**THE PROTECTIVE POTENTIAL OF ETHANOL EXTRACT OF MANGO PEEL ON NITROSOMETHYLUREA-INDUCED MAMMARY HYPERPLASIA IN FEMALE RATS**

**Abstract**

**Aims**: Breast cancer is the second leading cause of cancer death among women and Mammary gland hyperplasia is a frequent noninflammatory, nontumor condition that affects women of childbearing age. This syndrome is responsible for 75% of all breast diseases. The study investigated the effect of mango peel ethanol extract on mammary gland hyperplasia using Wistar rats induced with N-nitromethylurea

**Methodology:** Standard methods were used to analyze the proximate, mineral and vitamin composition of the mango peel extracts. In the *invivo* study, 30 female Wistar rats were used for this study and were grouped into control and treatment groups (200 mg/kgbw and 400 mg/kgbw) for a period of 28 days. Liver and kidney function, *Invivo* enzymatic and non – enzymatic compounds present in breast tissues were evaluated as well as the histopathological evaluation of the mammary gland.

**Result:** The results showed that mango peel extract had a moisture content of 9.97%, ash content of 4.51 %, crude fiber of 60.45%, and crude protein content of 4.65%. The minerals present were iron, sodium, zinc, and calcium. Calcium and sodium levels were significantly higher than other minerals (822 and 328 ppm respectively) . Vitamin content ranged between 0.102 mg/100g for vitamin A and 0.456 mg/100g for vitamin B2 and 0.10 mg/100g for vitamin B9. in the *in vivo* study result showed a significant increase (P<0.05) in MDA levels in NMU control group, while normal control group and MPE treated groups had a significantly lower MDA level. The antioxidant activity of the MPE in the female rats was also analyzed in these enzymes; Superoxide dismutase, Glutathione S-transferase, reduced glutathione and catalase. It was observed in the results that the level of antioxidants enzyme activity in the female rats treated with NMU was lower while the control and treatments group had a significantly higher antioxidant enzymatic activity. Histopathology evaluation on the mammary gland was also carried out to fully understand the effects of NMU on the mammary gland of the female rats. Findings from the study showed inflammation in the NMU only group while normal breast tissues were observed in the control and treatment groups.

**Conclusion**: This study showed that the ethanol extract of mango peel has notable mammary gland protective effects in female rats with NMU-induced mammary gland hyperplasia and this effect could be attributed to the ability of mango peel to increase antioxidant enzyme levels in the treated groups

**Keywords: mammary gland, enzymes, antioxidant, hyperplasia.**

# INTRODUCTION

The burden of breast cancer (BC) is rising in Nigeria. The International Agency for Research on Cancer (IARC) recorded 28,380 new BC cases in Nigeria in 2020, representing 22.7% of new cancers and accounting for the highest proportion of all cancers types (Hyuna et al., 2021). Mammary gland hyperplasia is a frequent noninflammatory, nontumor condition that affects women of childbearing age. This syndrome is responsible for 75% of all breast diseases (Gao et al., 2021). Mammary gland hyperplasia is associated with endocrine abnormalities, which are mostly caused by an imbalance of estrogen and progestin (MacLean & Hayashi, 2022). Hyperplasia of mammary epithelial cells can also be caused by cholesterol and its oxidative product, cholesterol epoxide (Ontsouka et al., 2017). There is currently much interest in phytochemicals as bioactive components of food. The roles of fruit, vegetables and red wine in disease prevention have been attributed, in part, to the antioxidant properties of their constituent polyphenols which are able to quench oxidative products (Parcheta et al., 2021). Mango peel which is a waste product of the mango fruit have recently been observed to be rich in polyphenols (Suleria et al., 2020). In this study, animal models of mammary gland hyperplasia will be established via the administration of N-Nitrosomethylurea to female Wistar rats. Using this model, the protective effect, of Mango peel extract (MpE) in Nitrosomethylurea mammary gland hyperplasia will be investigated.

