 2.5 folds and 3 folds, respectively, when compared to the disease group (Figure 3).

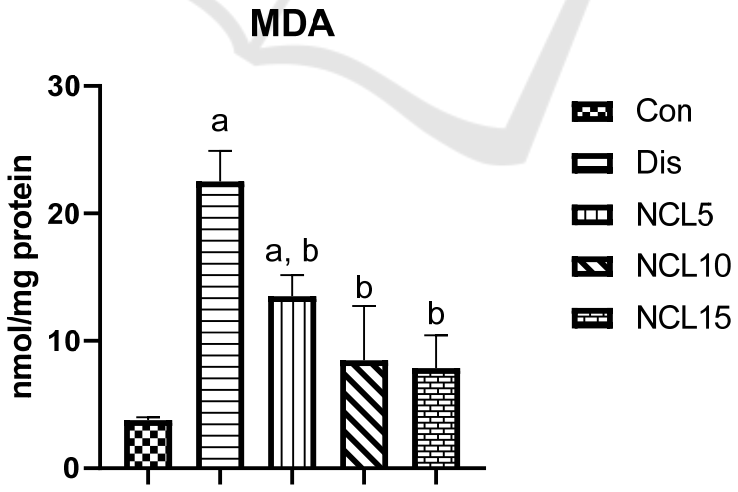

Figure 1: Effect of niclosamide on MDA. Data are presented as mean ± SD of 6 rats per group, a: significant difference from control group, and b: significant difference from disease group at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. Con, control group; Dis, disease group; NCL5, 5 mg niclosamide group; NCL10, 10 mg niclosamide group; NCL15, 15 mg niclosamide group.

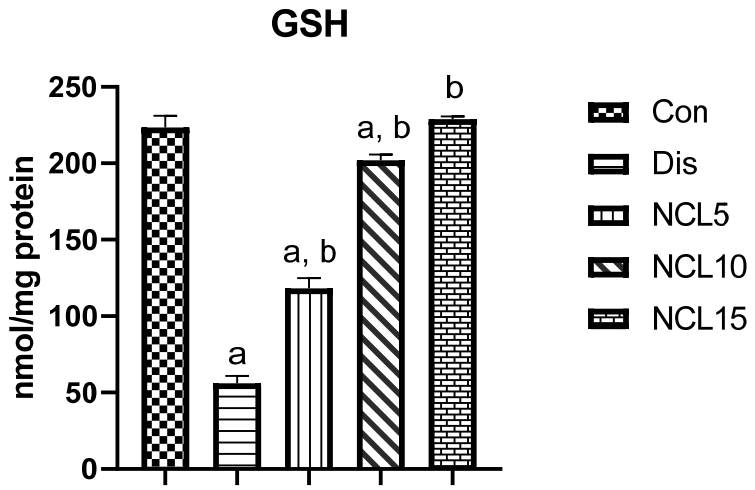

Figure 2: Effect of niclosamide on GSH. Data are presented as mean ± SD of 6 rats per group, a: significant difference from control group, and b: significant difference from disease group at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. Con, control group; Dis, disease group; NCL5, 5 mg niclosamide group; NCL10, 10 mg niclosamide group; NCL15, 15 mg niclosamide group.

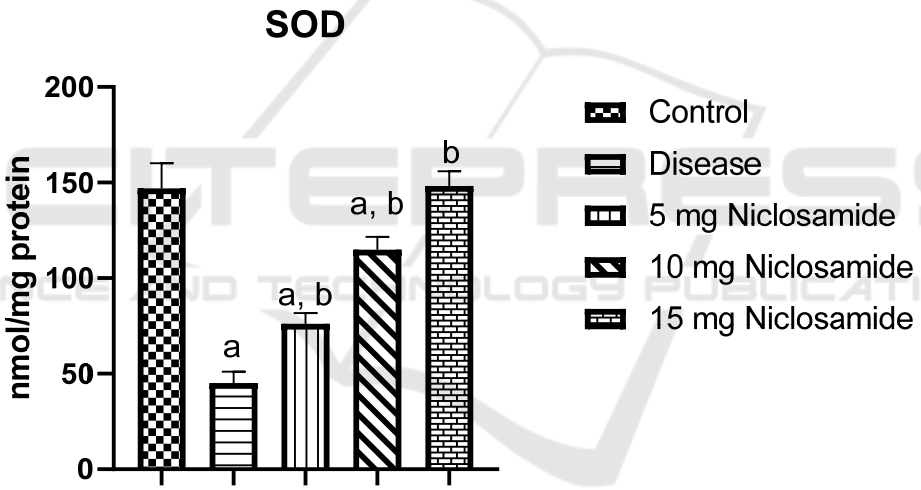

Figure 3: Effect of niclosamide on SOD. Data are presented as mean ± SD of 6 rats per group, a: significant difference from control group, and b: significant difference from disease group at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. Con, control group; Dis, disease group; NCL5, 5 mg niclosamide group; NCL10, 10 mg niclosamide group; NCL15, 15 mg niclosamide group.

