Volume 6. Issue 2, 2025

Trends in
Natural Products Research

Evaluation of Antitumor Activities of Trimethoxy Benzaldehyde Substituted Chalcone (C5) in 1-Methyl Nitrosourea-Induced Mammary Tumor in Rats

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Abstract

Chalcones have been identified as potential antitumor agents, and beta-tubulin has been reported as a novel target for anticancer agents. This study evaluated the antitumor activity of the synthesized 2,3,4- trimethoxy benzaldehyde chalcone (C5) as a potential tubulin inhibitor. The LD_{50} of C5 was estimated using the OECD-425 guidelines in rats. A mammary tumor was induced by a single subcutaneous administration of 65 mg/kg of 1-methyl nitrosourea (MNU). The rats were palpated weekly to determine tumor size. Eight weeks after MNU administration, the rats were divided into five groups of six each and treated with graded doses (12.5, 25, and 50 mg/kg) of C5and paclitaxel (10 mg/kg) for six weeks. Before treatment, three rats were randomly selected and sacrificed, and mammary gland samples were subjected to histological assessment to confirm tumor induction. At the end of the treatment period, the rats were euthanized, and blood samples, mammary glands, liver, and kidneys were collected and subjected to hematological, biochemical, and histological evaluations. The compound was relatively safe, with an LD_{50} > 2000 mg/kg. MNU-induced mammary tumor rats treated with C5 produced a significant decrease in tumor diameter compared with the untreated group, and histological slides of the mammary gland displayed fewer signs of hyperplasia and small numbers of connective tissue with larger lobules compared with the untreated group. Treatment with C5 did not produce significant differences in organosomatic indices, and the biochemical and hematological parameters were within the normal range. The synthesized chalcone demonstrated remarkable antitumor activity against MNU-induced mammary tumors in rats.

Keywords: Mammary tumor, Chalcones, MNU.

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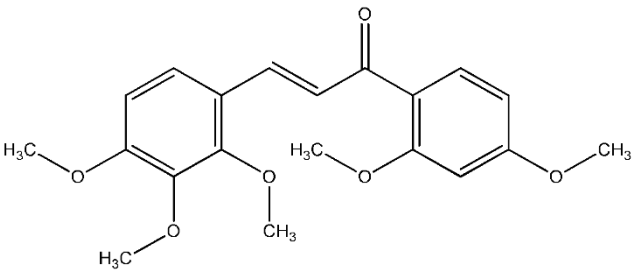
https://doi.org/10.61594/tnpr.v6(2).2025.122
Page No.: 60-69
Volume: Volume 6 Issue 2, 2025
Trends in Natural Products Research

Introduction

Cancer is a major health problem affecting millions of people worldwide (Ferlay et *al.*, 2021). Chemotherapy remains the main intervention for the treatment of cancer. Despite the discovery of new anticancer drugs, the treatment process is still inadequate and unsatisfactory because of its effects on normal human cells combined with cell resistance to anticancer drugs (Yang *et al.*, 2018). As a result, there is need for the discovery and development of new anticancer drugs with lower toxicity and better efficacy against the growth rate of anti-cancer drug resistance (Parkin *et al.,* 2021). Microtubules are hollow cylindrical assemblies of tubulin heterodimers that participate in many processes, such as cell division, where they form the mitotic spindle or intracellular border, where they form the paths along which microtubule-based neurons move (Perez, 2009), making them an attractive target for anti-cancer drug design (Jordan and Kamath, 2007). Currently, numerous microtubule inhibitors (MTIs) such as taxanes, *vinca* alkaloids, and epothilones are used in the management of many solid and hematological cancers (Bhalla, 2024). Microtubule inhibitors have been demonstrated to produce a high level of anti-cancer activity in clinical treatment; however, their effectiveness is limited by high toxicity and the development of drug resistance (Kavallaris, 2010). Colchicine was the first identified tubulin-destabilizing agent (Lockwood, 1979), but it has not been approved for the treatment of cancer because of its low therapeutic index and high toxicity, and many efforts have been made to develop colchicine-binding site inhibitors (CBSIs). Favorable factors for most CBSIs (for example. chalcones) have little or no multidrug resistance (MDR) issues, have simple structures, and are easy to synthesize (Muhammad *et al.,* 2023a). Research has revealed that another possible way to prevent MDR is to develop irreversible binding agents, as suggested by Buey *et al.*, who reported that a natural small molecule (cyclostreptin) retains its activity in cells that are resistant to paclitaxel by upregulating P-glycoprotein through covalent binding to β-tubulin (Buey *et al.,* 2007). The authors suggested that resistant tumor cells cannot eliminate the effect of compounds irreversibly binding to them by decreasing their affinity for the target or by increasing drug outward, and suggested that designing a compound that irreversibly binds to tubulin could be an effective way to prevent drug resistance. The compound: 2,3,4- trimethoxy benzaldehyde chalcone (C5), is a synthetic compound. It was synthesized at the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria. The 2-dimentional structure, IUPAC name, and molecular formula of C5 are shown in Figure 1. Compound (C5) is a milky powder that is slightly soluble in water. The compound was designed to bind to the colchicine-binding site on the tubulin protein to have good anticancer activity. This study aimed to evaluate the antitumor activity of a synthesized chalcone (C5) with less toxicity and a greater ability to fight drug resistance.



