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Calotropis Procera Extract Ameliorated Thyroid Toxicity Of Magnetic Iron Oxide Nanoparticles In Rats Via TLR4/MYD88/NF-κB Inflammatory Pathway

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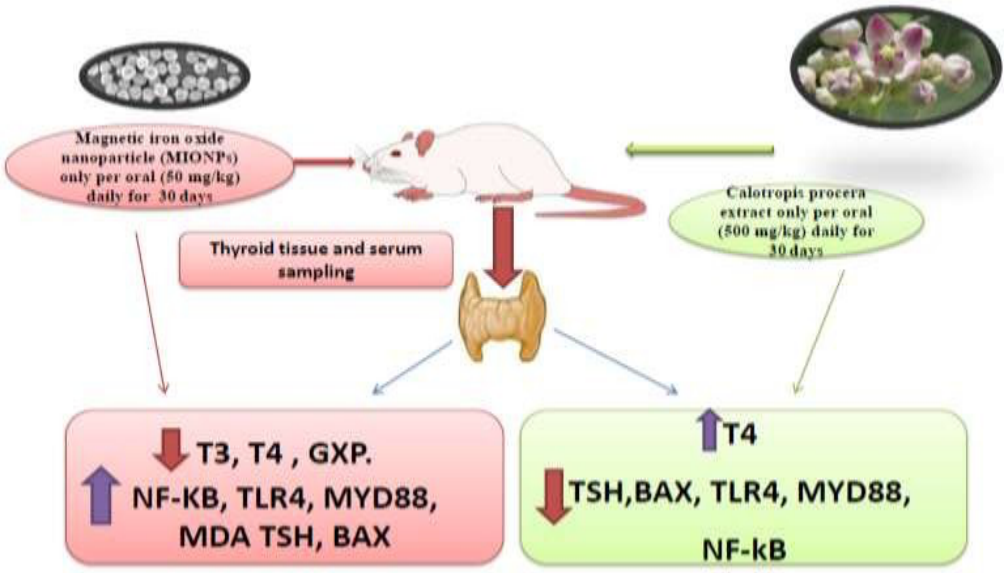
GRAPHICAL ABSTRACT



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Abstract:

Background: *Magnetic iron oxide nanoparticles (MIONPs) are widely used in many fields of life including the medical field, food and textile industries due to their unique properties. The long-term effects of MIONPs on the body are not fully studied. In addition natural products have emerged in the medical field as protective agents against toxic substances.*

Objective: *The purpose of our investigation was to determine the effectiveness of Calotropis procera (CP) extract on MIONPs induced thyroid function alternation in a rat model.*

Methods: *The study was conducted on eighty male Wistar rats (100-150g). Rats were allocated randomly into 4 groups of 20 rats; group (1) control (only tape water), group (2) CP extract only per oral (500 mg/kg Bw) daily, group (3) MIONPs only per oral (50 mg/kg Bw) daily and group (4) MIONPs (50 mg/kg Bw) plus CP extract per oral (500 mg/kg Bw) daily. At the end of the 30th day, Animals were sedated, and samples were obtained right away for assessment of tissue levels of MDA and GPX, Quantitative analysis of NF-κB, Western blotting with Protein-tec Kits, and serum hormone evaluation in addition to evaluation of apoptosis in thyroid tissue. Data were collected, tabulated and statistically analyzed.*

Results: *MIONPs intoxication caused a non-significant decrease in serum T3, T4 and tissue GXP. Also significant increase resulted in serum NF-κB, TLR4 and MYD88 proteins expression and tissue MDA level, while non-significant increase was noticed in serum TSH and BAX gene expression. After treatment with CP extract, there was non-significant increase in serum T4 but non-significant decrease was in serum TSH and BAX gene expression. TLR4 /MYD88/NF-κB axis showed significant decrease after treatment with CP. No conclusive results were noticed from Bcl_2 measurement.*

