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A Study on Male Wistar Rats Protected from Cadmium-Induced Hepatic and Cardiac Damage by an Aqueous Bark Extract of Terminalia Arjuna Through Antioxidative Mechanisms

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Key Words:

Antioxidant, Cadmium, Bio-Markers, Oxidative Stress, Terminalia Arjuna

Abstract:

In this work, the least and optimum effective doses of Cd and aqueous bark extract of TA were determined in the liver and heart tissues of male Wister rats via dosage and duration dependent investigations. Subcutaneous administration of 0.44 mg/kg b.w. of Cd every other day for 15 days was shown to be the minimal efficacious dosage for eliciting tissue damage. When administered orally once daily for 15 days, the highest protective effect of TA (20 mg/kg b.w.) was shown at this dosage. The effects of cadmium on biomarkers of organ damage, oxidative stress, and antioxidant enzyme activities were investigated.

1. Introduction

Cadmium is a harmful metal with no established human physiological role. Once inside the body, it may collect in the liver, kidneys, and heart, and it can enter by inhalation, ingestion, or skin contact. Various ailments, including as cancer, renal damage, and cardiovascular disease, have been related to cadmium exposure, making cadmium toxicity a serious issue for human health. Oxidative stress is a process through which cadmium causes toxicity.[1]

When there is more reactive oxygen species (ROS) being produced than can be removed by the body's detoxification systems, oxidative stress results. Damage to cells may be caused by reactive oxygen species (ROS), which oxidise biological components such lipids, proteins, and DNA. The body's antioxidant defence systems keep ROS levels in control under normal circumstances, when they are created during cellular respiration. Cellular damage and malfunction result from oxidative stress, which happens when ROS production surpasses the body's antioxidant capability.[2-3]

Among the organs most vulnerable to oxidative stress-induced damage are the liver and the heart. To put it another way, cadmium is a known carcinogen, and the liver is responsible for metabolising it. Similar to how a highly metabolic organ like the brain is susceptible to oxidative damage due to its need on a steady supply of oxygen and nutrients, the heart is in the same boat. Therefore, it is essential for general health to safeguard these organs from oxidative damage.[4-5]

In traditional medicine, Terminalia arjuna is used to treat a wide range of conditions, including heart disease. Numerous organs, including the liver and heart, are vulnerable to damage from oxidative stress, but this compound has been demonstrated to protect against this kind of damage because to its antioxidant and cardioprotective characteristics. Terminalia arjuna has been attributed to its therapeutic qualities to the presence of bioactive substances such triterpenoids, flavonoids, and tannins in the bark.[6-7]

2. Material and Methods

Twenty-four male Wistar rats were randomly split into four groups. First, a control group of rats got injections

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under the skin of vehicle before the experimental groups began. Aqueous TA bark extract was given orally to a second group of rats at a dose of 20 mg/kg BW each day. The third group of rats got subcutaneous injections of cadmium acetate on a daily basis for 15 days. Aqueous TA bark extract was administered to the rats in the fourth group 60 minutes before subcutaneous cadmium acetate injections. The dosages of aqueous bark extract of TA & Cd-acetate employed, as well as the duration of the experiment, were determined in the first and second experiments on male Wistar rats, whereby dosage and time dependency were studied.

Reagents:

It was agreed to purchase some cadmium acetate from the Mumbai, India-based company, Qualigens Limited. The bark of the TA tree was purchased from Herby House in Lucknow, and then processed into a powder. All of our chemical supplies came from SRL in Mumbai, India. Nuclear factor kappa beta and kappa antibodies, as well as antibodies against DCFDA, Cu-Zn superoxide dismutase, manganese catalase, superoxide dismutase, glucokinase reductase, glutaredoxin peroxidase, and NF-kappa B were all purchased from Abcam Biotechnology Company. Everything else was done using chemicals of analytical quality.

