

Research Article

Comparative Effects of Withania Somnifera Root and Rhodiola Rosea Extractions on Liver Enzymes, Neurotransmitters and Antioxidant Parameters in Stressed Induced Male Rabbit Model by MnCl₂.

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Abstract

This study intended to evaluate the comparative effects of Withania somnifera vs Rhodiola rosea extractions in reducing the toxic effects of manganese in male New Zealand rabbits. 40 male New Zealand white rabbits were divided into four equal groups. Group 1: as a control group left without treatments (only 1 ml of normal saline/ animal/ day). Stress induction for groups 2, 3 and 4 : by administering MnCl₂ (150 mg / kg (bw) body weight) intraperitoneally every day for 4 weeks. Then only groups 3 and 4 were administered 150 mg/kg/day of WS extract and 150 mg/kg/day and Rhodiola extract respectively, all the duration of treatment was 8 weeks (including 4 weeks of treatment post MnCl₂ exposure). Then the following criteria were investigated: liver enzymes (ALT, AST, ALP and GGT) , Neurotransmitters (serotonin, dopamine and GABA in the mid brain), and oxidation indicators SOD,CAT and GSH in serum). Results: exhibited significantly decreasing of ALT, AST,ALP and GGT levels in WS and Rhodiola groups compared to MnCl₂ and control groups. Both WS and Rhodiola extractions significantly increased serotonin, dopamine and GABA and restored them almost to the normal levels also improved levels of SOD,CAT and GSH antioxidant enzymes. Conclusion, The study demonstrates that both WS (Ashwagandha) and Rhodiola provide a significant neuroprotective, antioxidant, and hepatoprotective effects against MnCl₂-induced stressed male rabbit models with no differences except for a few significance.

Keywords: Serotonin, dopamine, male rabbit, oxidative stress.

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Introduction

Withania somnifera, known as Ashwagandha, and Rhodiola rosea, referred to as "roseroot" or "golden root," are two adaptogenic herbs recognized in traditional medicine for their possible ability to improve physical and mental resilience. Previous research indicates that these herbs might provide neuroprotective benefits, affect neurotransmitter levels, and boost antioxidant defenses. (Panossian and Wagner 2005). *Withania somnifera* (WS) is categorized as an agent that

promotes homeostasis of the entire body by eliciting complex responses in addition to one specific pharmacological mechanism because it can revitalize the body and promote the

health of all tissues (Balasubramani, et al.,2011) and (Panossian, et al. 2021). The majority of the extracts investigated came from the WS root. (Priyanka;et al., 2020) so extraction methods and test preparations in this search used alcoholic extracts (Dey; et al.,2016)

Rhodiola rosea is extensively researched for its role in reducing fatigue and enhancing mood. It is believed to affect neurotransmitter levels and may have antioxidant and liver-protective effects. However, there is a lack of systematic studies that measure these effects and compare the impacts of both herbs on neurotransmitter levels, antioxidant status, and liver enzymes in controlled animal studies (Panossian, and Wagner 2005). Manganese compounds or Mn in production are widely used in the manufacture of many foods. Though, wide manufacturing use of Mn compounds or Mn in production of paints, glass ceramics, steel, fertilizers, dry cell batteries, certain pesticides, in leather industries and texture and as antiknock agent in gasoline as well as smelting operations and mining of Mn may obtained exposure of workers to immoderate amount of this metal (Santamaria 2008). Manganese chloride (MnCl₂) exposure is associated with neurotoxicity, oxidative stress, and hepatotoxicity, making it a relevant model for studying environmental and occupational exposure to heavy metals. The interference caused by MnCl₂ involves changes in neurotransmitter levels, oxidative damage to cells, and liver function impairment (Brown and Miller 2019). Adaptogenic herbs like Ashwagandha and Rhodiola are thought to lessen these effects due to their neuroprotective and antioxidant characteristics. In this study, stress is induced in male rabbits using MnCl₂ at a dosage of 150 mg/kg body weight. The goal is to compare the

individual effects of Ashwagandha and Rhodiola on liver enzymes, neurotransmitter levels, and antioxidant defenses in stressed male rabbit models induced by MnCl₂, and to evaluate whether Ashwagandha or Rhodiola can influence these parameters, which are important for cognitive function, mood stability, and antioxidant action.

