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Effect of niclosamide ethanolamine on the serum oxidative stress markers in cardiac injury

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Abstract
Present study explored potential of anthelmintic niclosamide ethanolamine, known for its antioxidant, and anti-inflammatory properties, in mitigating cardiac injury brought on by isoprenaline. Cardiac injury was induced viasubcutaneous administration of isoprenaline (ISP) @ 20mg/kg body weight for 14 days. Niclosamide ethanolamine was administered @ 3 mg/kg b.w. (intraperitoneally) on alternate days with daily dosing of ISP in the treatment group. Mice were sacrificed on day 15th, and serum samples were used for estimation of oxidative stress parameters in different groups. The study reveals that isoprenaline-induced oxidative stress results in decreased antioxidants coupled with increased lipid peroxidation marked by elevated malondialdehyde (MDA) levels which have been improved by niclosamide ethanolamine administration. In conclusion, niclosamide ethanolamine showed the potential to attenuate the isoprenaline-induced oxidative stress, suggesting its role in limiting the progression of cardiac injury via its antioxidant ability.

Keywords: Cardiac, isoprenaline, niclosamide ethanolamine, antioxidant

Introduction
Cardiovascular diseases, encompassing conditions affecting the heart and vascular system contribute to a significant global health burden. World Health Organisation (WHO) has reported that these conditions accounted for 32% of total global mortality, causing approximately 17.9 million deaths in 2019 (WHO, 2021) [1]. Isoprenaline, a synthetic β-adrenoceptor agonist induces oxidative stress by generating free radicals, causing progressive damage to mitochondria and disrupting the biochemical homeostasis of heart, eventually leading to cardiac injury (Allawadhi *et al.,* 2018) [2]. Oxidants generation and anti-oxidant activities equilibrium in cell is crucial for the physiological homeostasis of cells. Excessive production of reactive species *viz*., ROS beyond the endogenous antioxidant capacity of cells results in oxidative stress. (Kurutas *et al*, 2016) [3]. These oxidative stress-mediated processes contribute to myocardial damage (Allawadhi *et al.,* 2018; Dhalla *et al.,* 2022) [2, 4]. Attenuation of the isoprenaline-induced oxidative stress could be beneficial in limiting cardiac injury progression.
Niclosamide ethanolamine demonstrated efficacy in ameliorating the hepatic steatosis mice fed with high-fat diet by activating the Nrf-2 pathway, indicating its anti-oxidant activity (Park *et al.,* 2019) [5]. Protective action of niclosamide by limiting oxidative stress in liver fibrosis mediated by cholestatic, suggesting its action as an anti-oxidant has been reported (Esmail *et al.*,2021) [6]. Furthermore, the nephroprotective action of niclosamide ethanolamine in c kidney disease may be attributed to its modulation of the redox balance (Han *et al.,* 2019) [7]. Although the anti-oxidant activity of niclosamide has been documented in various conditions. However, no study has investigated for its use in isoprenaline-induced cardiac injury associated with serum oxidative stress. Therefore, the current study was undertaken to explore impact of niclosamide ethanolamine on serum oxidative stress in cardiac injury brought on by isoprenaline in mice.

Material and Methods
Experimental animals: Healthy adult male mice were procured from the Laboratory Animal Resource Section at ICAR-Indian Veterinary Research Institute, Izatnagar, U.P.

Experimental animals were kept under 12-12 hours dark-light cycle and provided unrestricted access to food and water. After acclimatization, all experimental procedures were conducted in accordance with the guidelines approved by the Institutional Animal Ethics Committee (IAEC).

Vehicle in control group mice (Group-I) for 14 days and in the Group II, niclosamide ethanolamine (Cayman) alone was administered at a dose of 3 mg/kg body weight (in DMSO that further diluted in peanut oil) on alternate day for 14 days through intraperitoneal route. In the cardiac injury group (Group III), isoprenaline (Sigma) was administered at a dose of 20 mg/kg body weight (in distilled water) via subcutaneous route for 14 days. In the treatment group (Group IV), isoprenaline was given (S/C) for 14 days, while niclosamide ethanolamine was administered intraperitoneally on alternate days.

