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The Protective Effect of *Moringa oleifera* Leaves Extract on Paracetamol Hepatotoxicity in Male Rats

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ABSTRACT

In recent years, there has been an increase in concern regarding the effects of paracetamol poisoning on liver tissues, particularly when consumed in large amounts. Some studies have estimated that paracetamol is involved in 56% of acute liver diseases, whereas 0.4% of paracetamol overdose cases result in fatality. In this study, the effects of *Moringa oleifera* on paracetamol toxicity in the liver were explored. It has been demonstrated that *Moringa oleifera* is highly nutritious, contains bioactive molecules, and is therapeutically beneficial. Many studies have shown that *Moringa oleifera* leaves possess a wide range of biological properties, including antioxidant, tissue protection, analgesic, antihypertensive, and immunomodulatory activities. This study highlights the protective role of *Moringa oleifera* on handling possible paracetamol hepatotoxicity in male rats.

1. INTRODUCTION

Liver dysfunction represents a primary population-based healthcare challenge, particularly in both established and developing nations. The main reason is that the liver plays a central role in facilitating all bodily systems, which implies that the onset of an underlying liver disease greatly affects an individual's quality of life [1]. The susceptibility of the liver to damage can be attributed to its ability to concentrate, biotransform, and excrete various forms of xenobiotics. Another context is the anatomical proximity of the liver to the blood flow within the gastrointestinal tract. Selecting rats for this evidence-based study is significant, as there is an ease of chemically induced liver damage in the study subjects to mimic possible forms of liver illnesses [1].

Paracetamol acts as a largely renowned analgesic drug in population settings, as it is deemed safe and capable of initiating the desired quality healthcare outcomes. Nevertheless, if paracetamol is administered in large doses, it increases the chances of extensive centrilobular necrosis, which increases mortality rates in both people and experimental study subjects [1].

An example of a scenario that causes centrilobular necrosis is when paracetamol is administered in

large doses during continued fasting. Another risk factor is the postponement of hospitalization when one presents worrying symptoms after taking paracetamol, together with a lack of population-wide health literacy on the potential harm of paracetamol to the liver [1].

This implies that the ease of attainability of paracetamol as an over-the-counter drug singularly or amalgamated with other medications increases the chances of high protein levels and albumin, globulin, and A/G ratios.

In 2023, [2] did a study which found that approximately 6% of poisoning cases worldwide were caused by excessive use of paracetamol. Moreover, paracetamol has been identified as the underlying factor in 56% of cases of sudden liver injury, and 0.4% of instances in which excessive amounts of paracetamol were administered have resulted in fatalities. The likelihood of paracetamol-induced liver damage increases in the presence of additional risk factors. Several factors contributing to this problem include delayed hospitalization or postponement of hospitalization after symptom manifestation, improper use of high-dose modified-release paracetamol, use of drugs that stimulate enzymes such as carbamazepine and isoniazid, and smoking [2].

The lack of awareness regarding the potential dangers associated with the incorrect utilization of paracetamol is worrisome. There is a specific fallacy held by certain people that the consumption of over-the-counter medicines, such as Panadol and Fevadol, is not hazardous. In addition, some individuals fail to peruse medicine package inserts [3]. Given the function of the liver as the main barrier against toxins and its involvement in metabolizing pharmaceuticals into harmless substances, drug development should consider the potential hepatotoxicity of treatments [4].

Existing medications for liver disease are ineffective, resulting in unfavorable side effects and exorbitant expenses. Hence, it is necessary to investigate feasible and effective alternatives with therapeutic or protective characteristics [5]. Recently, there has been a notable increase in the interest in natural products, mostly because they are more affordable and have a higher level of safety with fewer negative effects. This study was conducted to investigate the efficacy of herbal medicines in preventing paracetamol toxicity.

Cardamom and aloe vera are natural remedies that possess hepatoprotective effects and exhibit antioxidant activities. Studies have demonstrated its ability to enhance antioxidant levels. In addition, ginger has shown efficacy in eradicating free radicals [6]. *Moringa oleifera*, an organic plant, has been widely used as a food resource and therapeutic agent, owing to its well-documented physiological benefits. *Moringa oleifera*, which originates in northwest India, is farmed worldwide.