Materials and Methods

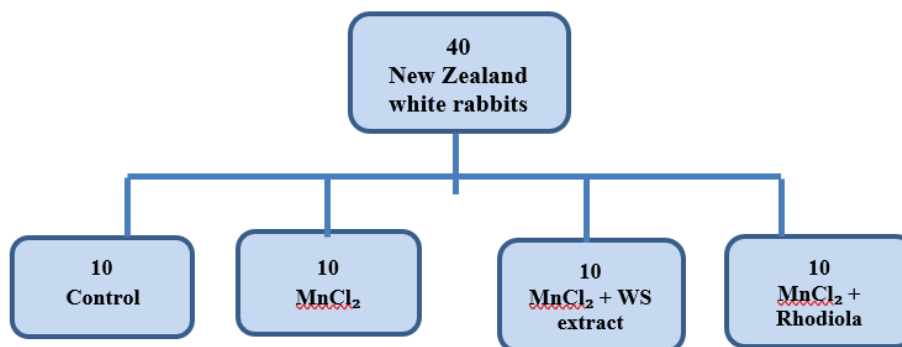
Experimental Animals

Male New Zealand white rabbits (Harlan Breeders, IN), 6 months old and weighing between 2.5–3.0 kg were kept under standard laboratory conditions (22 ± 2°C; 12-hour light/dark cycle) with ad libitum access to food and water. The rabbits were randomly subdivided into four groups (n = 10 per group). The study was approved by the Institutional Ethical Committee in the college of veterinary medicine, department of physiology, University of AL-Shatrah, Thiqar, Iraq, and all experimental protocols were conducted in accordance with the guidelines for the care and use of laboratory animals .

Herbal extract preparation and dose

The roots of Withania somnifera were hydroalcoholic extraction prepared. Rhodiola extract was prepared similarly. Both extracts were standardized for their active components, with (WS) containing 5% withanolides and Rhodiola containing 3% rosavins.

Experimental design



1. Control : group without treatment
2. MnCl₂
3. MnCl₂ + WS extract.
4. MnCl₂ + Rhodiola extract.

3. MnCl₂ + WS group: MnCl₂ exposure, administered 150 mg / kg daily of WS extraction.
4. MnCl₂ + Rhodiola group: MnCl₂ exposure, administered 150 mg /kg/ daily of Rhodiola extraction.

The animals were housed under standard laboratory conditions with unrestricted access to food and water. Stress Induction for groups 2, 3 and 4 : Neurotoxicity was induced by administering MnCl₂ (150 mg/kg body weight) intraperitoneally daily for 4 weeks.

Treatment:

1. Control group: No MnCl₂ exposure, received normal saline only.
2. MnCl₂ Control group: Received MnCl₂ only.

Duration: 8 weeks (including 4 weeks of treatment post MnCl₂ exposure).

Twenty-four hours after the last treatment, animals were sacrificed, drawing blood directly from the heart without anesthesia using a puncture heart, after that animals were killed by subjected to decapitation, the head was placed in an ice dissecting basin, the skull was opened, the brain was extracted and washed by using cooled normal saline solution, then dry it with filter paper, removed the cerebellum and olfactory buds, and the remaining brain were placed in dry, clean plastic containers and kept at a temperature of -80 until

tests are carried out. The drawn blood was placed in dry and immaculate glass test tubes free of anticoagulant, left at laboratory temperature for 15-20 minutes, then samples were centrifuged at 3000 rotations / minute a duration of fifteen minutes in order to separating serum, then isolating serum and preserving it at a temperature of $-20^{\circ}C$ until use.