Conclusions: *MIONPs induced a toxic effect on thyroid gland through increased the levels of thyroid tissue MDA, NF-κB in addition to the protein expression of TLR4 and MYD 88. CP extract could ameliorate the MIONPs induced thyroid toxicity viaTLR4 /MYD88/NF-κB inflammatory pathway.*

Key words: Magnetic iron oxide nanoparticles, Nano toxicity, Nanoparticles, Thyroid, Calotropis procera, Health care

1. BACKGROUND:

Air pollution by industrial particulate matter and the advancement in nanotechnology field take toxic exposure to nanoparticles to the front **(1).** A Number of nanoparticles inflicted toxic effects including inflammation, growth rate retardation, neurobehavioral malfunction, ulceration, viability defects and causing malformation in plants and cell lines plus animal models too. The nano toxic effects is incriminated to their physiochemical properties as great surface ratio comparing to their small size, dose, attenuation in body, target organ toxicity, immunogenicity, metabolism and excretion in living body **(2)**. MIONPs are incorporated into biomedical, electronics, transportation, cosmetics products and industrial applications; as drug delivery systems, MRI imaging, as industrial thermoplastics and catalysts; become aerosolized during these activities forming a portion of circulating air; however, the negative consequences of occupational exposure to MIONPs are yet unclear **(3)**. Conflicting research findings about the toxicity of MIONPs have been reported. For instance, Karlsson et al. (4) observed a mild level of toxicity accompanied by the generation of intracellular reactive oxygen species (ROS). Conversely, Karlsson et al. (5) found no evidence of toxicity at lower levels. Nevertheless, the administration of elevated doses of MIONPs leads to a reduction in cellular physiological processes predominantly as a result of oxidative DNA damage (6), impairment of mitochondrial function, regulation of gene transcription, and disruption of calcium-dependent signaling pathways (7). Previous studies have documented the adverse impacts of MIONPs on the development of animal models, including delayed hatching, embryonic abnormalities, and mortality in zebrafish embryos (8). Additionally, poor development and delayed maturation have been observed in mice exposed to MIONPs (9).

CP of Family Apocynaceae is distributed plant in the whole world; along roadsides, watercourses and sand dunes; particularly the Arabian Peninsula and the Middle East **(10)**. Traditional medicine used CP as a therapy for common cold, asthmatics, joint and bony aches, skin irritation and GIT problems **(11)**. Pharmacological evaluation of medicinal effects of CP extracts found curing action on cancer, inflammation, DM, fever, pain, oxidative stress, helmintic infestations, hyperlipidemia, microbial infection, and even convulsion. Their protective medicinal properties have also been attributed to phytoconstituents like, flavonoids alkaloids, saponins, glycosides, triterpenes and tannins **(12)**. The toxicity of MIONPs has received little exploration especially with increased industrial emissions in the whole world and in Egypt. Egyptian ecospecies is widespread, properly studied, available and cheap. The purpose of our investigation was to determine the effectiveness of CP extract on MIONPs induced thyroid function alternation in a rat model.

2. MATERIALS AND METHODS

2.1. Calotropis procera (CP) extration

Aerial parts of CP were harvested from an Egyptian desert land at Cairo-Ismailia Road. Plant Extraction was according to **Hifnawy et al., (13)**.

2.2. Magnetic Iron Oxide Nanoparticles (MIONPs):

In this study were produced by co-precipitation technique **(14).** In the FTIR spectrum of produced MIONPs, the vibration of the iron oxide group (Fe-O), hydroxyl group and atmospheric carbon dioxide are the functional groups that stand out the most. The surface charges of the prepared nanoparticles were measured by zeta potential analysis. The results show that they have zeta potential values of 0000 mV and 0000 mV. The transmission electron micrograph of examined MIONPs represented the outline with cubic like- shape. The average particle size is 20 ±5 nm in diameter.

