

## Protective Effects of Silver Nanoparticles of *Moringa Oleifera* Leaves against Acrylamide-Induced Blood Toxicity in Rats

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### Abstract

The present study aimed to indicate the harmful effects of Acrylamide (ACR) on blood cell of rats and the protective role of silver nanoparticles (Ag-NPs) derived from *Moringa oleifera* (*M. oleifera*) leaves against this effects. Ag-NPs were produced and subsequently examined using Transmission Electronic Microscope and Dynamic Light scattering measurements. Twenty adult male albino rats were randomly divided into four groups (five per each): Control group; ACR group (50 mg/kg b.wt, in drinking water); MO-NPsACRS group (50 mg/kg b.wt, orally at the same time of each treatment) and Protective group, (MO-NPs/ACR group), MO-NPs for 3 weeks (50 mg/kg b.wt, orally) then ACR for 3 weeks (50 mg/kg b.wt). At the end of the experimental period, blood samples were collected in clean tubes containing anticoagulant for blood cells count.

Red blood cells counts (RBCs), hemoglobin levels (HB), the percentage of hematocrit value (HCT), Mean Corpuscular Volume (MCV), and Lymphocytes (LYM) were significantly decreased after administration of ACR. On the other hand, white blood cells counts (WBCs), Platelets (PLTs), MID cells and Granulocytes (GRAN) were significantly increased when compared with control group. Treatment with the nano-extract of *M. oleifera* significantly reduces the toxic effect of ACR on the counts of WBCs, PLTs, MID cells and GRAN cells. Furthermore, a significant increase and influence on the hematological counts of RBCs, LYM cells, level of HB, percentage of HCT and MCV was observed after administration of nano-extract of *M. oleifera* ( $p < 0.05$ ). These results indicate that nano-extract of *M. oleifera* leaves enhances the protection against the hematological toxicity of ACR in male rats..

**Keywords:** *Moringa oleifera*, Silver Nanoparticles, Acrylamide, Blood Toxicity.

### Introduction

Food products derived from raw materials that are low in proteins, rich in carbohydrates, and

heat treated at high temperatures ( $>120^{\circ}\text{C}$ ), are the main source of acrylamide (ACR), which has been shown to have carcinogenic effects in animals, causing genotoxicity, neurotoxicity, and reproductive toxicity. For this reason, ACR

may have neurotoxic effects on humans (**Ahn et al., 2002; Jamshidi & Zahedi, 2015**). According to several research, humans consume between 0.3 and 0.6 g/kg of body weight of ACR on a daily average. Children and teenagers are more at risk than other age groups due to their propensity to consume more foods containing ACR, such as french fries, chips, and crackers (**Kadawathagedara et al., 2018**). After exposure to ACR via ingestion, inhalation, or intradermally, accumulation is highest in concentration in the blood than in any other tissues (**Shipp et al., 2006**).

It has also been shown that ACR can be metabolised into glycidamide via the cytochrome P450 pathway. This can then form a DNA-reactive epoxide that may induce changes in the signal pathway and cellular function (**Tornqvist, 2005**). Furthermore, ACR causes a disturbance in hematological parameters, represented by a decrease in the rate of red blood cells (RBCs), a delay in the synthesis or destruction of hemoglobin (HB), and increased membrane resistance (**Ghorbel et al., 2017**). Imbalance between the reactive production of increased oxygen species and antioxidant capacity increases oxidative stress. This, in turn, plays an important role in ACR-induced toxicity via increased lipid peroxidation and disruption of endogenous antioxidant biomarkers (**Abdel-Daim et al., 2015**). Reactive oxygen species are produced in RBCs in a process known as the redox cycle and catalysed with transition metals such as Fe<sup>2+</sup> and Cu<sup>2+</sup> (**Prakash et al., 2007**).

In another study, to produce cells that are protected from oxidative damage, natural dietary antioxidants were used by removing free radicals. *M. oleifera* is of the Brassica order and belongs to the Moringaceae family, a single genus of approximately thirteen species (**Mahmood et al., 2010**). In tropical and subtropical countries, *M. oleifera* has become familiar as a native Indian medicinal herb (**Fahey, 2005**). Numerous studies have shown that *M. oleifera* leaves and seed extract are effective antioxidants that protect against the harmful effects of free radical attack and oxidative stress (**Prakash et al., 2007**). In addition, *M. oleifera* is rich in a variety of essential phytochemicals (enzymes) such as catalase, polyphenol oxidase, ascorbic acid oxidase, total phenols, and vitamins (**Aboulthana et al., 2021a**). Interestingly, it has been shown that the antioxidant efficiency of *M.*

*oleifera* leaf extract, which is rich in phytoconstituents, can be enhanced by incorporating silver nanoparticles (Ag-NPs) (**Aboulthana et al., 2021b**). Ag-NP applications in different areas, such as chemistry, pharmaceuticals, electronics, and catalysis, are well known. Ag-NPs can be produced using different methods. Among these, biological methods for their synthesis are environmentally friendly, as non-toxic chemicals are used in their production. More specifically, plant extracts, proteins, enzymes, triglycerides, antioxidants, glycoproteins, flavonoids, terpenes, and tannins are used to reduce and stabilise the nanoparticles (**Ramaswamy et al., 2019**). The purpose of this study is indicate whether there are harmful toxic effects caused by ACR on certain blood parameters of rats and to determine the possible protective role of Ag-NPs derived from *M. oleifera* leaves against the toxic effect of ACR.