Experimental protocol

Antioxidant ability in serum was investigated. Superoxide dismutase was determined following method outlined by Mahesh and Balasubramanian [8]. In brief, reaction mixture having phosphate buffer saline, MTT (1.25 mM), serum sample and pyrogallol were incubated for 5 minutes and absorbances of samples were measured at 570 nm wavelength after halting reaction with DMSO. SOD units/mg of proteins used to express results. Method outlined by Aebi (1984) [9] was used to estimate catalase activity in the serum. Samples were added in phosphate buffer. After adding hydrogen peroxide (H_2O_2), absorbance was recorded at 240 nm for 60 seconds at every 15 seconds interval. Catalase activity expressed as per mg of protein. The protein of serum was estimated (Bradford, 1976) [10]. The non-enzymatic antioxidant activity, GSH in serum was determined by method as outlined by Sedlak and Lindsay (1968) [11]. In this procedure serum sample were mixed in distilled water, trichloroacetic acid and incubated for 15 minutes and further subsequent steps were followed. Absorbance was recorded within 5 minutes at 412 nm to quantify GSH. LPO was assessed as per previous method (Buege and Aust, 1978) [12]. Serum was added in TBA-TCA-HCl and incubated for 15 minutes. Subsequently, absorbance of supernatant was recorded. MDA was calculated and expressed in nmol/ml of serum.

Results

Effect of niclosamide ethanolamine on SOD activity in the serum samples in cardiac injury

The serum SOD activity in the isoprenaline-induced cardiac injury group was significantly (p<0.001) reduced (4.27±0.11 units/mg of protein; n=10) compared to control group (5.44±0.19 units/mg of proteins; n=7). The SOD was found significantly (p<0.001) higher in the mice of the niclosamide ethanolamine-treated cardiac injury group (5.49±0.16 units/mg of proteins; n=7) in comparison to the isoprenaline-alone administered group (4.27±0.11 units/mg of protein; n=10). No significant alteration in SOD was noted between the control group and the niclosamide ethanolamine-alone administered group (4.99±0.17 units/mg of proteins; n=7) (Fig 1).

Effect of niclosamide ethanolamine on catalase in serum in cardiac injury

The serum catalase activity was significantly reduced (p<0.05) in cardiac injury group (3.58±0.89 mmol H_2O_2/min/mg of proteins; n=7) in comparison to the control group (9.71±1.25 mmolH_2O_2/min/mg of proteins; n=6). In the niclosamide ethanolamine-treated cardiac injury group (9.48±1.49 mmolH_2O_2/min/mg of proteins; n=6), activity of catalase was significantly elevated (p<0.05) compared to the isoprenaline-alone administered group (3.58±0.89 mmolH_2O_2/min/mg of proteins; n=7). Catalase activity of the niclosamide ethanolamine-alone administered group (10.51±1.62 mmolH_2O_2/min/mg of proteins; n=6) was almost comparable to control group (Figure 2).

Effect of niclosamide ethanolamine on the serum reduced glutathione levels in cardiac injury

A significant (p<0.05) lower GSH level in serum was noted in the cardiac injury group (0.72±0.04 fold change; n=10) compared to control group (1.00±0.09 fold change; n=7). The GSH level was significantly (p<0.05) higher in the niclosamide ethanolamine-treated cardiac injury group (1.02±0.06 fold change; n=6) *vs* isoprenaline-alone administered group (0.72±0.04 fold change; n=10). No significant difference was observed betweencontrol group and the niclosamide ethanolamine-alone administered group (1.05±0.08 fold change; n=8) (Fig 3).

Effect of niclosamide ethanolamine on the MDA levels in the serum samples of cardiac injury

A significant (p<0.01) elevation in the serum MDA level was observed in the isoprenaline-induced cardiac injury group (6.01±0.32 nmol/ml of serum; n=6) compared to control group (3.64±0.30 nmol/ml of serum; n=7). MDA was moderately decreased in the niclosamide ethanolamine-treated cardiac injury group (4.28±0.41 nmol/ml of serum; n=6), than the isoprenaline-alone administered group (6.01±0.32 nmol/ml of serum; n=6). The MDA level in the niclosamide ethanolamine-alone administered group (3.41±0.64 nmol/ml of serum; n=6) was almost comparable to the control group (3.64±0.30 nmol/ml of serum; n=7) (Fig 4).