2.3. Procedures for using animals in the experiment

Eighty male Wistar rats (weighing 100–150g) were kept according to conventional housing guidelines of animal treatment universal regulations in Animal House of Faculty of Pharmacy, El Nahda University, Beni-Suef, Egypt. All experimental procedures were approved and conducted according to the guidelines for the care and use of laboratory animals established by the Animal Research Ethical Committee at Beni Suef University, Egypt. Then, rats were subjected to a random allocation process, resulting in their division into four groups, each consisting of 20 rats. This division was carried out in order to assess the toxicity treatment. Over a period of 30 days, rats in each group received daily doses as specified below:

Group 1: control (only tape water).
Group 2: CP only per oral (500 mg/kg Bw) daily.
Group 3: MIONPs only per oral (50 mg/kg Bw) daily.
Group 4: MIONPs per oral (50 mg/kg Bw) plus CP per oral (500 mg/kg Bw) daily.

On the 30th day of the experiment, the animals were subjected to anesthesia, following which blood samples were promptly obtained from their cardiac regions. Subsequently, serum was acquired through the process of blood centrifugation, with 3500 acceleration applied for duration of 10 minutes. The thyroid samples were first separated, followed by homogenization, and subsequently subjected to centrifugation at 2000 for duration of 10 minutes. The supernatants were subjected to a second round of centrifugation at a speed of 10,000 for duration of 10 minutes. Subsequently, the supernatants were preserved at a temperature of -80 °C until they were utilized for biochemical assessments. Thyroid tissues necessary for analysis of gene expression were stored at a temperature of -80 °C in a specific solution.

2.4. Tissue homogenization for biochemical analysis

A 10% tissue homogenate was prepared according to **Verma and Kumar, (15).**

2.5. Tissue levels of MDA and GPX

The method described by **Ellman (16)** was employed to determine the concentration of glutathione peroxidase (GPX) in thyroid tissue.

The level of Malonyldialdehyde (MDA) in thyroid tissue was determined according to the method of **Okhawa et al. (17)**.

2.6. Quantitative analysis of NF-κB

NF-κB in thyroid tissue was determined using ELISA. ELISA kits were purchased from, Reagent Genie, Ireland.

2.7. Western blotting

Thyroid tissue was processed to obtain whole-cell extracts with Protein-tec Kits (USA) according to **Yue et al., (1)**. Data were normalized to β-actin.

2.8. Serum Hormone evaluation

The serum samples were subjected to analysis for T3, T4, and TSH using an ELISA Reader Stat Fax–2100.

Enzyme Immunoassay Kits provided by DRG International Inc., USA were utilized for this purpose. The method was described by **Klee and Hay (18)**.

2.9. The assessment of apoptosis in thyroid tissue

The gene expression analysis of Bcl-2 and BAX in thyroid tissue was conducted using the Real-time PCR iCycler® apparatus (Bio-Rad, Hercules, CA) along with sequence-specific primer pairs designed for the targeted genes. The actin gene, which is commonly used as a housekeeping gene, was employed for the purpose of normalization. The processing steps were conducted in accordance with the methodology outlined by **Spampanato et al. (19).**

Table (1) Primers for quantitative real-time PCR

Genes	Forward primer	Reverse primer
Bcl-2	5′ACTTCTCTCGTCGCTACCGTCGC3′	5′AGAGCGATGTTGTCCACCAGGG3
BAX	5′CCAGGACGCATCCACCAAGAAG3′	5′CCCAGTTGAAGTTGCCGTCTGC3

2.10. Statistical analysis

The data that was collected was analyzed using the SPSS program (SPSS for Windows Version 22, SPSS Inc., Chicago, USA). Statistical significance was assessed through the utilization of variance analysis, specifically the ANOVA test, to identify significant differences between groups. When there were statistically significant differences ($p<0.05$), the Duncan's multiple range post-hoc test was conducted. The mean values were found to be statistically significant at a significance level of $p<0.05$. The data are presented as mean values with standard deviation (SD) indicated.