## Material and Methods

### *Chemicals and Plant Materials*

The ACR used for this research was obtained from Sigma Chemical Company, England, UK. Fresh, loose leaves of *M. oleifera* plant (Family - Moringaceae) were collected from Damietta City, Egypt and were identified and confirmed by the Botany Department of Damietta university. The leaves were washed three times using distilled water, then dried and ground into powder.

### *Animals and their Housing*

The current study was conducted on 20 adult male Wister albino rats. The weight of each rat was approximately 150 grams, and they were purchased from Helwan Animal Station. The animals were housed in separate cages (five rats in each group) and were subjected to cycles of 12 hours light / 12 hours dark at 25±2 °C. They were acclimatised for 14 days before the experiments began and were allowed free access to food and water under the standard conditions.

### *Preparation of Moringa oleifera Extract (MO-extract)*

The *M. oleifera* Extract (MO-extract) was

prepared by adding 10 grams of powder to 100 mL of distilled water. This was subsequently mixed to dissolve the powder, which was then boiled for 10 minutes until the colour of the extracted solution changed to a light green colour. The extract was filtered through filter paper and stored at 4 °C for future use as (MO-extract) (**Abdel-Rahman et al., 2022; Nilanjuna et al., 2014**).

#### *Synthesis of Silver Nanoparticles (Ag-NPs) Carried by Moringa oleifera (MO-NPs)*

In this experiment, 10mL of MO-extract was added to 190 mL of 2 mM silver nitrate solution with continuous stirring. In order for the solution to turn dark brown, the mixture is stirred in a magnetic stirrer at room temperature for four hours and then incubated in the dark for 24 hours. At the same temperature, the solution was centrifuged at 5000 rpm for at least 20 minutes to allow the solution to condense into pellets. The supernatant was then discarded, and the granules were re-dispersed in distilled water and left to dry at a room temperature before being stored for future use (**Abdel-Rahman et al., 2022; Nilanjuna et al., 2014**).

#### *Characterization of Ag-NPs*

##### *Transmission Electronic Microscopy (TEM)*

Using TEM (JEOL JEM1230, Tokyo, Japan), the morphology and particle size of the chosen Ag-NPs formulation were assessed. An inspection required staining a drop of the diluted material with 2% (w/v) phosphotungstic acid and placing it on a copper grid. The experiment was carried out at room temperature, and an appropriate magnification was used to capture the image on the micrograph (**Shousha et al., 2019**).

##### *Measurements of dynamic light scattering (DLS) and zeta potential*

Using Dynamic Light Scattering (DLS) (Malvern Instruments), the hydrodynamic particle size, Polydispersity Index (PdI), and zeta potential of the phytosynthesized nanoparticles were determined. The processes for preparing the sample involved diluting it with Milli Q water and then sonicating it for 20 minutes in an ultrasonic bath to ensure that the suspended nanoparticles were properly

dispersed. The machine's temperature parameter was adjusted at 25<sup>0</sup> C (**Gupta et al., 2020**).

#### *Experimental Design*

The rats were randomly divided into four groups of equal numbers (five rats per each) as follows- :

1. Control group (CT): rats received nothing additional to their normal diet.
2. ACR group (ACR): rats were given 50 mg/kg body weight in their drinking water daily for three weeks (**Gawesh et al., 2021; Richard et al., 2006**).
3. MO-NPsACRS group (MO-NPsACRS): rats were given 50 mg/kg body weight of ACR and received MO-NPs 50 mg/kg body weight orally, daily, for three weeks at the same time (**Malathi et al., 2018; Ramaswamy et al., 2019**).
4. MO-NPs /ACR group (MO-NPs/ACR): rats received 50 mg/kg body weight of MO-NPs daily for three weeks and were given 50 mg/kg body weight of ACR daily for three weeks.

#### *Collection of Blood Samples*

At the end of experimental period, rats were anesthetised by exposing them to chloroform. All blood samples were then collected from the heart using 5 mL syringes. The blood samples were deposited into clean tubes containing Ethylenediamine tetraacetic acid (EDTA) as an anticoagulant for measurement of blood count.

#### *Blood Cell Counts*

An Automated Hematology Analyzer (URIT-3060) was used for the measurement of the following blood parameters: red blood cell count (RBCs), hemoglobin (HB), hematocrit value (HCT), Mean Corpuscular Volume (MCV), white blood cell count (WBCs), Platelets (PLTs), Lymphocytes (LYM), Combined value of the other types of WBCs (MID) cells and Granulocytes (GRAN).

#### *Statistical Analysis*

Software SPSS Version 25 was used to carry

out the statistical analysis. One-way variance analysis tests (ANOVA) were used to examine all findings, which were presented as mean (M)  $\pm$  standard deviation (SD) (N=5), with  $p < 0.05$  being regarded as statistically significant.

## Results and Discussion

The study was planned to indicate the effectiveness of MO-extract after incorporation of Ag-NPs to protect against ACR-induced blood toxicity. It was found that MO-NPs may indeed have a protective effect on hematological levels during administration ACR.