# MATERIAL AND METHODS

## Sample Collection and Extract Preparations.

Edible fresh mangoes *Magnifera indica L* were collected from Sayedero market Ilaro, Ogun State, Nigeria. The mangoes were thoroughly washed and the skin was peeled, cleaned and oven dried at 50oC, and milled, 1kg of milled sample was soaked in 5L of absolute ethanol for 3 days at room temperature with occasional stirring and shaking. The mixture was then decanted and the filtrate was concentrated under reduced pressure at 60oC in a rotary evaporator to yield extract. The concentrated extract was kept in a water bath at 60oC to evaporate the ethanol residue yielding crude mango peel extract.

## Proximate, Mineral and Vitamin Composition

Proximate analysis was carried out according to the procedure of the Association of Official Analytical Chemist for moisture, ash, fat, crude fibre and crude protein content. Standard operating procedure for metal determination using AAS (Atomic Adsorption Spectroscopy) while HPLC (High-Performance Liquid Chromatography) was used to determine the vitamin composition according to (AOAC 2019).

## Sample Collection

Rats were fasted overnight before being sacrificed by cervical dislocation following the final treatment (Day 28). Ocular puncture was used to draw blood into ordinary centrifuge tubes, which were then left to stand for two hours before being centrifuged to extract serum. Animals' mammary glands were promptly removed and cleaned in a cold 1.15% KCl solution. To extract the post-mitochondrial supernatant fraction (PMF), which was utilized for the measurement of antioxidant indices, the tissues were homogenized using four volumes of 5 mM phosphate buffer, pH 7.4, and centrifuged at 10,000 rpm for 0.25 hours. For histopathology, the tissues were also preserved in formaldehyde solution.

## Blood Biochemistry

Using an automated analyzer Mindray BS Series, Chema Diagnostica Italy, serum biochemical tests were performed, including the detection of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine level, serum urea, albumin, and bilirubin. The ISE 5000 electroanalyzer from SFRI Medical Diagnostics in France was used to measure the serum electrolytes (Na, K, Cl, and HCO3).

## Antioxidant Parameters

Bovine serum albumin (BSA) was used as a reference and the Buiret technique (Gornall et al., 1949) was used to calculate total protein. The capacity of superoxide dismutase to prevent the auto-oxidation of adrenaline was measured by the rise in absorbance at 480 nm, as reported by (Sun & Zigman, 1978). We measured catalase activity in accordance with (Sinha et al., 2005). It was shown as µmoles of H2O2 consumed/min/mg protein at 250C and measured colorimetrically at 620 nm. The reduced glutathione (GSH) concentration of mammary gland tissue as non-protein sulphydryls was determined using the technique published by (Sedlak & Lindsay, 1968). To the homogenate, 10% TCA was added and centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was measured at 412 nm. (Buege & Aust, 1978) approach was used to determine malondialdehyde (MDA), which is a lipid peroxidation index. 1.0 ml of the supernatant was mixed with 2 ml of (1:1:1) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl, and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent, which was heated at 100oC for 15 minutes and allowed to cool. Flocculent materials were removed by centrifugation at 3000 rpm for 10 minutes. The supernatant was removed, and the absorbance was measured at 532 nm against a blank. The activity of glutathione-S-transferase was measured using the technique described by (Habig et al., 1974). This is predicated on the fact that all known glutathione-S-transferases have reasonably high activity with 1-chloro-2,4-dintrobenzene (CDNB) as the second substrate. As a result, the typical glutathione-S-transferase activity test uses 1-Chloro-2,4-dintrobenzene as a substrate. When this substrate is coupled with reduced glutathione (GSH), the absorption maximum moves to a longer wavelength. The absorbance rises at the new wavelength of 340nm, allowing a direct measurement of the enzyme process.