Natural remedies such as cardamom and aloe vera are known to have antioxidant and hepatoprotective effects. Their ability to increase the antioxidant levels has also been demonstrated. According to [6], ginger can also remove free radicals. Organic *Moringa oleifera* has several well-documented physiological benefits, which have led to its widespread use as both a food source and a therapeutic cure. Moringa plants, which originate in the northwestern region of India, are widely grown worldwide.

Moringa oleifera (MO) has demonstrated significant therapeutic advantages, leading to its informal designation as the "miracle tree" [7, 8].

Moringa oleifera is considered a potential remedy for over 300 medical conditions, owing to its abundant collection of bioactive components, including alkaloids, flavonoids, phenolic acids, and carotenoids. Multiple inquiries were conducted to assess treatment effectiveness. Various studies have demonstrated that MO possesses antidiabetic properties that are effective in managing both type 1 and type 2 diabetes. Specific studies have shown that MO enhances glucose absorption and glycogen synthase function in the body.

There is some evidence that MO may be beneficial to cardiovascular health, owing to the inclusion of phytochemicals that can reduce blood pressure. Another study conducted by [9] in 2021 indicated that 13 different MO subspecies have antibacterial properties. The bioactive components of *Moringa oleifera* (MO) are responsible for its therapeutic properties, including its ability to reduce oxidative stress [10]. In this study, M. oleifera was selected because of its hepatoprotective properties against paracetamol-induced liver damage and the antioxidant activity of the extract in reducing oxidative stress.

2. RESEARCH OBJECTIVE

a) To evaluate the efficacy of moringa leaf extract in tackling oxidative stress by reviewing published scientific papers on this extract.

b) To examine probable hepatoprotective effects of *Moringa oleifera* extract on liver function and tissue in a paracetamol-induced liver damage model by analyzing and studying scientific papers published on this topic.

3. PARACETAMOL

Paracetamol, often known as acetaminophen or N-acetyl-p-aminophenol, is a widely prescribed drug for illnesses, such as osteoarthritis and back pain (See **Table 1** and **Figure 1**). It can be accessed by anyone without prescription. It serves as a dual-purpose medicine, effectively decreasing temperature and relieving pain. Therefore, it is strongly recommended as the primary treatment choice for a range of unpleasant conditions. Paracetamol is employed, either in its pure state or in conjunction with other substances, such as codeine and ibuprofen, to alleviate mild-to-moderate pain and fever. Paracetamol is primarily administered orally, although there is a growing trend towards intravenous administration of paracetamol. Paracetamol, although similar to conventional nonsteroidal anti-inflammatory medications (NSAIDs), lacks anti-inflammatory properties and hence does not fall within the classification of NSAIDs [11, 12].

4. HISTORY OF PARACETAMOL

This analgesic medication has a historical origin that can be traced back to 1889 when it was first identified in the United Kingdom. Trigesic, which consists of paracetamol, aspirin, and caffeine, was first released in 1959. Agranulocytosis occurs in a subset of patients who are trigesic, resulting in its removal from the market. However, there is no established association between paracetamol and this disease. Consequently, paracetamol was reintroduced to the market in 1956 as a prescription-only medication under the name panadol. As a safe alternative to aspirin, which has been associated with Reye's syndrome in children, it was commercially available in the USA in 1983. Paracetamol is currently regarded as a highly efficient, secure, and affordable antipyretic [13, 14].

4.1. Pharmacokinetics

The gastrointestinal (GI) tract absorbs paracetamol quickly, enabling it to reach peak blood concentrations within 90 minutes of consumption. Paracetamol has a plasma half-life ranging from 1.5 to 2.5 hours when taken at the prescribed dosages. During an overdose, the body's metabolic processes are disrupted, resulting in a longer half-life of 4 - 8 hours. This extended half-life can lead to liver damage [11, 12].