Biochemical Assessments Analysis:

Liver Enzyme Assays: Sera were analyzed for alanine aminotransferase (ALT); aspartate aminotransferase (AST), alkaline phosphatase (ALP); and Gamma - Glutamyl Transferase (GGT); effectiveness using kits for standard enzymatic assay .**Neurotransmitter Assays :** Brain tissue was collected post euthanasia and analyzes for serotonin, dopamine and GABA levels were performed using the high-performance liquid chromatography (HPLC) method. Serum catalase (CAT) and malondialdehyde (MDA) were estimated by the method used by Wang (Wang et al., 2009). The concentration of glutathione (GSH) in serum was measured by using the modified method of Sedlak (Sedlak and Lindsay1968), the method is based on the use of Ellmans reagent-5,5 (dithio bis(2-Nitrobenzoic acid) DTNB,(which reacts with glutathione

to form a colored product whose absorbance is read at 412 nm. The technique suggested by Sun et al. was used to measure SOD activity (Sun et al, 1988).

Statistical Analysis

One-way ANOVA was used to determine significant differences between groups. Differences between data were compared using the least significant difference (LSD). Mean standard deviation is displayed for all data. All statistical tests were completed using the statistical program SPSS (version 21.0) for its version at the significance of ($p \leq 0.05$) (Bryman and Cramer 2011).

Results

Liver Enzyme Activities

Results showed a significant decrease in ALT , AST and ALP levels in rabbits of WS and Rhodiola groups compared to $MnCl_2$ ($p \leq 0.05$), but levels of GGT in Rhodiola group were significantly reduced than both control and WS as well as group of $MnCl_2$. The impact on liver enzymes is shown in Table 1.

Table 1: Effects of WS and Rhodiola Treatment on Liver Enzymes in $MnCl_2$ -Stressed Male Rabbits

Groups	ALT (U/L) mean $\pm$ SD	AST (U/L) mean $\pm$ SD	ALP (U/L) mean $\pm$ SD	GGT(U/L) mean $\pm$ SD
Control	50 $\pm$ 4.1 b	55 $\pm$ 2.1 b	90.6 $\pm$ 6.2 b	9.1 $\pm$ 2.1 b
$MnCl_2$	75 $\pm$ 3.1 a	80 $\pm$ 4.2 a	102.5 $\pm$ 2.1 a	11.4 $\pm$ 1.3 a
$MnCl_2$ + WS	38.7 $\pm$ 2.8 c	45 $\pm$ 3.2 c	84.3 $\pm$ 5.1 c	8.3 $\pm$ 1.7 c
$MnCl_2$ + Rhodiola	42 $\pm$ 3.5 c	48 $\pm$ 3.5 c	89.4 $\pm$ 4.8 c	7.9 $\pm$ 1.2 d

The significant differences at ($p \leq 0.05$) indicated by different letters.

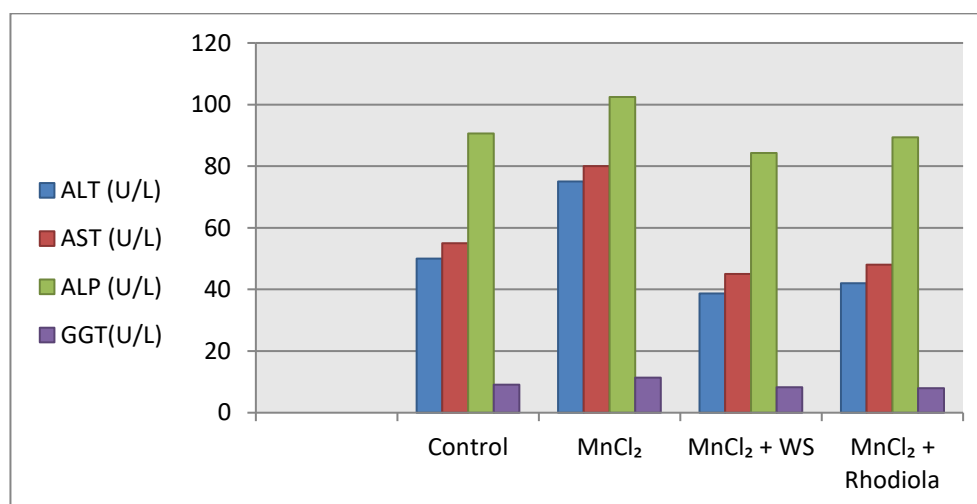


Fig. 1. Effects of WS and Rhodiola Treatment on Liver Enzymes in $MnCl_2$ -Stressed Male Rabbits.