Discussion

Appropriate functioning of sympathetic system is vital for preserving cardiovascular homeostasis. The initiation and progression of cardiovascular conditions are associated with overstimulation of this system. Prolonged overstimulation of this system by administering isoprenaline leads to cardiac injuries through the generation of reactive species causing oxidative stress, inflammation, and apoptosis (Hasan *et al.*, 2020) [13]. Oxidative stress brought on by isoprenaline is characterized by a reduction in the inherent antioxidant reserves of cells *viz*., superoxide dismutase, glutathione and catalase activity along with an increase in the oxidative and nitrative stress markers, including malondialdehyde, nitric oxide (Ulla *et al.*, 2017; Sumi *et al.*, 2019; Hasan *et al.*, 2020) [14, 15, 13]. In the present study, enzymatic antioxidant activity, including SOD and catalase and non-enzymatic endogenous anti-oxidant *viz*., GSH were significantly declined in the isoprenaline-administered cardiac injury group. Furthermore, in cells malondialdehyde serves as a crucial biomarker for oxidative stress (Cordiano *et al.*, 2023) [16]. In the current investigation, the isoprenaline-induced cardiac injury group exhibited a significantly elevated level of MDA. However, the administration of niclosamide ethanolamine in the injury group significantly improved the endogenous enzymatic (SOD and catalase) and non-

enzymatic (GSH) antioxidant activity. Furthermore, lipid peroxidation (MDA level) was also improved in niclosamide treated injury group. These findings are concordant to previous study which showed nephroprotective action of niclosamide in kidney disease by regulating the mitochondrial production and H_2O_2 scavenging by increasing the catalase (Han *et al.*, 2019) [7]. Another study also reported the niclosamide ethanolamine treatment increased the catalase and SOD2 protein expression and restored the renal redox imbalance in systemic lupus erythematosus (Han *et al.*, 2020) [17]. Additionally, Zeki and Al-Gareeb (2021) [18] have also documented that prior administration of niclosamide in mice with liver injury caused by methotrexate resulted in elevated levels of SOD and GSH, accompanied by a decrease in MDA levels.