4.2. Paracetamol Metabolism

Paracetamol is primarily metabolized by the liver, with a minor portion being processed in the kidneys and intestines. When PCM is administered at a therapeutic dose, three routes are implicated: inactive paracetamol glucuronide (APAP-GLU) is generated by conjugation with glucuronide, and approximately 30% - 40% of PCM undergoes conjugation with sulfate in the second pathway [12]. The enzyme Cytochrome P450, CYP2E1, converts 5% - 10% of paracetamol into N-acetyl-p-benzoquinone imine (NAPQI), which is responsible for the liver-damaging effects of paracetamol. Owing to its interaction with the sulfhydryl group of glutathione (GSH), NAPQI is rendered inactive by APAP-GSH. This compound is then eliminated from the body through the urine in the form of cysteine and mercapturic acid conjugates. The reference [17] was provided. Overdosing leads to an increased production of NAPQI metabolites, which saturate the metabolic pathway, deplete GSH, and cause liver damage [18].

4.3. Mechanisms of Action

Despite its extensive history, the exact mechanism of action of paracetamol remains unclear; however,

Table 1. Chemical and physical properties of the PCM [15, 16] Chemical and physical properties of paracetamol (PCM).

Molecular Formula	$C_8H_9NO_2$ or $HOC_6H_4NHCOCH_3$
Molecular Weight	151.16
Physical Appearance	odorless crystalline powder
Color	white
Melting Point	169˚C - 170.5˚C
Boiling Point	>500˚C
Solubility	Easily soluble in hot water, but less soluble in cold water (1:70, 1:20 at 100˚C)
Dosage	It is recommended that adults take 500 - 1000 mg orally per day. In children, the recommended dose is 10 - 15 mg/kg bw; no more than five doses should be administered over 24 h. The recommended dose for children is 10 - 15 mg/kg bw, administered no more than five times a day.
Vapor Pressure	6.29×10^{-5} mm Hg at 25˚C

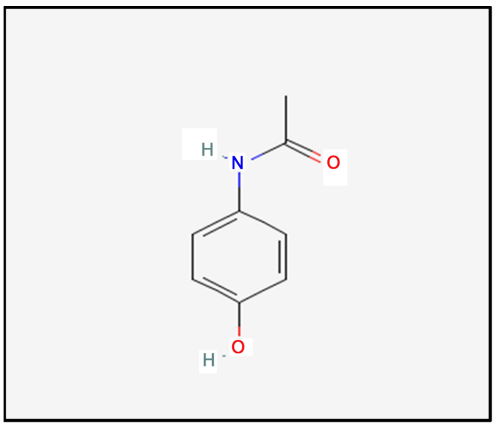

Figure 1. Structural formula of paracetamol [16].

some mechanisms have been proposed. Paracetamol, which is categorized as a multi-target medicine, operates through various mechanisms. The most probable method involves inhibition of COX isoforms (COX-1 and COX-2), which blocks the synthesis of prostaglandin E (PGE) from arachidonic acid (AA). Studies conducted by [12, 19], and [20] demonstrated that PCM exhibits a significantly higher inhibitory effect on PGE2 in the brain than in the spleen. This indicates that the PCM can penetrate the blood-brain barrier and effectively hinder the creation of cyclooxygenase in the central nervous system. The references cited are [12] and [21].

4.4. Paracetamol Hepatotoxicity

Paracetamol overdose is a frequent cause of abrupt liver failure in both the United States and United Kingdom. The accumulation of NAPQI metabolites occurs when an individual overdoses PCM, leading to the depletion of glutathione. Protein adducts that increase oxidative stress are created when NAPQI binds specifically to mitochondrial proteins and ion channels, in response to reduced GSH levels. Because of

this, mitochondrial function is compromised, and cells die. Researchers have examined the processes through which APAP causes cell death on multiple occasions.