3.3 Effect of Niclosamide on Inflammatory Markers

There was a significant increase in the levels of IL-6, TNF-α and NF-kB by 4.9, 5.6, 6.5 folds in the disease group compared to the control group. On the other hand, treatment using niclosamide in group 3 (5 mg/kg/day) caused significant reduction on IL-6, TNF-α and NF-kB by 39%, 42% and 49% respectively, when compared to the disease group. Also, treatment using niclosamide in group 4 (10 mg/kg/day) caused a significant reduction on IL-6, TNF-α and NF-kB by 58%, 60% and 71% respectively, when compared to the disease group. In addition, treatment using niclosamide in group 5 (15 mg/kg/day) caused a significant reduction on IL-6, TNF-α and NF-kB by 63%, 67% and 76% respectively, when compared to the disease group (Figures 4, 5 and 6).

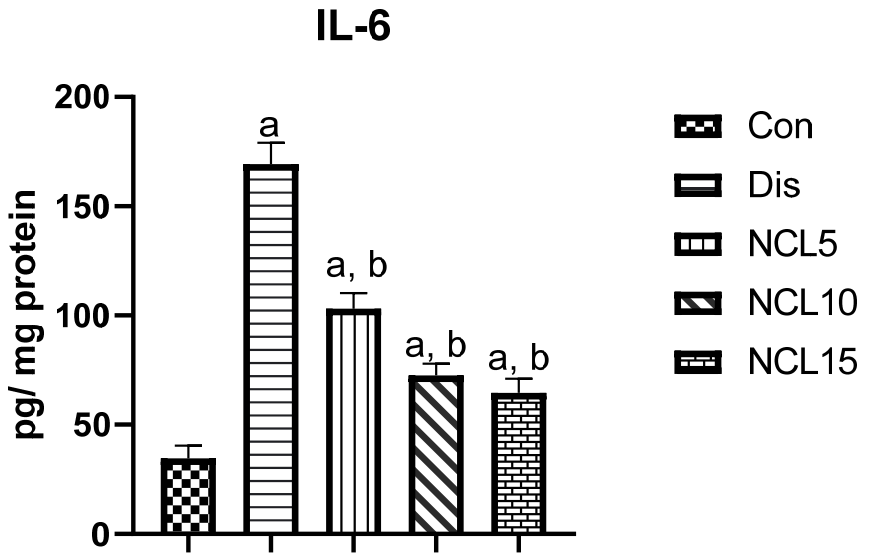

Figure 4: Effect of niclosamide on IL-6. Data are presented as mean ± SD of 6 rats per group, a: significant difference from control group, and b: significant difference from disease group at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. Con, control group; Dis, disease group; NCL5, 5 mg niclosamide group; NCL10, 10 mg niclosamide group; NCL15, 15 mg niclosamide group.

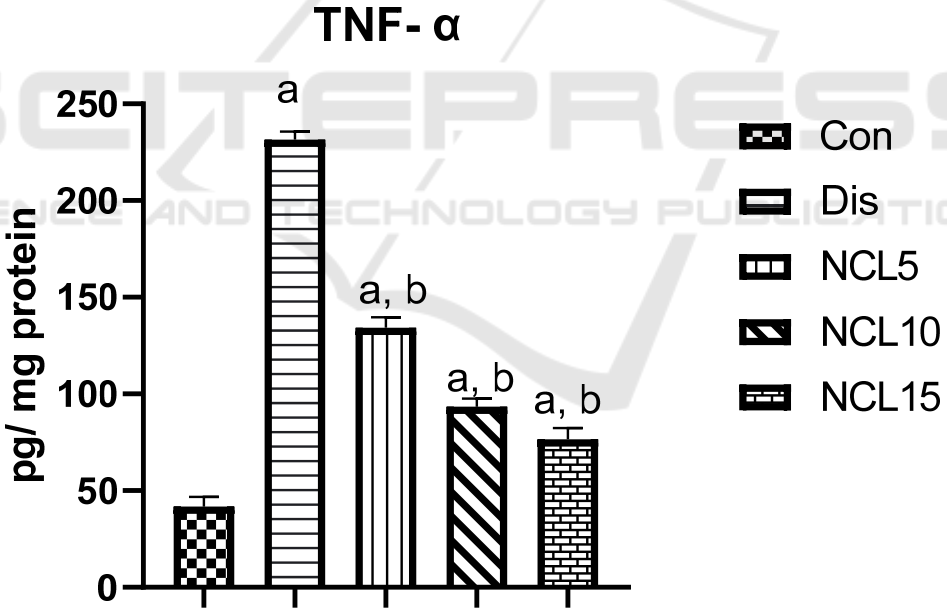

Figure 5: Effect of niclosamide on TNF-α. Data are presented as mean ± SD of 6 rats per group, a: significant difference from control group, and b: significant difference from disease group at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. Con, control group; Dis, disease group; NCL5, 5 mg niclosamide group; NCL10, 10 mg niclosamide group; NCL15, 15 mg niclosamide group.