## Histology

Formaldehyde solution was used to fix the mammary gland tissue that was taken from the test and control groups. The tissues underwent paraffin embedding, xylene clearing, and progressive ethanol dehydration. The paraffin block was sliced into portions that were 3–4 µm thick. After that, the sections were cleaned, deparaffinized, and put on a microscope slide. Hematoxylin and eosin (H&E) staining was applied to the slides, and optical microscopy was used to check for any pathological alterations. (Feldman & Wolfe, 2014)

## Animals and Treatment

Thirty sexually matured female rats were purchased. Every rat used was maintained using a containment structure, which provided a clean atmosphere. The rats were chosen at random and divided into five categories with six rats each.

**Table 1: Grouping of Experimental Rats**

|  |  |
| --- | --- |
| Group | Treatment |
| 1 | Normal control (Water and food only) |
| 2 | Negative control (NMU 50 mg/kg) |
| 3 | 400mg/kg Mango Peel extract |
| 4 | NMU 50 mg/kg and 200 mg/kg Mango Peel extract |
| 5 | NMU 50 mg/kg and 400 mg/kg Mango Peel extract |

## Statistical Analysis

The mean ±SD was used to express all data. The experimental groups' values were contrasted with the control values. GraphPad Prism 8.01 (GraphPad Prism Software Inc., La Jolla, USA) was used to do a one-way analysis of variance and a post-hoc analysis of Turkey. P<0.05 was the threshold for values to be deemed statistically significant.

# RESULTS AND DISCUSSION

**Table 2: Proximate Composition of MPE**

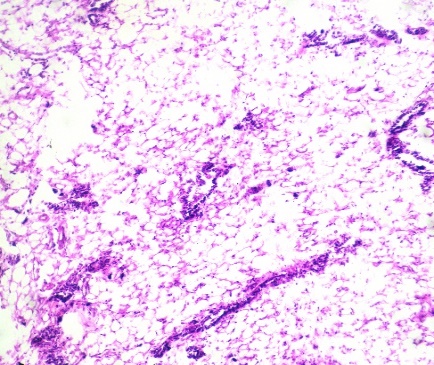
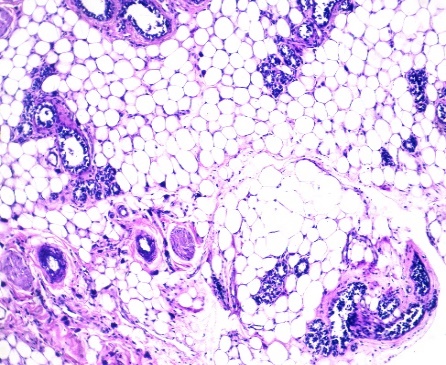
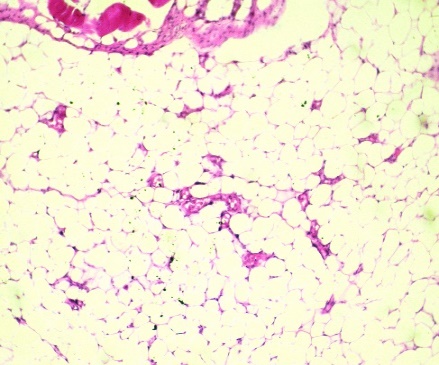
|  |  |  |
| --- | --- | --- |
| S/N | TEST | RESULT (%) |
| 1 | Appearance | Brown |
| 2 | Oduor | Unobjectionable |
| 3 | Moisture Content | 9.970 |
| 4 | Ash Content | 4.505 |
| 5 | Fat | 2.512 |
| 6 | Crude Fibre | 60.45 |
| 7 | Protein | 4.649 |

**Table 3: Mineral Composition of MPE**

|  |  |  |
| --- | --- | --- |
| S/N | TEST | RESULT (ppm) |
| 1 | Iron | 10.974 |
| 2 | Cadmium | 0.0021 |
| 3 | Arsenic | 0.0038 |
| 4 | Copper | 0.9473 |
| 5 | Sodium | 328.66 |
| 6 | Zinc | 7.8642 |
| 7 | Calcium | 822.78 |