Research has focused on how mitochondrial protein adducts trigger mitochondrial oxidative stress, which in turn activates JNK, causes the mitochondrial matrix to enlarge, and ultimately causes DNA damage. Activation of the MAPK cascade, particularly c-Jun N-terminal kinase (JNK), intensifies oxidative stress triggered by PCM adducts linked to mitochondrial proteins. Cell death occurs as a result of matrix elongation, opening of the mitochondrial membrane permeability transition pore (MPTP), and an increase in reactive oxygen species and peroxynitrite produced by JNK translocation to the mitochondria [22, 23].

5. *MORINGA OLEIFERA*

Moringa oleifera is a monogeneric tree species widely used in culinary applications. The tree has more names than the radish or horseradish. This plant is grown worldwide because of its high agricultural value despite its natural range in the sub-Himalayan zones of Afghanistan, Pakistan, Bangladesh, and India. Many edible parts of moringa have a long history of human consumption for a wide range of reasons, including traditional medicine and industrial processes. Moringa leaves can be cooked into biogas and used as green manure, and wood can be converted into blue dye and gum. There are other uses for flower nectar besides making honey and sugar. Ground seeds are a clarifying agent for cane juice. In addition to its numerous other uses, moringa contains several components that can be used in pharmaceutical manufacturing [24].

5.1. *Moringa oleifera* Scientific Classifications

Moringa oleifera has many beneficial nutrients. (See **Table 2**) Numerous minerals and vitamins, including vitamin A, C, calcium, potassium, zinc, magnesium, and iron, are found in the foliage of this plant. Furthermore, at 64 calories per serving, the leaves were perfect for weight loss plans. Moringa contains a wealth of phytochemicals such as tannins, sterols, and flavonoids. It is also well known that moringa possesses hypoglycemic and anticancer properties.

Pods have been used to treat gastrointestinal ailments because of their fibrous composition. Moringa plants possess several components that contain varying quantities of amino acids. More precisely, the leaves contained 44% of the substance, pods contained 30%, and blooms contained 31%. Studies have demonstrated that moringa seed oil contains 76% polyunsaturated fatty acids (PUFAs). PUFAs are polyunsaturated fatty acids including linoleic, linolenic, and oleic acids. Studies have demonstrated that these acids exert a substantial influence on decreasing cholesterol levels in the body. Thus, it is an excellent substitute for olive oil [26].

5.2. Bioactive Components in *Moringa oleifera*

a) Vitamins

Moringa oleifera acts as a profound source of Vitamin A important for good vision, reproduction, and embryonic growth. Vitamin A also aids in development, immunity competence, and underlying cell differentiation activity. This is because MO has the ability to generate carotenoids that steer Vitamin A potency. Additionally, MO has high levels of Vitamin E, which acts as an antioxidant and an enabler for cell proliferation [27].

b) Polyphenols

Moringa oleifera produces polyphenol compounds that amalgamate flavonoids and phenolic acids. Flavonoid acids tend to be produced as a reaction to microbial infections, upholding the benzo-γ-pyrone ring, which forms the primary structure. Flavonoids are essential for the fortification of various chronic illnesses linked to oxidative stress [27].

In particular, quercetin acts as an effective antioxidant that extends various therapeutic capabilities such as limiting hyperlipidemia and atherosclerosis with high-end cholesterol. Additionally, Quercetin

Table 2. *Moringa oleifera* scientific classification [25].

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family	Moringaceae
Genus	Moringa
Species	*M. oleifera*
Binomial name	*Moringa oleifera*

protects insulin-generating pancreatic β-cells against streptozotocin, which tends to steer oxidative stress coupled with apoptosis, especially in rats as study subjects [27].

Phenolic acids, on the other hand, represent a subset of phenolic compounds generated from hydroxybenzoic acid and hydroxycinnamic acid. Phenolic acids also exhibit anti-inflammatory, antimutagenic, and anticancer activities. An example of phenolic acids is chlorogenic acid, which acts as an ester of dihydrocinnamic acid and helps steer glucose metabolism activity [27].