### Characterization of Ag-NPs from *Moringa oleifera* (MO-NPs).

Ag-NPs that were generated by *M. oleifera* were assessed using DLS and the resulting hydrodynamic size distribution graphs are presented in (Fig 1c and 1d), respectively. The calculated average size of Ag-NPs is 127.7 nm based on the findings. Depending on the type of phytoconstituents used in the reduction of the metal nanoparticles, larger monometallic nanoparticles can occasionally be found (Banerjee et al., 2014). Ag-NPs were given a Polydispersity Index (PdI) of 0.394. (Table1). These numbers show that the synthesised nanoparticles are in a monodisperse phase and can avoid agglomeration (Nayak et al., 2015). Ag-NPs were found to have a -21.5 mV Zeta potential (Fig 1d). Ag-NPs that are mediated by leaf extract and are persistent over a wide pH range have more negative potential values (Dubey et al., 2010 ; Boote et al., 2013). One of the most important methods for examining the structural characteristics of created nanomaterials is the TEM. It was used to evaluate the morphology, size, and form of nanoparticles. In contrast to several of the other nanoparticles, which had variable shapes, the Ag-NPs were mostly spherical, as seen in (Figure 1a). Aggregations weren't found. Ag-NPs morphology determined by TEM analysis indicated that the nanoparticles were spherical and ranged in size from 2.88 to 15.14 nm with a mean = (7.11) nm (Figure 2a).

Table 1: DLS, PdI and Zeta potential of synthesized Ag-NPs From *M. oleifera*

Nanoparticle	DLS (nm)	PdI	Zeta potential (mV)
Ag-NPs	127.7	0.394	-21.5

DLS: Dynamic Light Scattering ; PdI: Polydispersity Index

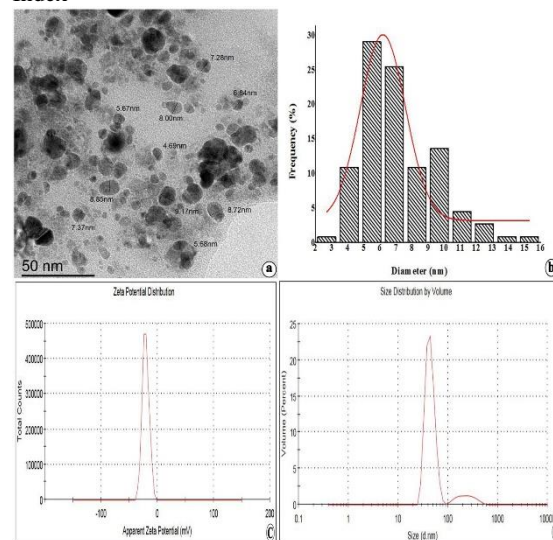


Figure1. a) Ag-NPs that were created and captured on TEM; b) Size distribution histogram of Ag-NPs of *M. oleifera* obtained from TEM micrographs; c) Zeta Potential Distribution ; and d) Size Distribution by Volume.

One of the most significant methods that is frequently used to characterise produced nanoparticles is TEM (Aboulthana and Sayed, 2018). It was discovered during the recently study that the Ag-NPs scattered throughout the particles ranged in size from 5 to 10 nm (Shousha et al., 2019). This was consistent with the findings of the experiment conducted by Ahmed and Ikram (2015). It was discovered that plant extracts contributed significantly to the synthesis of Ag-NPs. The amount of time that a plant extract and an AgNO<sub>3</sub> solution are incubated determines how quickly Ag-NPs are produced. Ag-NP production increased as incubation time was extended. The presence of the plant extract had no impact on how AgNO<sub>3</sub> was converted into Ag ions (Lakshmanan et al., 2018). The creation of nanoparticles using the plant extract was discovered to be an effective green reducing agent (Subramanian et al., 2013). As a result, using natural antioxidants to create nanoparticles appears like a good alternative, which may be because of their benign nature (Ahmad and Sharma, 2012). The potential stability of the particles in the colloidal suspension is measured by zeta potential. The majority of silver nanoparticles have a negative charge. *M. oleifera* was used to create silver nanoparticles that had a negative charge and were stable at room temperature (Arrieta et al., 2017). In the absence of steric

stabilisation, particles with zeta potentials larger than +30 mV and lower than -30 mV are regarded as stable for colloidal dispersion (Yue et al., 2008). Thus, it may be inferred that temperature has a significant impact on how nanoparticles form and remain stable (Nayak et al., 2015).

#### Hematological Changes of Blood Parameters (RBCs, HB, HCT and MCV) after Exposure to ACR and MO-NPs.

The results of this study showed that administration of ACR at a dose of 50 mg/kg body weight for three weeks resulted in a significant decrease in the counts of RBCs. Significant decreases were also observed in the level of HB, percentage of HCT and the volume of MCV when compared to the control group. In contrast, MO-NPsACRS and MO-NPs/ACR-treated groups showed a significant increase in terms of all hematological parameters when compared to the ACR group (Figure 2).