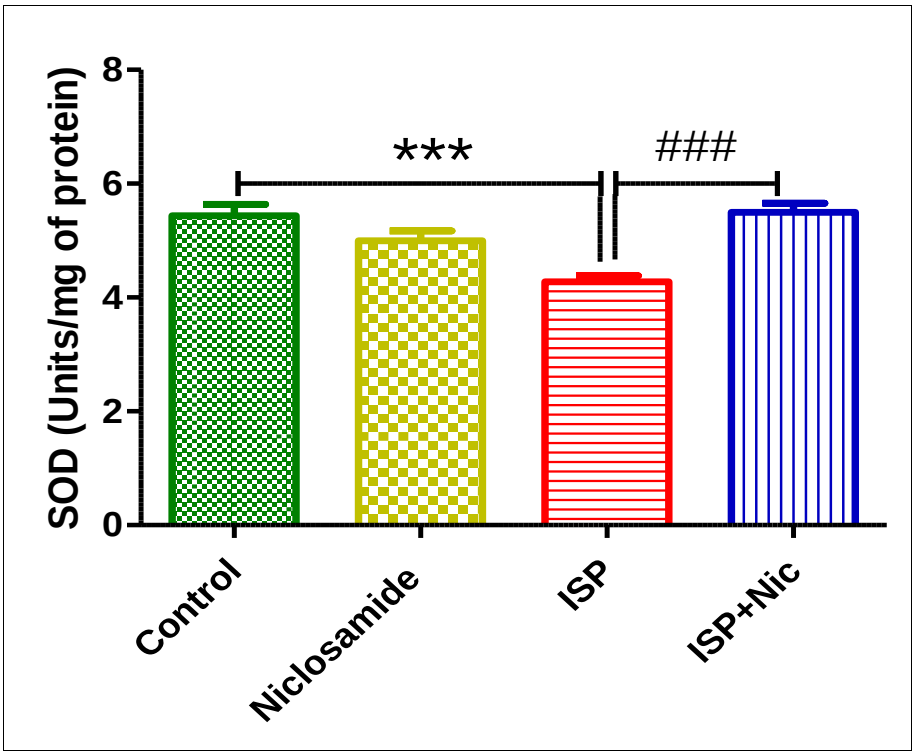

Fig 1: Effect of niclosamide ethanolamine on superoxide dismutase (SOD) activity in serum samples in cardiac injury. One-way ANOVA followed by Tukey's multiple comparison *post hoc* test. ***p<0.001 in comparison to control group; $^{###}p$<0.001 in comparison to ISP-alone group

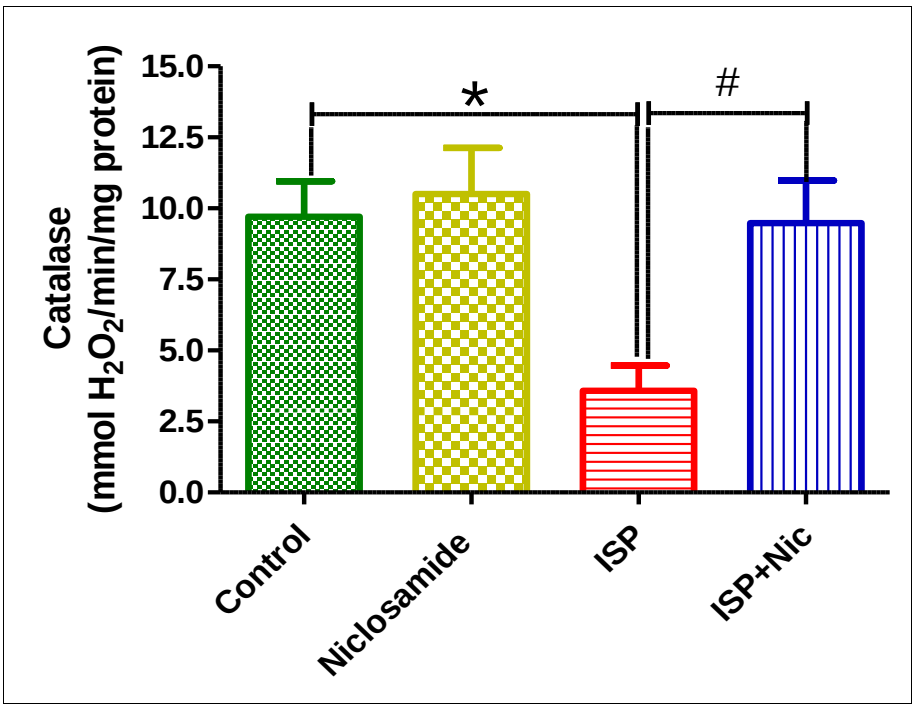

Fig 2: Effect of niclosamide ethanolamine on serum catalase activity in cardiac injury. One-way ANOVA followed by Tukey's multiple comparison *post hoc* test. *p<0.05 in comparison to control group; $^{#}p$<0.05 in comparison to ISP-alone group.