**Table 4: Vitamin Content of MPE**

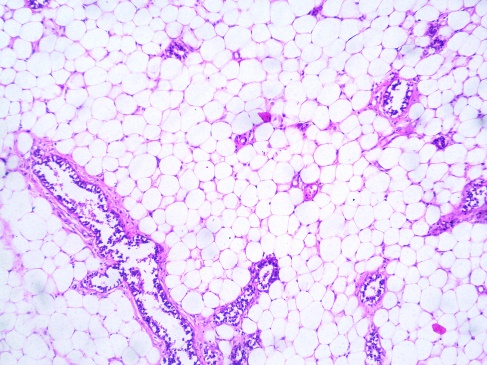
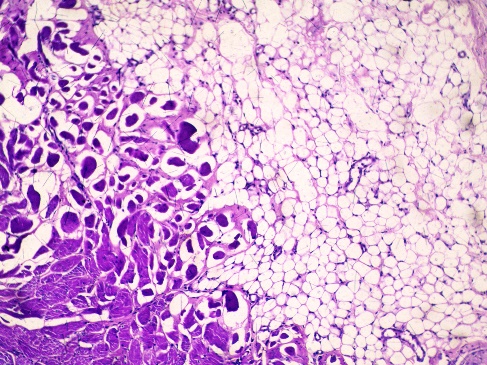
|  |  |  |  |
| --- | --- | --- | --- |
| S/N | TEST | RESULT (mg/100g) | |
| 1 | Vitamin A | | 0.102264 |
| 2 | Vitamin B1 | | 0.170722 |
| 3 | Vitamin B2 | | 0.455512 |
| 4 | Vitamin B9 | | 0.166274 |

 **** 

B

C

A

E

D

**Figure 1 : Effect of ethanolic extract of mango peel on the breast histology of female rats induced with Nitrosomethylurea**

**A (Control**) - Shows fibro-collagenous stroma with ducts and lobules lined by layers of cells along with predominantly adipose tissue. No malignant cells seen. NORMAL BREAST TISSUE

**B (Negative Control NMU 50 mg/kg)** - shows fibro-collagenous stroma with ducts and lobules lined by layers of cells along with adipose tissue. Also seen are dilatation of the mammary ducts, periductal fibrosis, and inflammation. No malignant cells were seen. INFLAMMATORY BREAST

**C (400 mg/kg MPE)** - shows some areas of fibro-collagenous stroma with ducts and lobules lined by layers of cells along with predominantly adipose tissue. No malignant cells were seen. NORMAL BREAST TISSUE

**D (NMU 50 mg/kg and 200 mg/kg MPE)** - shows fibro-collagenous stroma with ducts and lobules lined by layers of cells along with adipose tissue. Also seen are dilatation of the mammary ducts, periductal fibrosis, and inflammation. No malignant cells were seen. INFLAMMATORY BREAST

**E (NMU 50 mg/kg and 400 mg/kg MPE)** - shows fibro-collagenous stroma with ducts and lobules lined by layers of cells along with predominantly adipose tissue. No malignant cells seen. NORMAL BREAST TISSUE.

**Table 5: Effects of MPE on Oxidative Index in mammary gland tissue.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | MDA | CAT | SOD | GST | GSH |
| NORMAL CONTROL | 2.07±0.15b | 2.92±0.02b | 0.40±0.04b | 1.55±0.03b | 0.27±0.02 |
| NEGATIVE CONTROL | 3.15±0.19a | 1.63±0.05a | 0.20±0.02a | 1.05±0.02a | 0.26±0.04 |
| 400mg/Kg MPE | 1.70±0.14b | 2.13±0.02ab | 0.60±0.06ab | 1.91±0.06ab | 0.54±0.03ab |
| 200mg/Kg MPE + NMU | 2.03±0.08b | 2.22±0.02ab | 0.33±0.02 | 0.99±0.08a | 0.54±0.04ab |
| 400mg/Kg MPE + NMU | 1.78±0.09b | 2.22±0.02ab | 0.61±0.08ab | 2.05±0.05ab | 0.61±0.04ab |
|  |  |  |  |  |  |