3. RESULTS:

3.1. Effects of MIONPs and/or Calotropis procera extract (CP) on thyroid function test:

Table 2 showed that MIONPs intoxication (third group) caused non-significant decrease in serum levels of T3, T4 but increased TSH serum levels non-significantly. Treatment of the 4th group increased serum levels of T4 but decreased TSH serum levels non-significantly.

Table (2): Effects of Magnetic Iron Oxide Nanoparticles and/or Calotropis procera extract on thyroid function tests.

Variable /Group	Control	Calotropis procera extract (CP)	Magnetic Iron Oxide Nanoparticles (MIONs)	MIONs + CP	p-value
T3 (ng/ml)	2.11±0.25	1.96±0.32	1.65±0.44	1.36±0.34	0.063 (NS)
T4 (ng/ml)	5.48±0.81	5.47±0.36	5.42±0.85	6.00±0.54	0.248 (NS)
TSH (ng/ml)	1.84±0.88	1.96±0.69	3.30±1.26	2.33±1.13	0.205 (NS)

Means with different superscripts within the same row are significantly different at $p<0.05$, NS= non-significant differences between the different groups.

3.2. Effects of MIONPs and/or CP extract on oxidative stress biomarkers in rat thyroid homogenate:

Table 3 showed that MIONPs intoxication (third group) caused significant increase in MDA tissue levels (P-value <0.001) but non-significant decrease in GPX thyroid levels. In treatment group there were significant decrease in MDA tissue levels (P-value <0.001) comparing to MIONs intoxication group but no effect on GPX thyroid levels.

Table (3): Effects of Magnetic Iron Oxide Nanoparticles and/or Calotropis procera extract on oxidative stress biomarkers in mouse thyroid homogenate.

Variable /Group	Control	Calotropis procera extract (CP)	Magnetic Iron Oxide Nanoparticles (MIONs)	MIONs + CP	p-value
MDA (nmol/g)	0.70±0.15^{a}	1.09±0.13^{a}	1.81±0.28^{b}	0.76±0.19^{a}	<0.001**
GPX (U/g)	1.11±0.13	0.66±0.17	0.87±0.23	0.72±0.18	0.062 (NS)

Means with different superscripts within the same row are significantly different at $p<0.05$, NS= non-significant differences between the different groups.

3.3. Effects of MIONPs and/or CP extract on nuclear factor Kappa B (NF-κB) level in thyroid tissue:

Table 4 showed that MIONPs intoxication (third group) caused significant increase in NF-κB levels in thyroid tissue

(P value <0.001); and comparing to intoxication group; treatment group showed significant decrease in NF-κB levels back to control level (P value <0.001).

Table (4): Effects of Magnetic Iron Oxide Nanoparticles and/or Calotropis procera extract on nuclear factor Kappa B (NF-κB) level in thyroid tissue

Variable /Group	Control	Calotropis procera extract (CP)	Magnetic Iron Oxide Nanoparticles (MIONs)	MIONs + CP	p-value
NF-κB (ng/ml)	0.53 ± 0.12^{a}	0.61 ± 0.10^{a}	1.39 ± 0.22^{b}	0.58 ± 0.15^{a}	<0.001**

Means with different superscripts within the same row are significantly different at p<0.05.

3.4. Effects of MIONPs and/or CP extract on Gene expression of apoptotic markers in thyroid tissue:

Table 5 showed that MIONPs intoxication (third group) caused significant decrease in Bcl-2 gene expression in thyroid tissue (P value < 0.032) but in treatment group caused non-significant decrease in Bcl-2 gene expression in thyroid tissue (P value < 0.032) in comparison to MIONs group.

Intoxication group caused non-significant increase in BAX gene expression in thyroid tissue but after treatment caused non-significant decrease in BAX gene expression in thyroid tissue.