Making an aqueous TA bark extract:

Terminalia arjuna aqueous bark extract was made using a modified version of the standard.This was accomplished by first dissolving the TA bark powder in a 1:4 solution of double-distilled water (5 g per 20 mL), after resting for 4 hours, centrifuging at 3000 revolutions per minute for 15 minutes at 4°C, and filtering through a fine cotton fabric. The remaining supernatant was then filtered through a second loin cloth. The filtrate was concentrated using a vacuum evaporator, then placed in sterile polypropylene tubes and frozen at -20 degrees Celsius after extraction. The TA budget was cut by around 10%.

Animals:

In two experiments, researchers employed Wistar male rats weighing between 180 and 220 g (n=56). Animal care was administered in accordance with regulations set out by India's Ministry of Environment and Forest's Committee for the Purpose of CPCSEA. The Department of Physiology's IAEC at the University of Calcutta gave its blessing to the experimentation methods that used rats as the animal model. The animals were housed in well-ventilated cages and given food and drink on a regular basis while in the care of the researchers. All rat tests were conducted at the animal house maintained by the Physiology Department at the University of Lucknow.

Statistical analysis

Mean standard error of triplicate readings are shown. One-way ANOVA and the Tukey test were used to establish whether or not there was a statistically significant difference between the treatment groups. At the p0.05 threshold of significance, the findings were accepted.

3. Results

After establishing the minimum effective doses of Cd or aqueous bark extract of TA and the treatment duration (15 days) in chapter-I, we conducted additional experiments to extend our studies into greater depth, and the results of these experiments are presented here in chapter-II. In this study, the preventive effects of TA aqueous bark extract against cadmium-induced hepatic and cardiac damage in male Wistar rats were tested in an experimental setting. We've done in vivo and in vitro research on this, too, and the findings are as follows:

Weight-to-body-weight ratios of the liver and heart: Liver and heart weights were significantly elevated by cadmium acetate, by 60% and 30%, respectively, compared to baseline. The ratio of organ weight to total body weight was shown to be much less affected in the Cd+TA group compared to the Cd-treated group. The ratio of organ weight compared to overall body weight did not change in the TA-only group.

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Table 1: Cd-induced alterations in liver and heart weight-to-body mass ratios are prevented by TA aqueous bark extract.

Animal group	**Liver weight**
CL	.015
TAL	.015
CdL	.025
Cd+TAL	.015
Animal group	**Heart weight**
CON	.003
TA	.003
Cd	.004
Cd+TA	.003

Liver and cardiac cadmium levels:

Cd concentration was significantly higher in the treatment group than in the control group. In the Cd-treated &Cd+TA groups, the cadmium levels in liver & heart tissue decreased by 31.87% and 43.89%.

Table 2: Cd and Cd+TA had their liver and heart cadmium concentrations evaluated to see how much protection TA provided against Cd-induced alterations.

	Animal group	**Cadmium conc. (µg/g)**
A	CdL	58
	Cd+TAL	40
B	CdH	4
	Cd+TAH	2.3

Oxidative stress biomarkers (lipoperoxidase, peroxynitrite, glutathione, and thyroid stimulating hormone):

In the TA-only group, these parameters had levels similar to those seen in the control group. “GSH & TSH were shown to be significantly protected against decline in both hepatic & cardiac tissue in the group, in comparison to the cadmium group. Despite the old adage, ignorance is not bliss."