**Values are expressed as Mean ± SD. a Significantly different from Normal control group at p<0.05 b Significantly different from Negative Control group at p<0.05. MDA – Malondialdehyde, CAT – Catalase, SOD – Superoxide dismutase, GST – Glutathione-S-transferase, GPx – Glutathione peroxidase, GSH – Glutathione, NMU – Nitrosomethylurea, MPE – Mango Peel Extract.**

**Figure 2: Effect of MPE on liver maker enzymes.**

**AST – Aspartate aminotransferase, ALT – Alanine transaminase, ALP – Alkaline phosphatase. NMU – Nitrosomethylurea. MPE – Mango Peel Extract.**

**Table 6: Effect of MPE and Albumin and Bilirubin Concentration.**

|  |  |  |
| --- | --- | --- |
|  | ALBUMIN (µ /l) | BILIRUBIN (µmol/l) |
| NORMAL CONTROL | 30.47 ±2.77 | 3.70 ±0.27 |
| NEGATIVE CONTROL (NMU ONLY) | 31.37 ±2.20 | 3.20 ±0.53 |
| 400mg/kgbw MPE | 32.77 ±3.61 | 3.87 ±0.23 |
| 200mg/kgbw MPE + NMU | 34.20 ±1.31 | 3.27 ±0.46 |
| 400mg/kgbw MPE + NMU | 29.73 ±2.75 | 3.57 ±0.55 |

**Table 7: Effect of MPE on Serum Electrolytes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Na(mmol/L) | K(mmol/L) | Cl(mmol/L) | HCO3(mmol/L) |
| NORMAL CONTROL | 143.27 ±2.80 | 5.85 ±0.45 | 113.57 ±3.65 | 17.80 ±0.70 |
| NEGATIVE CONTROL | 144.13 ±1.78 | 5.68 ±0.58 | 116.00 ±3.14 | 17.30 ±1.58 |
| 400mg/kgbw MPE | 145.27 ±1.32 | 6.08 ±0.58 | 115.90 ±1.49 | 19.40 ±1.40 |
| 200mg/kgbw MPE + NMU | 139.67 ±4.60 | 5.31 ±0.77 | 106.40 ±5.59 | 22.17 ±2.02 |
| 400mg/kgbw MPE + NMU | 143.83 ±3.39 | 5.88 ±0.51 | 113.10 ±6.32 | 20.67 ±0.42 |

**Table 8: Effect of MPE on Kidney Markers**

|  |  |  |
| --- | --- | --- |
|  | UREA (mmol/L) | CREATININE (µmol) |
| NORMAL CONTROL | 4.83 ±0.47 | 39.53 ±0.57 |
| NEGATIVE CONTROL | 5.70 ±0.87 | 45.80 ±1.61 |
| 400mg/kgbw MPE | 5.00 ±0.20 | 40.57 ±2.40 |
| 200mg/kgbw MPE + NMU | 5.06 ±0.66 | 45.03 ±1.82 |
| 400mg/kgbw MPE + NMU | 5..47 ±0.31 | 44.40 ±1.18 |

**Values are expressed as Mean ± SD. a Significantly different from Normal control group at p<0.05 b Significantly different from Negative Control group at p<0.05. NMU – Nitrosomethylurea, MPE – Mango Peel Extract.**