Table (5): Effects of Magnetic Iron Oxide Nanoparticles and/or Calotropis procera extract on Gene expression of apoptotic markers in thyroid tissue

Variable /Group	Control	Calotropis procera extract (CP)	Magnetic Iron Oxide Nanoparticles (MIONs)	MIONs + CP	p-value
Bcl-2	1.73 ± 0.21^{b}	1.60 ± 0.26^{b}	1.20 ± 0.20^{a}	1.17 ± 0.31^{a}	0.032*
BAX	2.33±0.25	2.97±0.21	2.73±0.35	2.57±0.45	0.196 (NS)

Means with different superscripts within the same row are significantly different at p<0.05, NS= Non-significant differences between the different groups.

3.5. Effects of MIONPs and/or CP extract on protein expression of TLR4 and MYD88 in thyroid tissue:

Figure (3) showed significant increase in TLR4 protein expression in intoxicated thyroid tissue (P value <0.001) but significant decrease in TLR4 protein expression in thyroid tissue (P value <0.001) in treatment group in comparison to intoxication and control groups.

There is also a significant increase in MYD88 protein expression in intoxicated thyroid tissue (P value <0.001) but significant decrease in MYD88 protein expression in thyroid tissue (P value <0.001) in treatment group in comparison to intoxication group.

Figure (2): Western blot analysis of MYD88 and TLR4 proteins expression in the thyroid tissues of the four rat groups