This occurs through the inhibition of glucose-6-phospahte translocase, which limits hepatic gluconeogenesis alongside glycogenolysis. Additionally, Chlorogenic acid helps reduce postprandial blood glucose levels in rat study subjects [27].

c) Alkaloids, Glucosinolates, and Isothiocyonates

Alkaloids represent a class of chemical compounds that contain basic nitrogen atoms. Examples of upheld compounds within *Moringa oleifera* include N, α-l-rhamnopyranosyl vincosamide, phenylacetonitrile pyrrolemarumine, 4'-hydroxyphenylethanamide-α-l-rhamnopyranoside, and the underlying glucopyranosyl derivative. Glusinolates and Isothiocyanates represent a limited extent of metabolites within MO which upholds health-improving competencies [27].

d) Tannins

Tannins act as water-soluble phenolic compounds that trigger the steer precipitation of alkaloids, gelatine, and several proteins. The involved concentration level in MO range from 13.6 to 20.6 g tannin per kilogram which helps steer anticancer, antiatherosclerotic, anti-inflammatory, and anti-hepatoxic capabilities [27].

e) Saponins

Saponins constitute isoprenoidal-derived aglycones that are tied to a single sugar-based moiety that facilitates anticancer capabilities [27].

5.3. Pharmacological Characteristic of *Moringa oleifera*

Moringa oleifera upholds hypolipidemic properties through lipid regulation that helps inhibit possible pancreatic cholesterol esterase action, which limits cholesterol absorption, bile acid binding, insoluble complex development, and reduction of plasma concentration levels. MO also helps initiate antioxidant properties via β-carotene through the inactivation of lipid free radicals, while also halting possible hydroperoxide decomposition to free radicals via its redox nature [27]. MO further extends anti-inflammatory activity coupled with immunomodulatory capabilities by limiting human macrophage cytokine generation. The context is the stimulation of cellular and humoral immune reactions within cyclophosphamide-induced immunodeficient mice study subjects. This is based on an elevation in the total white blood cell number and neutrophils coupled with serum immunoglobulin concentrations [27].

Hepatoprotective Activity

Observations were made on The hepatoprotective benefits of extracts obtained from *Moringa oleifera*

leaves are either ethanolic or aqueous in nature.

[28] reported that the ethanolic floral extract of M. oleifera had a strong protective effect on the liver. This significant effect was mostly attributed to the high flavonoid concentration of the extract, particularly that of quercetin. In addition, *Moringa oleifera* has demonstrated hepatoprotective effects by decreasing the levels of liver enzymes AST, ALP, and ALT. Furthermore, multiple studies conducted in rodents have demonstrated that *Moringa oleifera* has a notable capacity to decrease lipid peroxidation in the liver. Therefore, the utilization of leaf extracts in guinea pigs has demonstrated that MO extracts effectively impede the development of non-alcoholic fatty liver illness (NAFD) [27].

Moringa oleifera upholds phytochemical components that facilitate the regulation of naturally occurring antioxidant enzymes, which aid in tackling the production of ROS. Additionally, the phytochemical components help normalize the increased levels of biochemical parameters due to paracetamol hepatoxicity on the functioning enzymes [29].

The enzymes involved included ALT, AST, ALP, serum proteins, and related oxidative stress biomarkers. Furthermore, MO helps in steering the amelioration of underlying liver cell necrosis, pertinent inflammatory changes, and preserves normal-level hepatic structural composition. There is also the generated aptitude of extended liver functionality by MO through inhibition of the underlying cellular stress signaling cascades coupled with under-regulation of MAPK-8, TRAF-4, and TRAF-6 expression. The underlined MAPK-8, TRAF-4, and TRAF-6 proteins are significant for facilitating inflammatory activity [29].

5.4. The Liver

The largest organ in the human body is the liver, which accounts for approximately 2.5 percent of a person's total weight. An organ is considered necessary because it is essential for the body to sustain life. The liver is an endocrine and an exocrine organ that performs more than 500 separate tasks [30]. A sleek exterior and dome shape characterize the building. The organ lies within the right upper quadrant of the abdomen and is shielded from damage by the diaphragm and the thoracic cage.