## Discussion of Results.

The dried mango peels had a dark brown appearance, and determining the proximate composition is essential for evaluating the sample's quality and functional qualities. Table 2 displays the proximate composition of mango peel extract (MPE). The average moisture content of dried MPE was 9.97%, this would confer a long shelf life on sample because of their low moisture content. It was also found to contain (60%) crude fibre, (Tariq et al., 2023) also reported a significant dietary fibre content which is good for gut health and metabolic disorders. The mango peel extract utilized in this research included a substantial quantity of minerals, Iron (10.9741), cadmium (0.0021), arsenic (0.0038), copper (0.9473), sodium (328.6629), zinc (7.8642), and calcium (822.7846). Minerals are necessary for proper growth, muscle and skeletal development (e.g., calcium), cellular activity and oxygen transport (copper and iron), fluid balance and nerve transmission (sodium and potassium), and acid-base regulation. Iron may help prevent anemia and other related disorders (Elstrott et al., 2019). Zinc stimulates protein synthesis, normal body development, and sickness healing (Willekens & Runnels, 2022). Deficiencies in several nutrients and minerals have been related to poor human health. Vitamins are a class of chemical components present in little quantities in foods that the body need for optimal metabolism (Akram et al., 2020)The research found that the mango peel extract included varied amounts of vitamins A (0.102264), B1 (0.170722), B2 (0.455512), and B9 (0.1662737). Vitamin A has been demonstrated to improve immune function by maintaining and activating white blood cells and other immune cells. It also helps to minimize free radicals and their negative effects. Vitamin B2 (riboflavin) complemented the other B vitamins. It is necessary for proper body growth and red blood cell formation. Vitamin B9 (folate) collaborates with vitamin B12 to generate red blood cells. It is essential for DNA synthesis, which controls tissue growth and cell function (Akram et al., 2020).

The liver and kidneys play a crucial role in various metabolic functions, including growth, energy production, reproduction, nutrient provision, disease resistance, metabolic homeostasis regulation, and drug-induced metabolites (Casotti & D’Antiga, 2019). Free radicals, medications, and viral infections can affect these organs. The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (BL), and albumin (ALB), Urea, creatinine and electrolytes can be used to assess liver and kidney function. These enzymes and substances can reach the circulation and serve as indicators of illness. The study found that nitrosomethyl urea (NMU) treatment caused a significant increase in serum ALT levels, which may suggest hepatocyte injury. However, pre-treatment with mango peel extract significantly lowered ALT levels, demonstrating that MPE may inhibit transaminase enzyme activity while promoting regeneration and maintaining liver cell membrane integrity. MPE also restored rats' ALP levels to normal, demonstrating its hepatoprotective characteristics. The study also found that the extract restored liver cell function, as liver bilirubin levels rose after extract administration, confirming its efficacy in restoring normal liver function. . Electrolyte levels which is a marker for kidney function seem to remain normal across all groups in this study which might indicate that NMU acute toxicity did not adversely affect the kidney. However, slightly elevated levels of creatinine and urea were observed in the negative control groups and were remediated in the treatment groups.