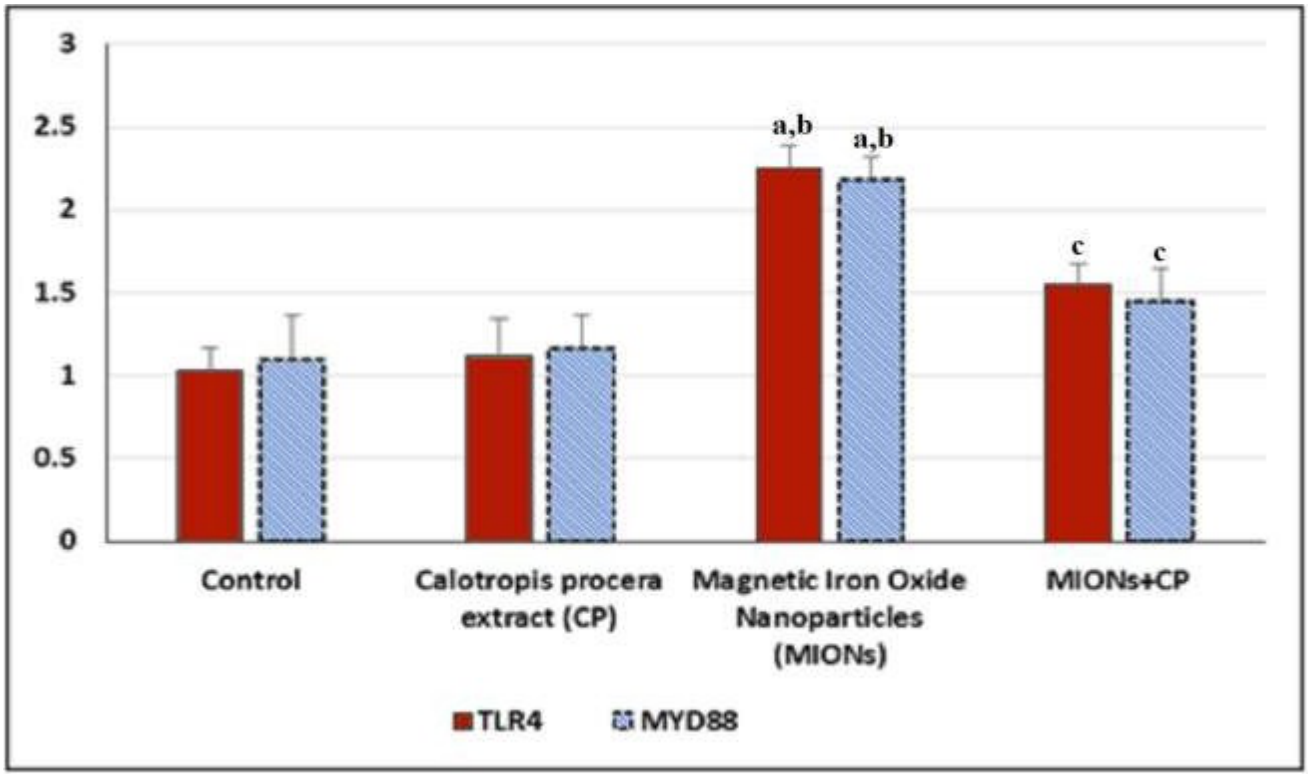

Figure (3): Effects of Magnetic Iron Oxide Nanoparticles and/or Calotropis procera extract on protein expression of TLR4 and MYD88 in thyroid tissue.

4. DISCUSSION:

In the field of medicine, magnetic iron oxide nanoparticles (Fe_3O_4NPs) have found widespread application in the diagnosis and treatment of tumors, as well as their application in MRI, biological catalysis, magnetic hyperthermia, magnetic targeting, magnetic separation, photo-responsive therapy, and drug-delivery (20).

The goal of this investigation is to study the effect that an extract of Calotropis procera has on alterations in thyroid function induced by MIONPs in a rat model.

According to the findings of this experiment, the effect that MIONPs have on the thyroid gland is evidenced by decreased blood levels of thyroid hormones (T3 and T4) and elevated serum levels of the hormone TSH. However, these changes were non-significant.

The effect of MIONPs on thyroid functions is not fully investigated with few publications about it. Akhtar et al. (21) conducted a research on this topic that assessed the effect of diet fortification with iron on thyroid functions found a significant impact on the thyroid function; as a consequence, this ultimately results in a decrease in the concentration of T4 in the serum. On the other hand, Jiang et al. (22) discovered that the thyroid functions of adult Wistar male rats was affected when they were given an oral dose of 150 µg /kg MIONPs. In contrast to the results obtained in our study, their study reported an increase in serum T3 levels. In their study, Fallahi et al. (23) employed a solution with a volume of 0.5 ml, containing concentrations of 100 and 200 ppm of Fe_2NiO_4Zn. Following duration of 14 days, the researchers observed the presence of enlarged and inflamed thyroid follicles, also reported a notable decrease in the concentration of thyroid-stimulating hormone (TSH), accompanied by a significant rise in serum thyroxin (T4) levels. However, the levels of triiodothyronine (T3) in the serum remained unaffected.

According to the findings of the current study, MIONPs are responsible for thyroid damage by triggering an inflammatory response. Induction of oxidative stress by magnetic iron oxide nanoparticles was demonstrated by the significant increase in tissue MDA levels seen in the experimental group in comparison to the control group. In addition, there was a decrease in the reduced glutathione tissue level; however, this difference did not show statistical significance.

Researchers investigating the oxidative stress caused by MIONPs in a variety of organs presented findings that were comparable to those found in this study. Following exposure to 2.3 nm MIONPs, levels of MDA increased, which is

suggestive of severe lipid peroxidation. This was reported by Wu et al., (24), who investigated the acute oxidative stress caused by MIONPs in different organs.

In a study that was quite similar, Dora et al., (25) studied the oxidative stress caused by MIONPs in the rat brain. The researchers observed a statistically significant difference in the levels of the lipid peroxidation product MDA between the MIONPs group and the control group. Specifically, the MIONPs group exhibited significantly higher levels of MDA. Furthermore, when comparing the MIONPs group to the control group, it was observed that the levels of GSH in the brain homogenate were significantly reduced ($p < 0.001$).

The production of ROS is a significant mechanism that contributes to the toxicity of MIONPs. This mechanism may result in oxidative stress, inflammation, and the consequent degradation of proteins, cell membranes, and DNA (26).