Neurotransmitter Levels:

Both WS and Rhodiola significantly increased serotonin and dopamine levels ($p \leq 0.05$), with WS demonstrating a slightly higher effect. $MnCl_2$ exposure significantly reduced serotonin

levels in contrast to the control. In this study $MnCl_2$ produced a notable decline in GABA levels in male rabbits. Both WS (Ashwagandha) and Rhodiola supplementation significantly restored GABA nearly to the standard levels..

Table 2: Effects of WS and Rhodiola Treatment on neurotransmitters in $MnCl_2$ -Stressed Male Rabbits

Groups	Serotonin (ng/mL) mean $\pm$ SD	Dopamine (ng/mL) mean $\pm$ SD	GABA(μ g/g) mean $\pm$ SD
Control	85 $\pm$ 1.6 b	165 $\pm$ 4.3 b	45.6 $\pm$ 2.1 a
$MnCl_2$	45 $\pm$ 2.4 c	95 $\pm$ 6.2 c	21.4 $\pm$ 1.7 c
$MnCl_2$ + WS	100 $\pm$ 5.8 a	175 $\pm$ 1.4 a	38.9 $\pm$ 2.1 b
$MnCl_2$ + Rhodiola	95 $\pm$ 3.7 a	169 $\pm$ 1.2 a	36.5 $\pm$ 1.8 b

The significant differences at ($p \leq 0.05$) indicated by different letters.

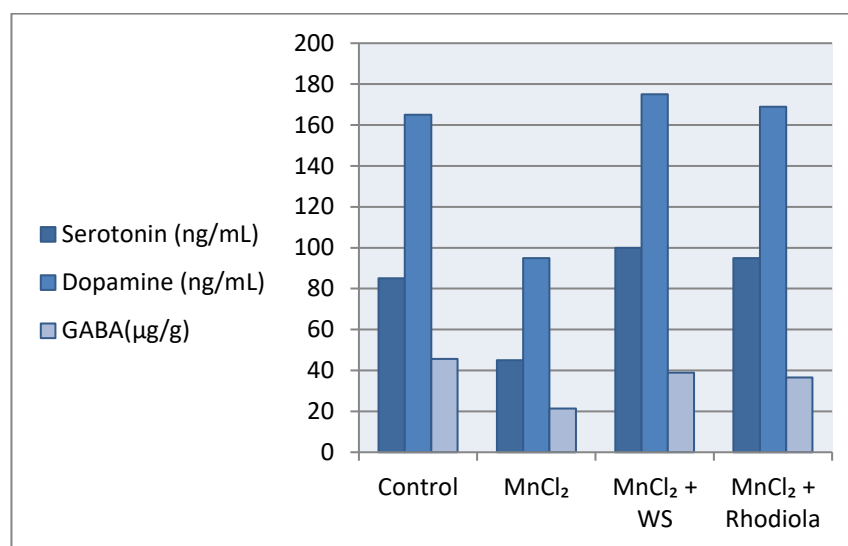


Fig. 2. Effects of WS and Rhodiola Treatment on serotonin and dopamine in $MnCl_2$ -Stressed Male Rabbits.

Antioxidant Parameters

$MnCl_2$ exposure caused the SOD and CAT activities to significantly decline., However (WS) and Rhodiola treatments improved levels of these antioxidant enzymes. GSH: The reduced glutathione levels were markedly lowered by $MnCl_2$

exposure. Treatments with Ashwagandha and Rhodiola individually improved GSH levels. The impact on neurotransmitters is shown in Table 3.

Table 3: Effects of WS and Rhodiola Treatment on Antioxidant Parameters in $MnCl_2$ -Stressed Rabbits

Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (μ mol/mg protein)
Control	24 $\pm$ 2.1 b	18 $\pm$ 1.4 b	10 $\pm$ 0.5 b
$MnCl_2$	10 $\pm$ 1.1 c	6 $\pm$ 0.6 c	4 $\pm$ 0.2 c
$MnCl_2$ + WS	32 $\pm$ 3.2 a	28 $\pm$ 2.1 a	15 $\pm$ 0.8 a
$MnCl_2$ + Rhodiola	30 $\pm$ 2.4 a	25 $\pm$ 2.2 a	13 $\pm$ 0.7 a

The letters representing the significant differences at ($p \leq 0.05$).