MDA is the end consequence of lipid peroxidation; hence, an increase in hepatocytes implies that lipid peroxidation occurred owing to antioxidant system failure, resulting in tissue damage (Tsikas, 2017) . The present research clearly shows that providing 50 mg/kg body weight of nitrososmethylurea in the negative control (nmu alone) dramatically elevated MDA levels in comparison to the normal control group, resulting in oxidative stress. However, rats pre-treated with mango peel (200 mg/kgbw and 400 mg/gbw) showed a substantial reduction in MDA (Table 5). Malondialdehyde (MDA) and reduced glutathione (GSH) levels have been seen to increase and decrease in a number of tissues under oxidative stress (Samarghandian et al., 2017). Natural compounds' antioxidant activities have been connected to increased levels of endogenous enzymes SOD CAT GST, and GSH, as well as exogenous antioxidant capacities (Aziz et al., 2019)Glutathione is a key endogenous antioxidant for liver maintenance. It is one among the most abundant non-enzyme free radical scavengers generated by the liver. It neutralizes free radicals such hydrogen peroxides, superoxide radicals, and alkoxy radicals while protecting membrane proteins. GSH is also a substrate for glutathione peroxidase (Chakravorty et al., 2020) In this research, nitrosomethylurea therapy led to a substantial drop in breast GSH levels. However, rats treated with mango peel extract at dosages of 200 and 400 mg/kgbw showed a considerable rise in GSH levels, as indicated in Table 5. Glutathione S-transferase (GST) is an important enzyme in detoxification and cytoprotection (Singh & Reindl, 2021) .As a phase II drug metabolizing enzyme, it catalyzes the conjugation of reduced glutathione to phase I modified substances such as pharmaceuticals and oxidative stress products, making them less toxic and simpler to excrete. In this study, nitrosomethylurea in the negative control (nmu alone) dramatically lowered GST levels compared to the normal control groups. However, providing mango peel extract to rats at 400 mg/kgbw resulted in a considerable rise in GST levels (Table 5). Superoxide dismutase (SOD) is the first endogenous antioxidant that can withstand radicals (Ighodaro & Akinloye, 2018). When rats are exposed to NMU, they require a greater dose of antioxidants. To combat oxidative stress, the body's SOD levels must be supplemented with exogenous antioxidants. The desire for more antioxidants and to promote endogenous SOD drives the use of mango peel extract as an exogenous antioxidant source. Table 5 indicates that group I, the untreated control, had the highest SOD level among groups at 0.40 µmol/ml/min/mg pro. Because there was no NMU therapy in Group I, there was no increase in the free radical level; hence, radical neutralization by SOD was unnecessary. Group II, the negative control, had the lowest SOD level (0.20 µmol/ml/min/mg pro) due to exposure to NMU without mango peel. The findings between the two controls show that NUM has an effect on reducing SOD levels. Treatment with 200 mg/kgbw of mango peel extract increased SOD concentration to 0.33 µmol/ml/min/mg pro in the treated group, while 400 mg/kgbw increased SOD concentration to 0.61 µmol/ml/min/mg pro, indicating that higher concentrations had a greater effect. In this investigation, the concentration of CAT in the breast tissues of the NUM-treated group dropped, indicating an increase in free radical generation caused by the NUM metabolic product's activities (Table 5). However, administering mango peel extract (200 mg/kgbw and 400 mg/kgbw) reduced NUM-induced toxicity by boosting CAT levels in groups treated with 200 mg/kgbw MPE + NMU and 400 mg/kgbw MPE + NMU, respectively. These findings also indicate that mango peel extract has antioxidant activity to combat free radicals produced by NMU exposure.

This study also examined the histological features in the breast tissue of rats that had been given nitrosomethylurea (NMU) and treated with mango peel extract. The histological examination indicated dilatation of the mammary ducts, periductal fibrosis, inflammation, and increased vascular abnormalities in the group treated exclusively with NMU, which is most likely indicative of breast hyperplasia (Hamwi & Winters, 2020). This discovery may be attributable to the carcinogenic qualities of NMU identified in this cohort, which are consistent with previous studies on NMU's capacity to induce breast cancer (Faustino-Rocha et al., 2015). The reduced vascular abnormalities observed in histological sections of breast tissue from NMU-induced experimental animals treated with varying concentrations of mango peel extract (Figure 2) could be attributed to the extract's anticancer properties, which supports (Lauricella et al., 2019) findings on the anticancer effects of mango peel extract in colon cancer cells.

# CONCLUSION

Mango peel extract contains vital elements such as protein, dietary fiber, ash, and fat. It includes health-promoting substances including vitamins and minerals, particularly calcium. Furthermore, this study discovered that mango peel extracts at 200 mg/kgbw and 400 mg/kgbw protected rats from nitrosomethylurea-induced mammary gland hyperplasia by increasing enzymatic antioxidants such as GST, SOD, and catalase, decreasing MDA production, and increasing the non-enzymatic antioxidant molecule GSH. Mango peel extract has excellent antioxidant activity and may be useful in protecting tissues from oxidative stress.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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