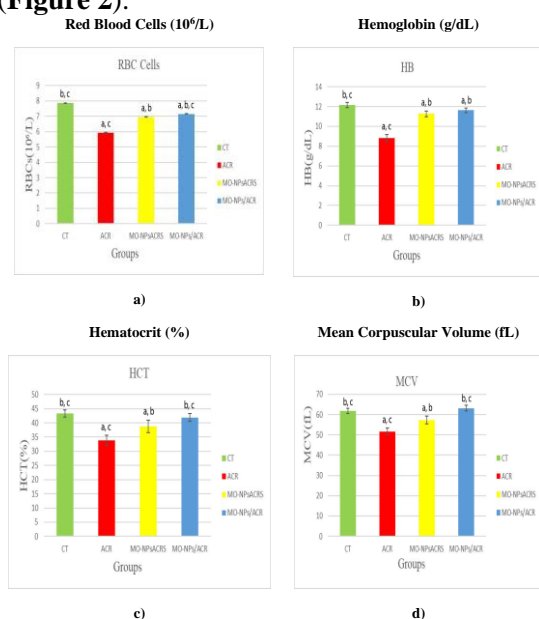


Figure 2. The effect of the administered ACR and the protective effect of MO-NPs on blood counts (a) CBCs, b) HB, c) HCT and d) MVC). The values are shown as the Mean  $\pm$  SD of five rats from each group ( $p < 0.05$ ).

CT: control group; ACR: acrylamide; MO-NPsACRS: silver nanoparticles of *M. oleifera* and ACR at the same; MO-NPs/ACR: silver nanoparticles of *M. oleifera* for three weeks, followed by ACR for 3 weeks. RBCs: Red Blood Cells ( $10^6/L$ ); HB: hemoglobin (g/dL); HCT: Hematocrit (%) and MCV: Mean Corpuscular Volume (fL). a : compared with control group, b : compared with ACR group and c: compared with MO-NPsACRS group.

The results of the present study concur with

the findings of previous studies, such as those of Rivadeneyra-Domínguez et al. (2018), who found that their ACR-treated group resulted in a decrease in levels of RBCs, HB, HCT, and MCV when compared to their control group (Rivadeneyra-Domínguez et al., 2018). These results agree with the conclusion that the administration of ACR causes a significant decrease in the levels of HB and the counts of HCT and RBCs (Ali et al., 2014; Ghorbel et al., 2017). Yet another study showed a significant reduction in the counts of RBCs, HCT percentage and HB concentration but no change in the volume of MCV in an ACR-treated group compared to the control group (Farouk et al., 2021).

RBCs, Hb, and HCT significantly decreased after receiving ACR. These alterations could be brought on by a reduction in erythrocyte acid resistance, an increase in lipid peroxidation, and a delay in the creation or destruction of HB (Barber et al., 2001). ACR is electrophilic and makes covalent bonds with the cysteine residues on HB. This causes the loss of the heme component of HB molecules, which in turn lowers the amount of HB in the blood and may also be the cause of anaemia that have been identified by low levels of HB, RBCs and HCT (Gargas et al., 2009). The valine residue of HB's (NH<sub>2</sub>-terminal) NH<sub>2</sub> group forms an adduct with ACR and its epoxide glycidamide, and ACR also forms an adduct with HB, which disrupts the levels of serum iron (Konings et al., 2003). However, the values of MCV measured in a previous study were significantly increased during the administration of ACR at a dose of 60 mg/kg b.wt, further increasing with an increase in the dose of ACR (Hammad et al., 2013). HCT is measured by the mass of RBCs, MCV and the volume of plasma. The modifications of HCT follow changes in RBCs distribution width when erythrocytes have a normal size (Ali et al., 2014). Results of previous studies show that administration of ACR at different doses in mice causes a significant decrease in HB and other hematological parameters (Lal et al., 2011).

The MO-NP-treated groups showed significant improvements of certain hematological parameters when compared to the ACR group. This outcome is in agreement with a study carried out by Aboulthana et al. (2021b), which indicated that *M. oleifera* nano-extract restored the levels of blood parameters to normal values in simultaneous and post-

targeted groups. In line with this result, Dhar and Gupta (1982) reported that *M. oleifera* improves blood condition in the body because its leaves are rich in various vitamins, iron and phytochemical compounds in sufficiently large amounts. It is known that these phytochemical components are hematopoietic factors, can directly affect the production of blood cells in bone marrow (Ramadhan, 2021). In rats that are anaemic, oral administration of MO-extract has the ability to enhance blood parameters such RBCs count, HB count, and packed cell volume, regardless of the dose (Ajugwo et al., 2017). In contrast to the Ag-NPs group, the AgNPs + *M. oleifera* group shown a considerable improvement in several haematological parameters. This is explained by the phytochemical elements, vitamins, and minerals that are included in the extract. The generation of blood cells in bone marrow is directly impacted by these phytochemical components, which are well-known to be hematopoietic factors (Ghareeb, 2021).

#### Hematological Changes to Blood Counts (PLTs, WBCs, LYM, MID and GRAN) after Exposure to ACR and MO-NPs.

The present study revealed that administration of ACR at a dose of 50 mg/kg body weight for three weeks resulted in extremely significant increase in the counts of PLTs. Another significant increase was observed in the counts of WBCs, MID cells and GRAN cells, but the counts of LYM cells significantly decreased when compared to the control group. In contrast, MO-NPsACRS and MO-NPS/ACR-treated groups showed a significant decrease in the previously cited hematological parameters (PLTs, WBCs, MID and GRAN) when compared to the ACR group, but LYM significantly increased when compared to the ACR group (Figure 3).