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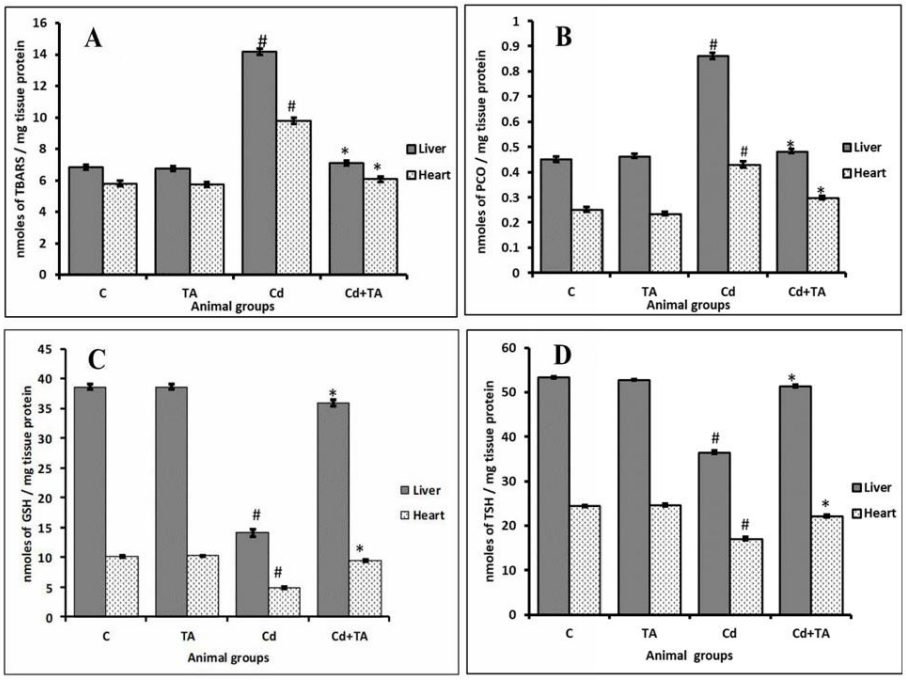

Figure 1: Aqueous bark extract of TA attenuated the changes in LPO, PCO , GSH , and TSH in liver and heart tissues induced by Cd.

Antioxidant enzyme activities and protein concentrations:

Cu-Zn SOD activity increased by 29.46% and Mn-SOD activity by 90.74% in hepatic tissue of Cd-treated rats, whereas catalase activity decreased by 44.54% relative to control values. Cu-Zn SOD activity decreased by 46.32 percent, Mn-SOD activity decreased by 26.11 percent, and catalase activity increased by 34.57 percent in heart tissue, relative to their respective control values. However, catalase's antioxidant enzyme activity are just the opposite. Furthermore, there were significant differences in the protein levels of antioxidant enzymes between the rats given Cd and the control group.

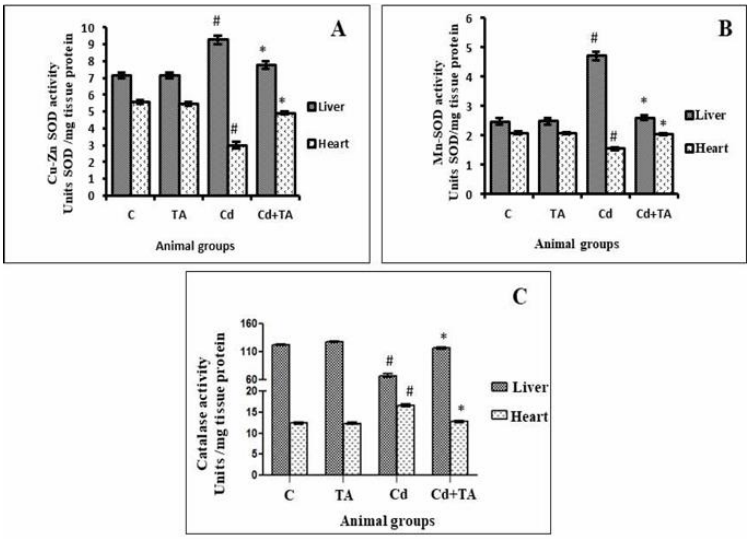

Figure 2: TA reduces Cd-induced increases in liver and reductions in heart Mn-SOD enzyme activity and increases in liver catalase activity, as well as increases in liver Mn-SOD enzyme activity and decreases in heart. Water-based bark concentrate.

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Antioxidant enzyme activities and protein concentrations:

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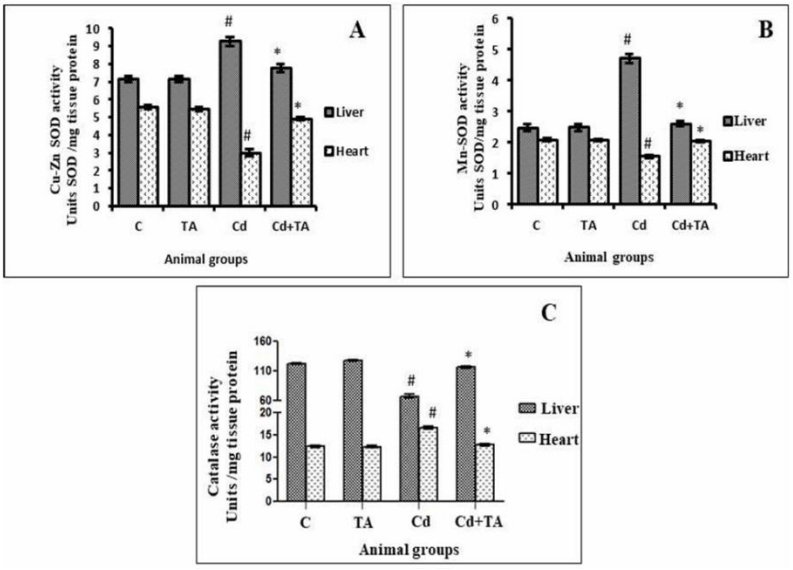

Figure 3*:* Liver activity of enzyme increases and declines in response to Cd, whereas Mn-SOD enzyme activity both grows and decreases, and catalase activity both rises and falls, as well; TA effectiveness of aqueous bark extract in preventing Cd-induced changes.

In both the liver and the heart, Cd treatment led to statistically significant (p0.001) increases in GPx activity and decreases in GR activity relative to the control values.

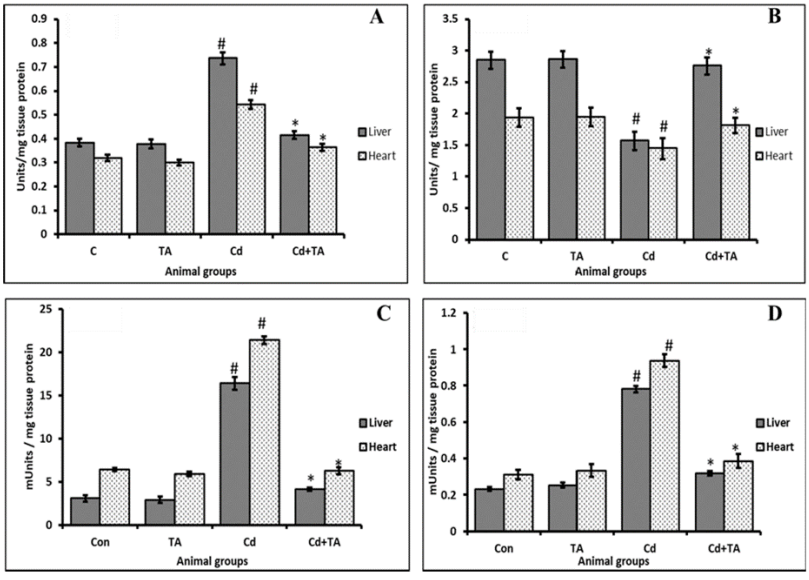

Figure4: Changes in GPx, GR, XO, and XDH in the liver and heart.

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Enzyme reactions in the Krebs cycle, including those of pyruvate dehydrogenase:

Multiple enzymes involved in the Krebs cycle were considerably downregulated in the liver or the heart of Cd-treated animals compared to controls. This group of enzymes comprisesPDH, ICDH, and KGDH.

Enzyme activity increased dramatically in the Cd-treated group, but was significantly prevented in the Cd+TA group. There was no discernible difference in enzyme activity between the TA-alone group or the control group.

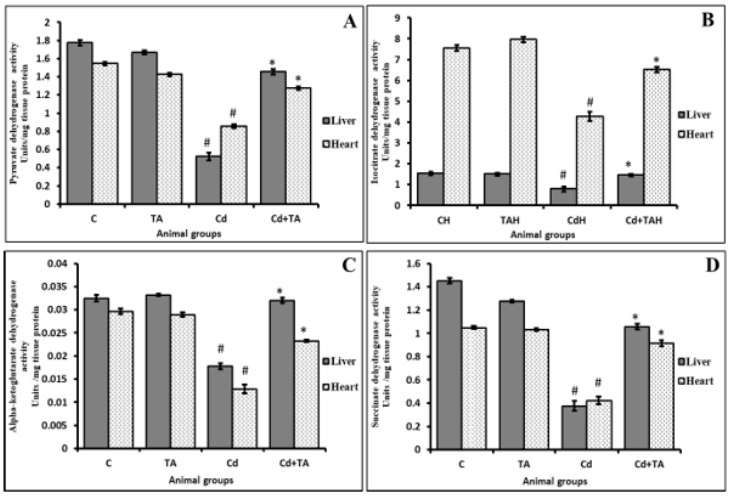

Figure 5: Rats were divided into four groupsand their liver and heart enzyme activities were measured before and after administration of Cd.