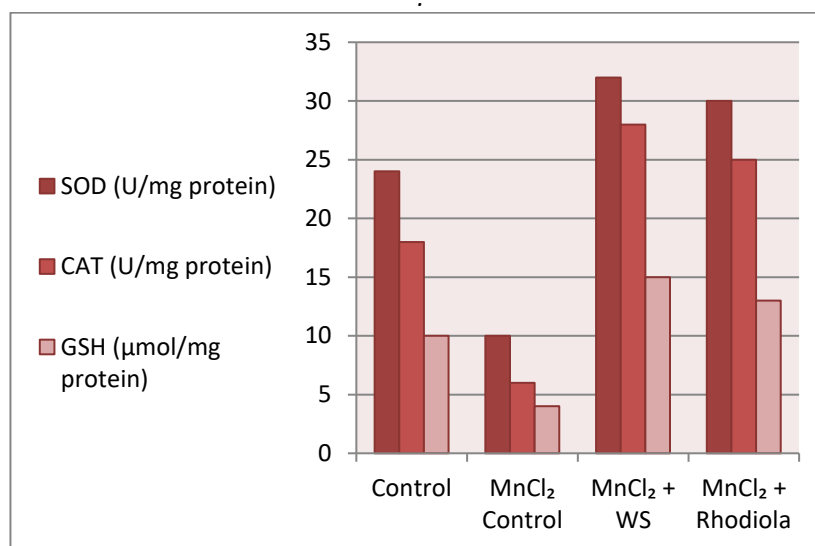


Fig. 3. Effects of WS and Rhodiola Treatment on SOD,CAT and GSH in $MnCl_2$ -Stressed Male Rabbits.

Discussion

Elevation of liver enzymes due to $MnCl_2$ exposure indicates liver damage (Gyan Chand Jain 2016). while enzyme levels in WS and Rhodiola groups indicate improved liver function and protection against liver injury. The consumption of Rhodiola extract had a significant effect in lowering GGT levels, indicating a strong hepatoprotective effect and a more intense effect on liver enzyme system compared to WS (Panossian, and Wagner 2005); (Kelly 2001).

The results for serotonin are consistent with the finding that $MnCl_2$ disrupts the serotonergic system (Blecharz et al., 2012), (Bouabid, et al., 2014). Treatment with WS and Rhodiola significantly increased serotonin levels, suggesting a compatible protective mechanism (Patel, and Singh, 2021). WS and Rhodiola found similar improvements in dopamine levels in both treatment groups, were effective, and the treatment was capable of restoring dopamine levels, which may be due to the complementary actions of the herb. (Rahman, 2021).

In this study, $MnCl_2$ significantly decreased GABA levels in male rabbits, suggesting a loss of GABAergic neurotransmission (Gupta and Kumar, 2020). Both WS (Ashwagandha) and Rhodiola supplementation significantly restored GABA levels compared to the $MnCl_2$ group, reflecting their neuroprotective activities known for its adaptogenic stress-reducing effects (Chaudhary 2023). Rhodiola caused a flight of GABA levels effectiveness, possibly through interactions with stress mechanisms and changes in neurotransmitter activity (Smith, and Patel 2022), (Verma, et al., 2021).

Both enhanced these activities, with significant restoration of antioxidant enzyme activities, indicating their ability to neutralize reactive oxygen species (ROS). Research findings $MnCl_2$ induces oxidative stress comes in line with previous studies (Brown and Miller 2019). Improved antioxidants after WS and Rhodiola treatment. enzyme activity; GSH levels and

effective antioxidants support their role in the pathway. The improved effect observed in combination therapy highlights how these adaptors can be used in combination to maximize their protective benefits (Patel and Singh 2021).

Conclusion

The study demonstrates that both WS (Ashwagandha) and Rhodiola provide significant neuroprotective, antioxidant, and hepatoprotective effects in $MnCl_2$ -induced stressed male rabbit models with no differences except for a few significance. Further studies including the combined treatment of WS and Rhodiola could show the most effective results, and could indicating a potential synergistic benefit in mitigating the adverse effects of $MnCl_2$ exposure.

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