Results from this study, alongside the agreement with results from previous studies, show a significant increase in the number of WBCs in ACR-treated rats, perhaps due to activation of the immune system (Ghorbel et al., 2017). Other results show that treatments induced an increase in WBCs (Ghorbel et al., 2015). A high-dose of ACR (50 mg/kg b.wt) has also been shown to significantly decrease the count of LYM cells ( $p < 0.05$ ) compared to the control group (Raju et al., 2015). Additional results have also shown a significant increase in

the number of WBCs ( $9.95 \pm 1.44$  and  $10.44 \pm 1.21$ ) in Groups 3 (received a regular diet of food and water containing 10 mg/L ACR) and 4 (received a regular food, mineral water, and one gavaged dosage of ACR over the course of 14 days, equivalent to the LD50 (150 mg/kg body weight), respectively, when compared to the control group (Benziane et al., 2019).

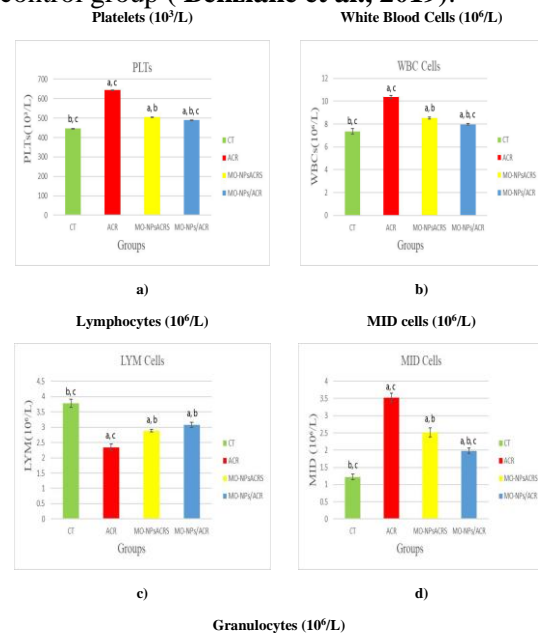


Figure 3. The effect of ACR administered and the protective effect of MO-NPs on blood counts (a) PLTs, b) WBCs, c) LYM cells, d) MID cells and e) GRAN cells). The values are shown as the Mean  $\pm$  SD of five rats from each group ( $p < 0.05$ ).

CT: control group; ACR: acrylamide; MO-NPsACRS: silver nanoparticles of *M. oleifera* and ACR at the same; MO-NPs/ACR: silver nanoparticles of *M. oleifera* for three weeks, followed by ACR for 3 weeks. PLTs: Platelets ( $10^3/L$ ); WBCs: White Blood Cells ( $10^6/L$ ); LYM: Lymphocytes ( $10^6/L$ ); MID: MID cells ( $10^6/L$ ); GRAN: Granulocytes ( $10^6/L$ ). a: compared with control group; b: compared with ACR group; and c: compared with ACR+MO-NPs group.

Similar findings have also been obtained by Hamed et al. (2013), in which the counts of WBCs were significantly higher ( $p < 0.001$ ) in different groups given ACR at doses of 10, 30 and 60 mg/kg body weight when compared to the control group (Hamed et al., 2013). In another study, treatment of young female rats with ACR for four weeks led to a significant decrease ( $p < 0.05$ ) in LYM cells and increased the counts of monocytes when compared to the corresponding controls (Sayed et al., 2012). In addition, there was a significant decrease in the number of LYM cells found in the ACR-treated

group compared to the control group in yet another study (Farouk et al., 2021). Also Ali et al., (2014), administration of ACR for 28 days caused a significant ( $p < 0.05$ ) decrease in LYM cells of rats when compared to the control group (Ali et al., 2014). ACR may be an immunosuppressive, so it can lead to an alteration in the WBCs count, perhaps due to a decrease in their production or rapid destruction of WBCs and/or redistribution from peripheral blood into the tissue (Debaun, 2005). On the other hand, results by Rivadeneyra-Domínguez et al. (2018), Farouk et al. (2021) and Sayed et al. (2012), showed a significant decrease in the level of PLTs and counts of neutrophils.

The MO-NPs treated groups evidenced significant changes to blood parameters when compared to the ACR group. In line with our result, Aboulthana et al. (2021b) found that in all *M. oleifera* nano-extract-treated groups, levels of PLTs and the count of WBCs and its differentials (LYM, GRAN and monocytes) decreased significantly ( $p \leq 0.05$ ) compared to a colon cancer-induced group. Levels returned to normal in the simultaneous and post-treated groups. Moreover, the MO-NP-treated group showed no significant change ( $p < 0.05$ ) in WBCs and PLTs compared to the control group (Aboulthana et al., 2021a). According to another study, adding *M. oleifera* to potato-based products is an appealing and effective way to lessen ACR production while maintaining products' acceptable sensory qualities. *M. oleifera* can be helpful as a natural liver protector, especially when added to dishes that include starch before frying. The method shown here could be viewed as a groundbreaking discovery of an easy and natural way to lower the levels of ACR in foods made with potatoes and its hazardous consequences, making it a desirable candidate for implementation in the food sector in the future (Rifai et al., 2020). In haematological diseases, co-administration of *M. oleifera* and ZnONPs had a notable recovery effect. Therefore, these results proved that *M. oleifera* has a protective effect against ZnONPs-induced haematological changes in male rats (Ramadhan, 2021).

## Conclusion

The results of this study clearly indicate that ACR cause numerous harmful effects in blood

cells. Moreover, the administration of nano-extract of *M. oleifera* enhances protection against the hematological toxicity of ACR in male rats.