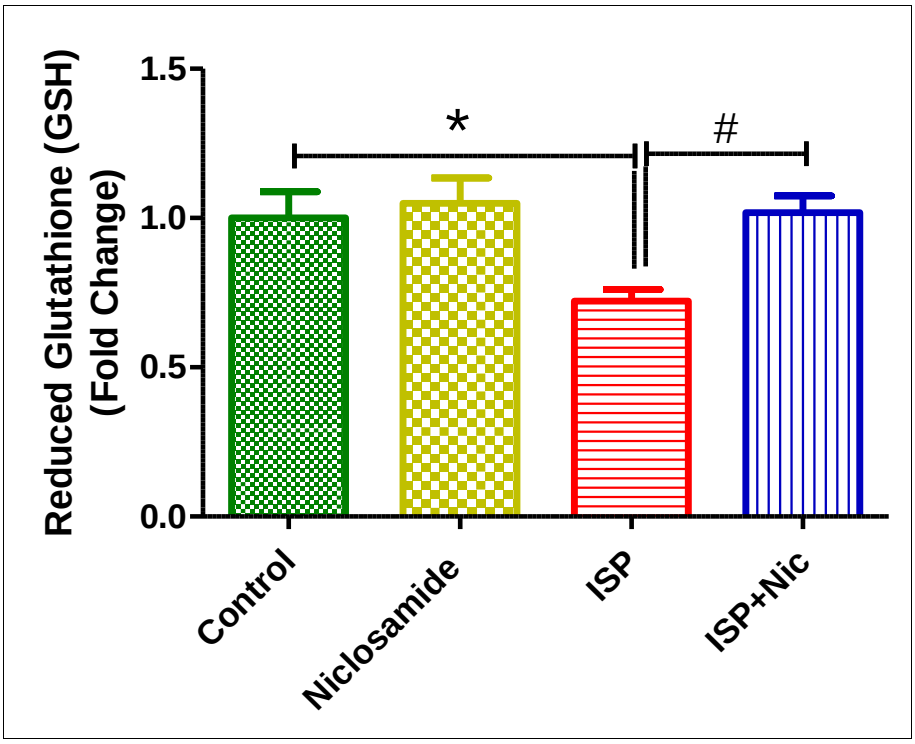

Fig 3: Effect of niclosamide ethanolamine on non-enzymatic antioxidant reduced glutathione (GSH) in serum in cardiac injury. One-way ANOVA followed by Tukey's multiple comparison *post hoc* test. **p*<0.05 in comparison to control group; $^{\#}$*p*<0.05 in comparison to ISP-alone group.

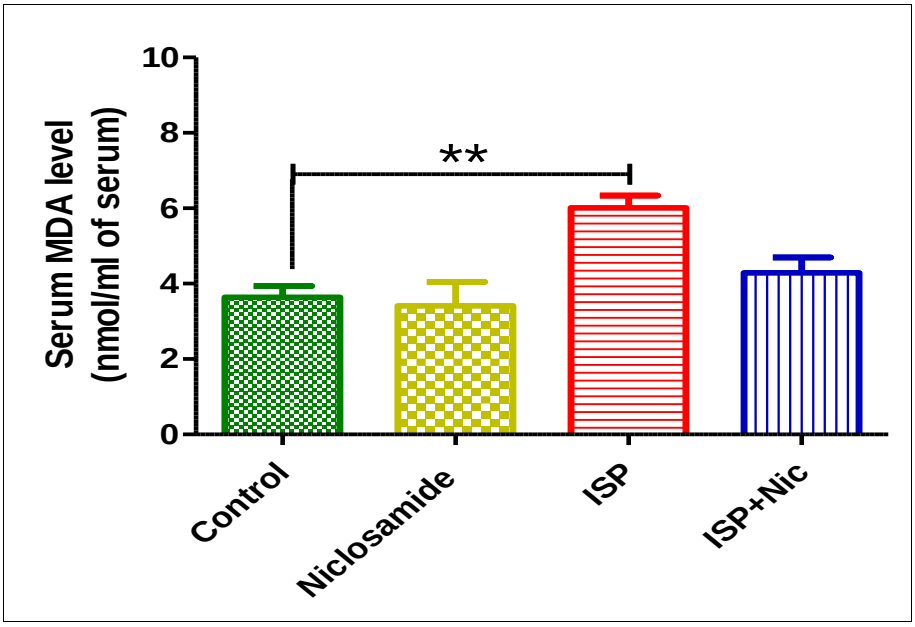

Fig 4: Effect of niclosamide ethanolamine on malondialdehyde (MDA) level in serum in cardiac injury. One-way ANOVA followed by Tukey's multiple comparison *post hoc* test. ***p*<0.01 in comparison to control group

Conclusion

The findings of this study indicate that anti-oxidants and MDA were changed in isoprenaline-induced cardiac injury. The administration of niclosamide led to decreased serum MDA coupled with an elevated levels of antioxidants *viz*., SOD, catalase, and GSH. Consequently, it is plausible to imply that niclosamide treatment may hinder the advancement of cardiac injury by exerting its antioxidant effects in the cardiac injury induced by isoprenaline.

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