The NF-κB signaling pathway activation and up-regulation of apoptotic marker gene expression may also play a role in the cytotoxic effects exerted by MIONPs on thyroid tissue. According to the findings of this study, MIONPs intoxication dramatically decreased the BAX gene expression in thyroid tissue compared to the control groups, while slightly increasing the amount of Bcl-2 gene expression. The TLR4/NF-κB signal pathway as well as inflammatory factor-related proteins was investigated with the use of the Western blotting technique. The animals that were subjected to exposure exhibited elevated levels of TLR4 receptor and MYD88 protein expression in their thyroid tissue. Based on the findings of a quantitative study, it was observed that the concentration of NF-κB in the thyroid tissue of rats subjected to MIONPs treatment exhibited a statistically significant increase compared to the control group.

This pathway was proven in different studies performed on other organs. According to the findings of Ebrahimpour and colleagues (27), who investigated the effects of MIONPs on the brains of rats, mentioned that the normal level of NF-κB activity that occurs during inflammation is generally beneficial and helps prevent further damage to cells. However, an increase in this activity is harmful and causes apoptosis to be triggered in the cells. A significant contribution from Bcl-2 and BAX is essential for apoptosis. MIONPs -exposed rats had significantly increased levels of BAX/Bcl-2 expression in their hippocampi, which ultimately led to cell death via apoptosis**.**

According to the findings of the research conducted by Yue et al., (1), the mechanism of airway inflammation caused by MIONPs was determined to be via an increase in the expression of mRNA and proteins for TLR2, TLR4, TRAF6, MYD88, and NF-κB.

The effects of MIONPs on mouse pulmonary inflammation, the toll-like receptor pathway, and the production of downstream cytokines were studied by Sun and colleagues (28). MIONPs substantially upregulated ($p < 0.05$) the expression of TLR2, TLR4, MYD88, TRAF6, NF-κB, and TNF-α compared to the control group.

The NF-κB signaling pathway and the concurrent expression of the TLR4/MYD88 proteins result in the synthesis of diverse inflammatory cytokines, chemokines, and transcription factors. These molecules play a crucial role in initiating and regulating inflammatory responses and modulating the host's reaction to tissue damage (29).

Because of the beneficial role that they play in maintaining human health, compounds derived from plants and microbes have played a significant role in the development of modern medicine. As a result of the anti-inflammatory and antioxidant properties possessed by a number of naturally occurring compounds, there is a promising possibility for future discoveries to be made in the field of nanomedicine that is based on natural sources (30).

The target of this investigation was to examine whether an extract of Calotropis procera could help to reduce the negative effects of MIONPs on the thyroid. When the extract of Calotropis procera was added with MIONPs, a protective effect was triggered in the thyroid. This was demonstrated by a non-significant rise in serum T4 levels and a decrease in TSH levels. It has been demonstrated that the extract of Calotropis procera had antioxidant effect. This was demonstrated by a significant reduction in the MDA tissue level (P-value = 0.001) when compared to the MIONPs-intoxication group. On the other hand, there was no significant effect on the GPX thyroid level.

It is possible that the impact that Calotropis procera has on the thyroid gland can be explained by the presence of compounds in the plant that have a relationship to the gland. It has been demonstrated that Rutin, which is one of its components, can improve thyroid function by raising production of the hormones T3, T4, and improving the uptake of iodide in the thyroid. There is a relation between the thyroid gland and the major component stigmasterol. Stigmasterol has been related to having thyroid-inhibiting effects, as indicated by research demonstrating a drop in blood levels of T3 and T4. The research conducted by Leila and her colleagues (31) showed that stigmasterol followed a similar mode of action in the thyroid**.**

The study conducted by Okwudiri Ihegboro et al. (32) aimed to assess the potential anti-diabetic and hepatoprotective properties of calotropis procera root extract in a group of wistar rats. The researchers observed a notable elevation in the levels of GSH and a considerable decrease in the levels of MDA within the liver tissue. These findings provide evidence for the antioxidant properties of the extract derived from CP. These findings are in line with the results of our study. A slight increase in catalase (CAT) activity was observed, while no discernible impact on the activity of superoxide dismutase (SOD) was detected.