## References

- Abdel-Daim, M. M., Abd Eldaim, M. A., & Hassan, A. G. (2015). Trigonella foenum-graecum ameliorates acrylamide-induced toxicity in rats: Roles of oxidative stress, proinflammatory cytokines, and DNA damage. *Biochemistry and Cell Biology*, 93(3): 192-198.
- Abdel-Rahman, L. H., Al-Farhan, B. S., El-ezz, A., Sayed, A. E., Zikry, M. M., & Abu-Dief, A. M. (2022). Green Biogenic Synthesis of Silver Nanoparticles Using Aqueous Extract of Moringa Oleifera: Access to a Powerful Antimicrobial, Anticancer, Pesticidal and Catalytic Agents. *Journal of Inorganic and Organometallic Polymers and Materials*, 32(4):1422-1435.
- Aboulthana, W. M., & Sayed, H. H. (2018). How to use green technology to enhance antioxidant efficiency of plant extracts: A novel strategy. *Journal of Applied Pharmacy*, 10(2), 264-267.
- Aboulthana, W. M., Youssef, A. M., Seif, M. M., Osman, N. M., Sahu, R. K., Ismael, M., ... & Omar, N. I. (2021a). Comparative study between Croton tiglium seeds and Moringa oleifera leaves extracts, after incorporating silver nanoparticles, on murine brains. *Egyptian Journal of Chemistry*, 64(4):1709-1731.
- Aboulthana, W. M., Shousha, W. G., Essawy, E. A. R., Saleh, M. H., & Salama, A. H. (2021b). Assessment of the Anti-Cancer Efficiency of Silver Moringa oleifera Leaves Nano-extract against Colon Cancer Induced Chemically in Rats. *Asian Pacific Journal of Cancer Prevention: APJCP*, 22(10): 3267.
- Ahmed S, Ikram S. (2015). Synthesis of gold nanoparticles using plant extract: an overview. *Nano Research and Applications*, 1:1 –64.
- Ahmad, N., & Sharma, S. (2012). Green synthesis of silver nanoparticles using extracts of Ananas comosus. *Green and Sustainable Chemistry*, 2, 141-147.
- Ahn, J. S., Castle, L., Clarke, D. B., Lloyd, A. S., Philo, M. R., & Speck, D. R. (2002). Verification of the findings of acrylamide in heated foods. *Food Additives & Contaminants*, 19(12):1116-1124.
- Ajugwo, A. O., Mounbegna, P. E., Kemajou, T. S., & Ofokansi, V. C. (2017). Effects of Moringa oleifera leaves extract on haematological parameters of phenylhydrazine anaemia induced wistar rats. *International Journal of Public Health*

- and Safety, 2(4): 139.
- Ali, M. A., Aly, E. M., & Elawady, A. I. (2014). Effectiveness of selenium on acrylamide toxicity to retina. *International journal of ophthalmology*, 7(4):614.
- Arrieta, E. C., Valdez, B., Carrillo, M., Curiel, M. A., Mateos, F. D., Ramos, R. A., ... & Bastidas, J. M. (2017). Silver nanoparticles biosynthesized by secondary metabolites from *Moringa oleifera* stem and their antimicrobial properties. *African Journal of Biotechnology*, 16(9), 400-407.
- Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, 1, 1-10.
- Barber, D. S., Hunt, J. R., Ehrich, M. F., Lehning, E. J., & LoPachin, R. M. (2001). Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. *Neurotoxicology*, 22(3): 341-353.
- Belhadj Benziane, A., Dilmi Bouras, A., Mezaini, A., Belhadri, A., & Benali, M. (2019). Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. *Drug and chemical toxicology*, 42(2):157-166.
- Boote, B. W., Byun, H., & Kim, J. H. (2013). One-pot synthesis of various Ag–Au bimetallic nanoparticles with tunable absorption properties at room temperature. *Gold Bulletin*, 46, 185-193.
- Debaun, M. R. (2005). Hydroxyurea as secondary prevention for stroke in children with sickle cell anemia. *The Journal of pediatrics*, 147(4):560-561.
- Dhar B, Gupta OP (1982). Nutritional value of *Moringa oleifera* Lam. *Bull. Med Ethnobotany Research Journal*, 3, 280-8.
- Dubey, S. P., Lahtinen, M., & Sillanpää, M. (2010). Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of *Rosa rugosa*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 364(1-3), 34-41.
- Fahey, J. W. (2005). *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for life Journal*, 1(5): 1-15.
- Farouk, S. M., Gad, F. A., Almeer, R., Abdel-Daim, M. M., & Emam, M. A. (2021). Exploring the possible neuroprotective and antioxidant potency of lycopene against acrylamide-induced neurotoxicity in rats' brain. *Biomedicine & Pharmacotherapy*, 138, 111458.
- Gargas, M. L., Kirman, C. R., Sweeney, L. M., & Tardiff, R. G. (2009). Acrylamide: Consideration of species differences and nonlinear processes in estimating risk and safety for human ingestion. *Food and chemical toxicology*, 47(4):760-768.
- Gawesh, E. S., Hamdey, E. S., Abdelreheem Elshoura, A. I., & Abbas, M. A. (2021). Protective Effect of Cinnamon and Ginger on Acrylamide Induced Hepatotoxicity in Adult Male Albino Rats. *International Journal of Medical Arts*, 3(1):1136-1144.
- Ghareeb, O. A. (2021). Toxic Effect of Silver Nanoparticles on Some Hematological Parameters and Possible Preventive Role of *Moringa Oleifera*: In Vivo. *Annals of the Romanian Society for Cell Biology*, 13796-13801.
- Ghorbel, I., Chaabane, M., Elwej, A., Kallel, C., Grati Kamoun, N., & Najiba, Z. (2017). Extra Virgin olive oil mitigates hematotoxicity induced by acrylamide and oxidative damage in adult rats. *Pharmaceutical and Biomedical Research*, 3(1):34-40.
- Ghorbel, I., Maktouf, S., Kallel, C., Chaabouni, S. E., Boudawara, T., & Zeghal, N. (2015). Disruption of erythrocyte antioxidant defense system, hematological parameters, induction of pro-inflammatory cytokines and DNA damage in liver of co-exposed rats to aluminium and acrylamide. *Chemico-biological interactions*, (236): 31-40.
- Gupta, S., Hemlata, H., & Tejavath, K. (2020). Synthesis, characterization and comparative anticancer potential of phytosynthesized mono and bimetallic nanoparticles using *Moringa oleifera* aqueous leaf extract. *Beilstein Archives*, 1, 95.
- Hammad, A. Y., Osman, M. E., & Abdelgadir, W. S. (2013). Histopathological assessment and hematotoxicity of dietary acrylamide on Wistar rats. *International Journal of Life Sciences*, 7(1):21-25.
- Jamshidi, K., & Zahedi, A. (2015). Acrylamide-induced acute nephrotoxicity in Rats. *International Journal of Scientific Research in Science and Technology*, 1(5):286-93.
- Kadawathagedara, M., Botton, J., de Lauzon-Guillain, B., Meltzer, H. M., Alexander, J., Brantsaeter, A. L., ... & Papadopoulou, E. (2018). Dietary acrylamide intake during pregnancy and postnatal growth and obesity: Results from the Norwegian Mother and Child Cohort Study (MoBa). *Environment international*, (113): 325-334.
- Konings, E. J., Baars, A. J., van Klaveren, J. D., Spanjer, M. C., Rensen, P. M., Hiemstra, M., ... & Peters, P. W. J. (2003). Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food and Chemical Toxicology*, 41(11):1569-1579.