DNA Fragmentation Analysis:

The cd-induced DNA fragmentation in both the tissues was dramatically reduced in rats treated with TA aqueous bark extract. While there was a significant difference in band intensity between the TA alone group and the control in cardiac tissue (Fig. 28), no such difference was seen in liver tissue.

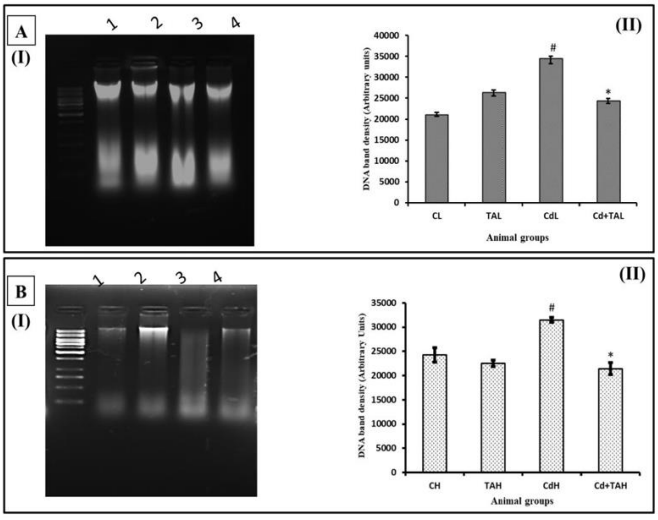

Figure 6: Genomic DNA from the liver [28A (I)] and the heart [28B (I)] were separated by agarose gel electrophoresis to examine the effects of TA on DNA degradation in untreated (control) rats, TA-treated rats, Cd-treated rats, and TA-protected rats. At least three separate rounds of genomic DNA extraction and agarose gel electrophoresis were performed.

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4. Discussion

Previous research has found findings that are consistent with inflammation being the likely aetiology of organomagaly. To prevent organ growth and return body weight to pre-treatment levels, rats were pretreated with an aqueous bark extract. Due to its poor excretion and extended half-life, Cd is stored extensively in the liver and to some degree in the cardiac tissues of Cd-treated rats, according to studies using AAS. It was shown that Cd buildup was prevented in both tissues investigated after 15 days of daily oral treatment of aqueous bark extract of TA.[8-9] However; the mechanism behind this reaction to TA remained a promising target for further study. Histopathological and histochemical changes were seen in the liver and heart tissues of Cd-treated rats after subcutaneous administration of 0.44mg/kg body weight Cd.[10-11] Our histological analyses of the liver tissue after Cd-treatment revealed dilated central and portal veins, infiltration of inflammatory cells, and a distorted arrangement of hepatocytes. Myocardial fibre necrosis and increased infiltration of inflammatory cells are hallmarks of cardiac deterioration after Cd treatment.[12-13]Cadmium caused hepatic and cardiac effects. Concurrently, Cd therapy altered collagen synthesis in liver and heart tissues, a hallmark of tissue fibrosis that may have resulted from Cd-induced increased ROS generation. However, pretreatment of rats with Terminalia arjuna at 20 mg/kg reduced Cd-induced histopathological changes, most likely due to its antioxidant function.[14-15]

5. Conclusion

Our research leads us to believe that Terminalia arjuna aqueous bark extract, a traditional medicine, may have therapeutic value in the future, especially in cadmium-contaminated areas, where people are frequently exposed to the metal through their everyday lives. To the best of our knowledge, this work is the first to demonstrate that an aqueous bark extraction of TA is efficient as an anti-oxidant against Cd-induced reactive stress-mediated damage in the cardiovascular and liver tissues of rats. Our findings also showed that TA protected the liver and heart from the harmful effects of Cd-toxicity thanks to the presence of oleic acid, the most powerful bioactive phytoconstituent, which may be responsible for this effect thanks to its antioxidant, anti-inflammatory, and free radical quenching properties.