($C_{20}H_{22}O_6$)

1-(2,4-dimethoxyphenyl)-3-(2,3,4-trimethoxyphenyl) prop-2-en-1-one

Figure 1: Chemical structure of C5

Materials and Methods

Materials

The compound (C5) was synthesized at the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University (ABU), Zaria. 1-methyl nitrosourea, Paclitaxel, weighing balance, animal cages/feeders, dissecting kid, formalin, chloroform

In Vivo Antitumor Study

Animal Management

Ethical approval was obtained from the ABU Committee on Animal Use and Care (ABUCAUC) (approval number ABUCAUC/2022/003). Sixty Wistar rats weighing–60-80 g (not more than 50 days old) was obtained and housed in the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Brazil. The animals were fed standard feed and provided with access to water *ad libitum.*

Acute toxicity studies in rats

The oral median lethal dose (LD_{50}) was determined according to the OECD 425 guidelines for rats. Three rats were fasted for 3 h before treatment. Food was withheld for two hours after administration of the new compound. A rat was administered 2000 mg/kg (limit test) and observed for 48 h. The first rat survived; therefore, two additional rats were administered 2000 mg/kg. The rats were observed for signs and symptoms of toxicity at least once during the first 30 min, periodically during the first 24 h, and daily for 14 days after dosing.

Induction of mammary tumor

Female Wistar rats 45-50 days old) were used in this study. Mammary tumors were induced according to the method described by Thompson *et al.* (1992) with minor modifications. Methyl nitrosourea was dissolved in normal saline and 65 mg/kg was administered by subcutaneous injection beneath the mammary glands of each rat. The rats were observed and palpated weekly to determine the development, localization, and size of the neoplasia in the mammary glands. Eight weeks after tumor induction, the rats were divided into five groups of six rats each. Rats in group I served as a negative control and received normal saline (1 ml/kg); Group II served as a positive control and received paclitaxel (10 mg/kg, *i. p.*) on alternate days. Groups III, IV, and V rats were treated with graded doses ofC_5(12.5, 25, and 50 mg/kg, respectively, orally) daily. The tumor diameter of each rat was measured before the commencement of treatment and weekly during the treatment with C_5. All animals were treated for six weeks.

Samples collection

At the end of the study, the rats were euthanized and blood samples were collected and subjected to hematological and biochemical analyses. Mammary gland, liver, and kidney samples were collected, and preserved in $10\%^{v}/_{v\ formalin}$ in normal saline for histological assessment.

Statistical Analysis

Statistical analysis was carried out using SPSS (Version 20), and the data obtained are expressed as mean ± SEM. Differences between means were analyzed using one-way or repeated measures analysis of variance (ANOVA), followed by Bonferroni post hoc test; for multiple comparisons, values with $P < 0.05$ were considered significant.

Results

In Vivo Study

Acute Toxicity Study

The oral median lethal dose (LD_{50}) of C5 was greater than 2000 mg/kg body weight in rats. This suggested that the compound was relatively safe.

Effect of the Synthesized Chalcones on Mean Tumor Diameter in MNU-Induced Mammary

Tumor Rat

There was a significant decrease in the mean tumor diameter of the group of rats treated with C5 at all tested doses when compared with the tumor diameter of the rats before treatment. There was an increase in the mean tumor diameter of the group of rats treated normal saline (Figure 2)

Effect of C5 on Hematological indices

There were no significant changes in the hematological indices of the rats treated with all the compounds at all tested doses when compared to normal control (figure 3)