- Lal, R., Arora, M., & Sharma, A. (2011). Acrylamide caused hematotoxicity on musculus through gavage. *Indian Journal. Fundam. Appl. Life Science*, (1):330-334.
- Lakshmanan, G., Sathiyaseelan, A., Kalaichelvan, P. T., & Murugesan, K. (2018). Plant-mediated synthesis of silver nanoparticles using fruit extract of *Cleome viscosa* L.: assessment of their antibacterial and anticancer activity. *Karbala International Journal of Modern Science*, 4(1), 61-68.
- LoPachin, Richard. M., Barber, David. S., He, Deke., & Das, Soma. (2006). Acrylamide inhibits dopamine uptake in rat striatal synaptic vesicles. *Toxicological Sciences*, 89(1), 224-234.
- Mahmood, K. T., Mugal, T., & Haq, I. U. (2010). *Moringa oleifera*: a natural gift-A review. *Journal of Pharmaceutical Sciences and Research*, 2(11): 775.
- Malathi, R., Chandrasekar, S., & Sivakumar, D. (2018). Assessment of toxicity study of ethanolic extract and synthesized silver nanoparticles of *Moringa concanensis* Nimmo leaves using wister albino rats. *International Journal of Current Research in Life Sciences*, 7(03):1250-1254.
- Nayak, D., Pradhan, S., Ashe, S., Rauta, P. R., & Nayak, B. (2015). Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. *Journal of colloid and interface science*, 457, 329-338.
- Nilanjuna, G., Samrat, P., & Piyali, B. (2014). Silver nanoparticles of *Moringa oleifera*—green synthesis, characterisation and its antimicrobial efficacy. *Journal of Drug Delivery and Therapeutics*, (11): 20-25.
- Prakash, D., Singh, B. N., & Upadhyay, G. (2007). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food chemistry*, 102(4):1389-1393.
- Raju, J., Roberts, J., Taylor, M., Patry, D., Chomyshyn, E., Caldwell, D., ... & Mehta, R. (2015). Toxicological effects of short-term dietary acrylamide exposure in male F344 rats. *Environmental Toxicology and Pharmacology*, 39(1): 85-92.
- Ramadhan, S. A. (2021). Hematological Alterations Induced by Zinc Oxide Nanoparticles and Preventive Role of *Moringa Oleifera*: In vivo. *Indian Journal of Forensic Medicine & Toxicology*, 15(2), 3403-3408.
- Ramaswamy, M., Solaimuthu, C., & Duraikannu, S. (2019). Antiarthritic activity of synthesized silver nanoparticles from aqueous extract of *Moringa concanensis* Nimmo leaves against FCA induced rheumatic arthritis in rats. *Journal of Drug Delivery and Therapeutics*, 9(3): 66-75.
- Rifai, L., Mohammad, M., Raafat, K., & Saleh, F. A. (2020). In vitro and In vivo Evaluation of the Protective Potential of Against Dietary Acrylamide-induced Toxicity. *The Open Medicinal Chemistry Journal*, 14(1).
- Rivadeneira-Domínguez, E., Becerra-Contreras, Y., Vázquez-Luna, A., Díaz-Sobac, R., & Rodríguez-Landa, J. F. (2018). Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. *Toxicology reports*, 5, 1124-1128.
- Sayed, M. R., Mohamed-Assem, S. M., Sohair, R. F., & Salma, A. E. A. (2012). Hazardous effects of acrylamide on immature male and female rats. *African Journal of Pharmacy and Pharmacology*, 6(18):1367-1386.
- Shipp, A., Lawrence, G., Gentry, R., McDonald, T., Bartow, H., Bounds, J., ... & Van Landingham, C. (2006). Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Critical reviews in toxicology*, 36(6-7):481-608.
- Shousha, W. G., Aboulthana, W. M., Salama, A. H., Saleh, M. H., & Essawy, E. A. (2019). Evaluation of the biological activity of *Moringa oleifera* leaves extract after incorporating silver nanoparticles, in vitro study. *Bulletin of the National Research Centre*, 43(1), 1-13.
- Subramanian, R., Subbramaniyan, P., & Raj, V. (2013). Antioxidant activity of the stem bark of *Shorea roxburghii* and its silver reducing power. *SpringerPlus*, 2(1), 1-11.
- Tornqvist, M. (2005). Acrylamide in food: the discovery and its implications. *Advances in experimental medicine and biology*, 561, 1.
- Yue, P. F., Yuan, H. L., Yang, M., You, R. H., Cong, L. B., Zhu, J., ... & Xiao, X. H. (2008). Preparation, characterization, and pharmacokinetic evaluation of puerarin submicron emulsion. *PDA Journal of Pharmaceutical Science and Technology*, 62(1), 32-45.