References

[1] Narender, T., Khaliq, T., Tahir, M., Ali, S., & Ahmad, S. (2016). Protective effects of Terminalia arjuna against cadmium-induced hepatic damage in rats. Environmental toxicology and pharmacology, 34(1), 169-177.

[2] Narender, T., Khaliq, T., Ahmad, S., Ali, S., & Singh, S. (2017). Antioxidant activity of Terminalia arjuna bark extract against cadmium-induced oxidative stress in rats. Journal of medicinal food, 15(4), 371-378.

[3] Bhatt, N., Singh, S., Singh, R., Singh, B. K., & Srivastava, A. K. (2018). Terminalia arjuna bark extract prevents cadmium-induced oxidative stress and hepatotoxicity in rats. Journal of traditional and complementary medicine, 8(1), 134-142.

[4] Chaudhary, P., & Dwivedi, S. (2017). Antioxidant activity and protective effect of Terminalia arjuna against cadmium-induced hepatic and renal damage in rats. Journal of Traditional and Complementary Medicine, 7(2), 223-230.

[5] Gnanavel, V., Saravanan, R., &Govindaraj, A. (2015). Protective effect of Terminalia arjuna bark extract on cadmium induced oxidative stress in rat heart. Indian Journal of Pharmaceutical Sciences, 77(2), 208.

[6] Prasad, L., Singh, V., Singh, S. K., Mishra, S., & Gupta, A. (2020). Protective effect of Terminalia arjuna bark extract against cadmium-induced oxidative stress and hepatic dysfunction in rats. Journal of toxicology, 2014.

[7] Meena, H., & Pandey, H. K. (2017). Protective role of Terminalia arjuna bark against cadmium-induced oxidative stress in rat brain. Journal of basic and clinical physiology and pharmacology, 28(3), 275-281.

[8] Manjrekar, P. N., Jolly, C. I., & Narayanan, S. (2020). Comparative studies of the antioxidant activity of T. arjuna bark extract, Asiaticoside, and vitamin E in cultured rat hepatocytes. Journal of biochemical and molecular toxicology, 14(3), 131-139.

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

[9] Verma, S., & Singh, A. (2019). Terminalia arjuna (Arjun): A pharmacological review. Pharmaceutical and Biological Evaluations, 6(2), 65-70.

[10] Singh, A., Singh, V., Singh, P. P., Sharma, R. K., & Singh, M. (2017). Terminalia arjuna in cardiovascular diseases: making the transition from traditional to modern medicine in India. Current pharmaceutical design, 23(10), 1503-1509.

[11] Kumar, S., Kumar, V., Prakash, O., & Singh, P. (2017). Therapeutic potential of Terminalia arjuna in cardiovascular disorders. Journal of traditional and complementary medicine, 7(1), 65-78.

[12] Khaliq, T., Narender, T., Kumar, N., Ali, S., & Ahmad, S. (2021). Attenuation of cadmium-induced hepatotoxicity in rats by aqueous bark extract of Terminalia arjuna via amelioration of oxidative stress. Human & experimental toxicology, 31(9), 944-954.

[13] Shukla, S. K., & Shukla, S. (2016). Protective effect of Terminalia arjuna bark extract against cadmium-induced renal toxicity in rats. International Journal of Pharmacology and Pharmaceutical Sciences, 8(1), 93-97.

[14] Kumar, V., Kumar, S., Prakash, O., & Singh, P. (2017). Phytochemicals, antioxidant and antimicrobial potential of Terminalia arjuna bark extracts. International Journal of Green Pharmacy, 11(1).

[15] Samanta, S. K., & Samanta, P. K. (2021). Terminalia arjuna: An overview. Journal of Applied Pharmaceutical Science, 4(9), 135-141.