Based on the research conducted by Bharti et al. (33), it was observed that the administration of a methanolic extract derived from a plant significantly elevated the levels of GSH and SOD activity in the gastric tissue of Wistar rats. These rats were induced with ulcers caused by alcohol or aspirin. Additionally, this treatment was found to be associated with a reduction in the levels of thiobarbituric acid reactive substances (TBARS).

According to Dayana and Manasa (34), the presence of triterpinoids such as calotropin, calotoxin, cardiac glycosides, and calactin in the Calotropis procera plant is responsible for its antioxidant activity.

The observed decrease in protein expression of TLR4 and MYD88 in thyroid tissue, when comparing the intoxicated group with the MIONPs group, suggests the existence of an additional mechanism. Furthermore, the significant decrease in NF-κB levels returning to the control level offers supporting evidence for a plausible mechanism. Following the administration of CP extract, a notable reduction in the expression of the Bcl-2 gene was observed in thyroid tissue. However, the decrease in the expression of the BAX gene was not statistically significant. This was compared to both the groups who were intoxicated and the control group.

Previous studies have provided evidence supporting the presence of various compounds, such as cardenolides, triterpenoids, alkaloids, resins, anthocyanins, sterols, saponins, and proteolytic enzymes, within the latex of the Calotropis procera plant. Moreover, the plant exhibits the capacity to amass flavonoids, tannins, sterols, and saponins. According to Ramadan et al. (35), beta-sitosterol is the primary sterol present in the leaves.

The study conducted by Guoying et al. (36) revealed that the saponin compound exhibited inhibitory effects on the expression of TLR4, MYD88, NF-κB, and IL-1 in the synovial tissue of rat models induced to develop gouty arthritis. During the course of acute gouty arthritis progression in rats, researchers observed a significant upregulation of TLR4, MYD88, NF-κB, and IL-1 expression in the synovial tissue of the experimental group. This phenomenon was consistently observed in the experimental group over the duration of the entire study. The protein expression levels of TLR4, MYD88, and NF-κB were significantly decreased in the high- and medium-dose groups treated with total saponin, as compared to the control group. This suggests that the mechanism of action of total saponin in treating gouty arthritis may involve the regulation of the TLR4/MYD88/NF-κB receptor signaling pathway.

The expressions of TLR4, MYD88, and NF-κB were suppressed in the saponin of Panax quinquefolius (70 and 140 mg/kg) groups compared to the myocardial ischemia group, as reported by Yu et al., (37). Saponin has been shown to significantly suppress TLR4/MYD88/ NF-κB signaling axis. In their investigation on the potential protective properties of methanol extract of latex derived from Calotropis procera, the researchers observed a noteworthy down-regulation of Bcl-2. This finding pertained to an experimental model of colorectal cancer. Conversely, Kumar et al. (38) observed an up-regulation of BAX gene expression in cancer cells. This was the case despite the fact that they found an increased expression of BAX genes in cancer cells. Because normal cells and cancer cells are not identical, it makes sense to differentiate between the two.

5. CONCLUSIONS:

Magnetic iron oxide nanoparticles have a negative influence on the thyroid gland via the oxidative stress route, as well as through enhanced protein expression of the TLR4/ NF-κB signal pathway and inflammatory factor-related proteins MYD88, as well as gene synthesis of apoptotic markers. This is due to the fact that these nanoparticles cause an increase in the production of apoptotic markers. Extract of calotropis procera, when combined with MIONPs, was found to minimize thyroid toxicity. It's possible that the favorable effect can be attributed to the reduced gene expression of apoptotic markers and the protein expression of the TLR4/ NF-κB signal pathway in Calotropis procera extract.

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