## الملخص العربي

## عنوان البحث: التأثيرات الوقائية لجزيئات الفضة النانوية لأوراق المورينجا أوليفيرا ضد سمية الدم المستحثة بالأكريلاميد في الجرذان

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أظهرت التجارب المعملية السابقة التأثير المسرطن لمادة الأكريلاميد (ACR)، وبينت العديد من الدراسات أن أوراق المورينجا أوليفيرا ومستخلص البذور هي مضادات أكسدة فعالة تحمي من الآثار الضارة لهجوم الجذور الحرة والإجهاد التأكسدي. هدفت الدراسة الحالية إلى تحديد التأثيرات السامة الضارة التي تسببها مادة الأكريلاميد على بعض مقاييس خلايا الدم لدى الجرذان والدور الوقائي المحتمل لجزيئات الفضة النانوية لأوراق المورينجا أوليفيرا ضد التأثيرات السامة للأكريلاميد. لقد تم إنتاج جزيئات الفضة النانوية لأوراق المورينجا أوليفيرا وفحصها باستخدام المجهر الإلكتروني النافذ (TEM) وجهاز تشتت الضوء الديناميكي ((DLS). تم استخدام عشرين جرذ أبيض من ذكور الجرذان البالغة لمدة ثلاثة أسابيع، حيث كان وزن كل جرذ حوالي ١٥٠ جرامًا. وقسمت عشوائياً إلى أربع مجموعات (كل مجموعة من خمسة جرذان): مجموعة ١: المجموعة الضابطة. مجموعة ٢: مجموعة الأكريلاميد، تم إعطاؤها الأكريلاميد (٥٠ مجم / كجم من وزن الجسم في مياه الشرب لمدة ثلاثة أسابيع)؛ مجموعة ٣: مجموعة الأكريلاميد + جسيمات الفضة النانوية من المورينجا أوليفيرا (٥٠ مجم / كجم من وزن الجسم لكلاً منهما عن طريق الفم في نفس الوقت) ومجموعة ٤: مجموعة الحماية، تم إعطاء جسيمات الفضة النانوية من المورينجا أوليفيرا لمدة ٣ أسابيع (٥٠ مجم / كجم من وزن الجسم عن طريق الفم) ثم إعطاؤها مادة الأكريلاميد لمدة ٣ أسابيع (٥٠ مجم / كجم من وزن الجسم). في نهاية الفترة التجريبية، تم جمع عينات الدم في أنابيب نظيفة تحتوي على مادة الـ EDTA كمضاد للتجلط لقياس عد الدم. أدى استخدام مادة الأكريلاميد إلى انخفاض كبير للغاية في عدد خلايا الدم الحمراء (RBCs)، ومستويات الهيموجلوبين (HB)، ونسبة الهيماتوكريت (HCT)، ومتوسط حجم الكرية (MCV) والخلايا الليمفاوية (LYM). من ناحية أخرى، زاد تعداد خلايا الدم البيضاء (WBCs) والصفائح الدموية (PLTs) وخلايا MID والخلايا الحبيبية (GRAN) بشكل ملحوظ عند مقارنتها بالمجموعة الضابطة. يقلل العلاج بجسيمات الفضة النانوية من المورينجا أوليفيرا بشكل كبير من التأثير السام لمادة الأكريلاميد على تعداد خلايا الدم البيضاء وخلايا PLTs وخلايا MID وخلايا GRAN. علاوة على ذلك، لوحظت زيادة معنوية على عدد كرات الدم الحمراء وخلايا LYM ومستوى HB ونسبة HCT و MCV بعد إعطاء جسيمات الفضة النانوية من المورينجا أوليفيرا ( $p < 0.05$ ). تشير هذه النتائج إلى أن جسيمات الفضة النانوية لأوراق المورينجا أوليفيرا يعزز الحماية ضد خلايا الدم المستحثة بالأكريلاميد في ذكور الجرذان.