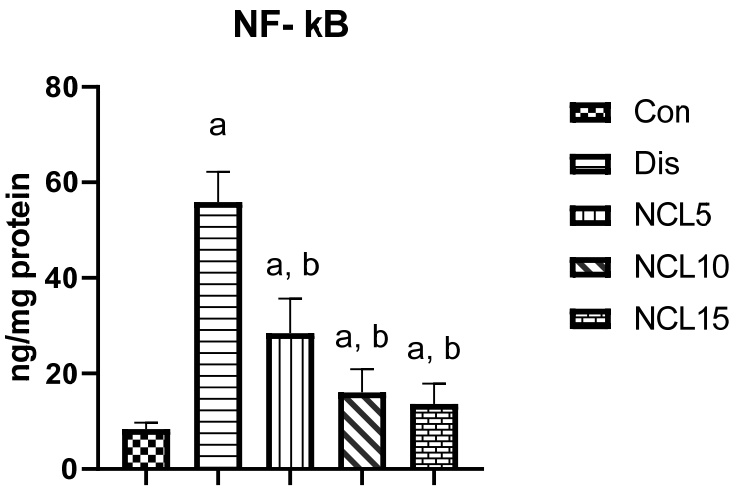

Figure 6: Effect of niclosamide on NF-kB. Data are presented as mean ± SD of 6 rats per group, a: significant difference from control group, and b: significant difference from disease group at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. Con, control group; Dis, disease group; NCL5, 5 mg niclosamide group; NCL10, 10 mg niclosamide group; NCL15, 15 mg niclosamide group.

4 DISCUSSION

Liver is an essential organ for regulation of the internal body environment. Therefore, any damage affects the liver due to any reasons can have a serious consequence. One example of the causes behind liver toxicity, is drug-induced toxicity by paracetamol. Paracetamol is used as analgesic and antipyretic drug at normal doses, however, in high doses it possesses a hepatotoxic effect. Moreover, paracetamol-induced hepatotoxicity was used as an experimental model for evaluation of hepatoprotective agents.

In this study, liver fibrosis in rodents was induced using paracetamol-induced hepatotoxicity model. There was elevation in the hepatic markers AST, ALT, ALP and GGT showing hepatic dysfunction[15]. Using different doses of niclosamide (5, 10 and 15 mg/kg), it displayed a significant decrease in the AST, ALT, ALP and GGT enzymes activity. The prominent effect was at dose 15 mg/kg, however, the effect of doses 10 mg/kg and 15 mg/kg was almost the same.

Paracetamol-induced liver toxicity showed before an increase in the ROS and depletion in the antioxidant reservoirs (Canayakin, 2016). Depletion of GSH stores and decrease in its value in this study was in accordance with a previously published results (Kushwah, 2014). Treatment using niclosamide different doses (5, 10 and 15 mg/kg) had a significant effect on restoring the GSH stores displayed increase of its values. Although there was a significant difference in the effect on GSH between the dose 5 mg/kg and 10 mg/kg but comparing the effect of dose 10 mg/kg to 15 mg/kg, the values were closely related. Same pattern was obvious with the SOD, where there was a significant decrease in SOD value in the disease group, correlated with the previous results (Madi, 2015). Co-treatment using niclosamide (5, 10 and 15 mg/kg) lead to the increase of SOD activity with the prominent effect at 10 mg/kg and 15 mg/kg. Free radicals cause tissue damage due to lipid peroxidation (Esterbauer, 1991). One of the lipid peroxidation products is MDA, which showed a surge in its values in this study due to paracetamol-induced hepatotoxicity in accordance with a recent study (Rašković, 2017). Treatment using niclosamide (5, 10 and 15 mg/kg) displayed a decrease in MDA values. Comparing the 3 different doses, the effect was the most with 15 mg/kg and the dose 5 mg/kg showed the lowest effect.

In addition, herein inflammatory markers such as IL-6 and TNF-α was significantly increased with paracetamol-induced liver toxicity. This elevation is correlated to recent studies (Karakus, 2013; James, 2003). Co-treatment using niclosamide (5, 10 and 15 mg/kg) decrease the activity of these markers, doses 10 mg/kg and 15 mg/kg showed almost same pattern with prominent effect if compared to dose 5 mg/kg. Also, the inflammatory marker NF-kB was significantly boosted with the paracetamol-induced toxicity, in alignment with the recent study (Jiang, 2021). Using niclosamide various doses (5, 10 and 15 mg/kg) caused significant reduction in NF-kB activity, such reduction was more obvious with the 10 mg/kg and 15 mg/kg doses.

5 CONCLUSION

The hepatoprotective effect of niclosamide against liver toxicity induced by paracetamol for four-weeks period was elevated by the author. Niclosamide significantly cause reduction in the hepatic enzymes AST, ALT, ALP and GGT. Moreover, niclosamide had the ability to ameliorate oxidative stress by significantly reduce MDA and increase SOD and GSH. Nevertheless, niclosamide had also part in inflammation reduction by significantly decrease the inflammatory markers TNF-α, IL-6 and NF-kB. Although further studies are needed for understanding the exact mechanism of niclosamide in healing liver toxicity, but niclosamide can be considered a promising hepatoprotective drug.

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