Hepatocytes, endothelial cells, Kupffer cells, and stellate cells are the four principal types of cells in the liver. Hepatocytes constitute almost 60% of the cellular makeup of the liver and play a key role in vital metabolic functions, such as protein synthesis and detoxification. Endothelial cells create a protective layer on the surfaces of sinuses, where they are involved in the filtration process and can assist in eliminating viruses and smaller particles. However, these cells lack the capacity to engulf particles via phagocytosis. Studies have demonstrated that Kupffer cells secrete inflammatory chemicals upon activation. Stellate cells not only engage in many functions but also function as a repository for vitamin A [30].

Liver as Detoxification Organ

The detoxification process in the liver necessitates the involvement of many enzymes that possess unique activities, control, and specificity towards different chemicals. The liver is the primary site for metabolism of xenobiotics, including chemicals and pharmacological agents. Although the kidneys and intestines can metabolize medications, they are less efficient than the liver is. The drug metabolism in the liver is not uniform. Certain substances are transformed into metabolites that dissolve in water, making them easy to eliminate in the urine. Drug-metabolizing enzymes (DMEs) facilitate other chemical reactions, leading to the production of fewer harmful substances. However, some of these reactions also generate highly reactive metabolic intermediates that can harm the tissues [31].

The enzymes responsible for drug metabolism and detoxification can be categorized into two groups: Phase I and phase II. Phase I encompasses the "cytochrome P450" enzyme system, which is a crucial mechanism involved in drug metabolism and detoxification. Cytochrome P450 is located in the endoplasmic reticulum (ER) of hepatocytes in the liver, where it metabolizes a diverse array of xenobiotics. The majority of medicines are metabolized by a single P450 enzyme, while certain medications require multiple phase I reactions followed by phase II metabolism prior to elimination. However, certain processes may generate reactive metabolites during metabolism [32].

6. *MORINGA OLEIFERA* EFFECT ON PARACETAMOL HEPATOXICITY

Several studies have demonstrated the protective effect of *moringa oleifera* and its antioxidant activities against paracetamol toxicity.

The results of a study conducted by [33] showed that *Moringa oleifera*, administered before or after paracetamol treatment, increased liver-reduced glutathione concentrations, demonstrating a protective effect against paracetamol exposure in male mice.

In another study conducted on isolated rat hepatocytes, M. oleifera was found to protect against paracetamol hepatotoxicity, and the results showed that pretreatment with *Moringa oleifera* extract boosted the viability of cells. They also observed decreased leakage of LDH, ALT, and AST; increased concentration of GSH; and decreased lipid peroxidation levels. Furthermore, the extract reduced DNA damage caused by paracetamol [34].

A similar study found that the combination of *Moringa oleifera* and N-acetylcysteine (NAC), a paracetamol overdose therapy, effectively reduced NAPQI production by suppressing CYP450 enzymes and increasing antioxidant enzyme levels to counter reactive oxygen species (ROS) [35].

7. CONCLUSIONS

It has been proven that *Moringa oleifera* ethanolic extract, taken orally, has a defensive effect on various hematological indicators. The liver was protected against PCM hepatotoxicity by *Moringa oleifera* ethanolic extract prior to paracetamol administration.

Several benefits of *Moringa oleifera* can be attributed to its nutritional, bioactive, and pharmacological properties, including antioxidants, anti-inflammation, hepatoprotective, as well as many other properties. To use the *Moringa oleifera* extract for medical and therapeutic purposes, clinical trials must be conducted to evaluate its efficacy.

AUTHOR CONTRIBUTIONS

Reham Al-Sultan and Noorah Al-Sowayan contributed to the design, conceptualization, and implementation of the research. Reham Al-Sultan contributed to data collection, analysis, and the writing of the original draft preparation. Noorah Al-Sowayan Authentication, editorial review, supervision, and project management conceived the original manuscript and edited it. All authors have read and agreed to the published version of the manuscript.

AVAILABILITY OF DATA

The authors certify that the paper and its supplementary materials provide the necessary information to support the conclusions of this study.

ETHICAL APPROVAL

The Qassim University's Scientific Research Ethics Committee approved the study protocol (protocol code: 21-24-02; date of approval: 31-7-2022).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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