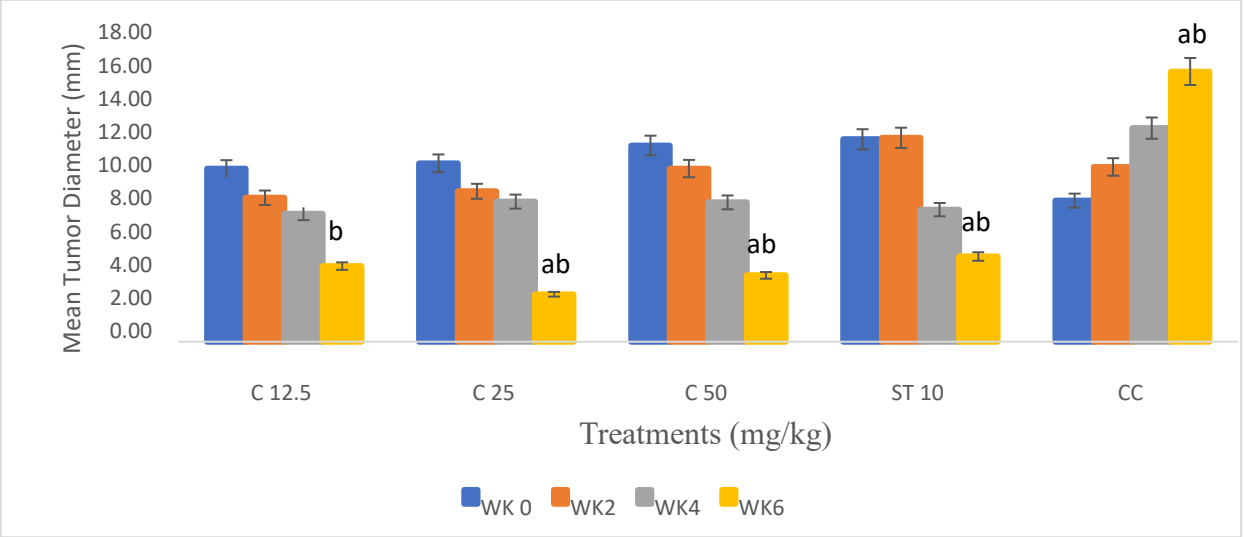


Figure 2: Effects of C5 administration on average tumor diameter in MNU MNU-induced mammary tumors. Values are expressed as mean ± S.E.M. Data was analyzed using repeated measures ANOVA followed by Bonferoni post hoc test. n=6, C = C5, ST = Paclitaxel CC =cancer control, 12.5, 25, 50 and 10 = doses in mg/kg, a = difference within, b = difference between the groups

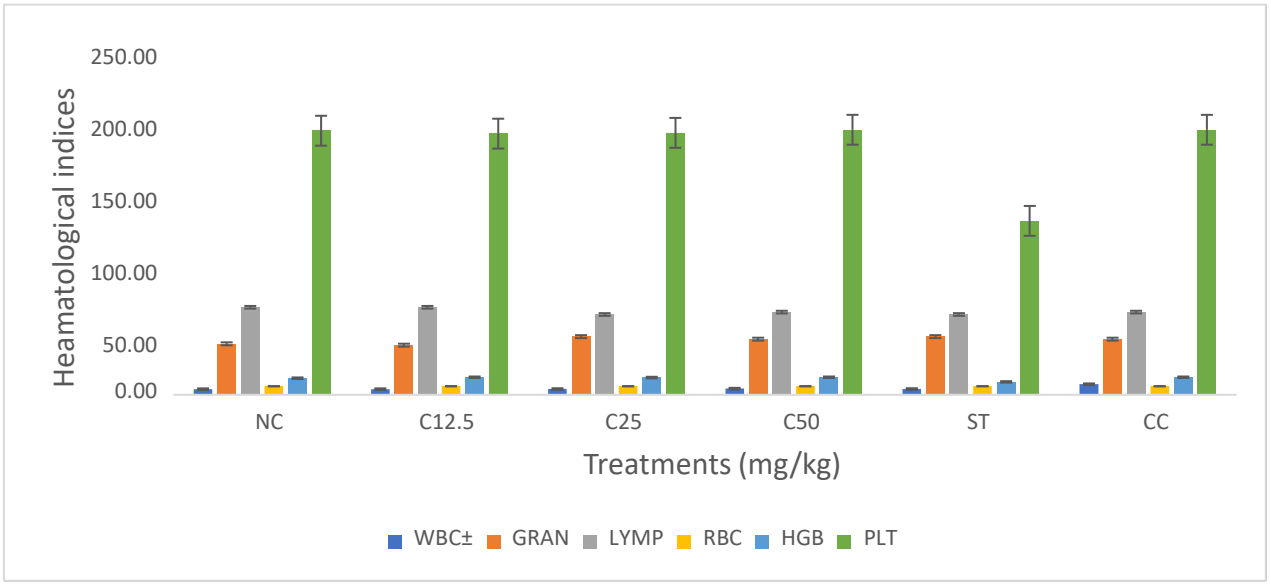


Figure 3. Effects of C5 on Hematological Parameters of Wistar Rats
Values are expressed as mean ± S.E.M. One-way ANOVA. n = 6, NM = Distilled water, WBC=White blood cells, GRAN=Granulocytes, LYMP=Lymphocytes, RBC=Red blood cells, HGB= Haemoglobin, PLT= Platelets, NC= Normal control, C = C5, ST = Paclitaxel CC = Cancer control, 12.5, 25 and 50 = graded doses of C5 (mg/kg)

Effect of the Synthesized Chalcones on Histological Examination in MNU-Induced Mammary Tumor Rat

The mammary tissues of control rats had normal fibrous connective tissue, well-differentiated ducts, and tiny lobules. The induction of mammary gland tumors, characterized by dilated ducts filled with tumor cells, extreme hyperplasia of mammary lobules, and decreased connective tissue, occurred in MNU-induced mammary tumor rats. Rats with MNU-induced mammary tumors treated with C5 displayed fewer signs of hyperplasia and fewer connective tissues with larger lobules when compared with the untreated group. The group of rats treated with (25 mg/kg) showed no signs of hyperplasia, ducts were well differentiated, and tiny

lobules that were almost the same as those in the control group were observed (Figure 4).

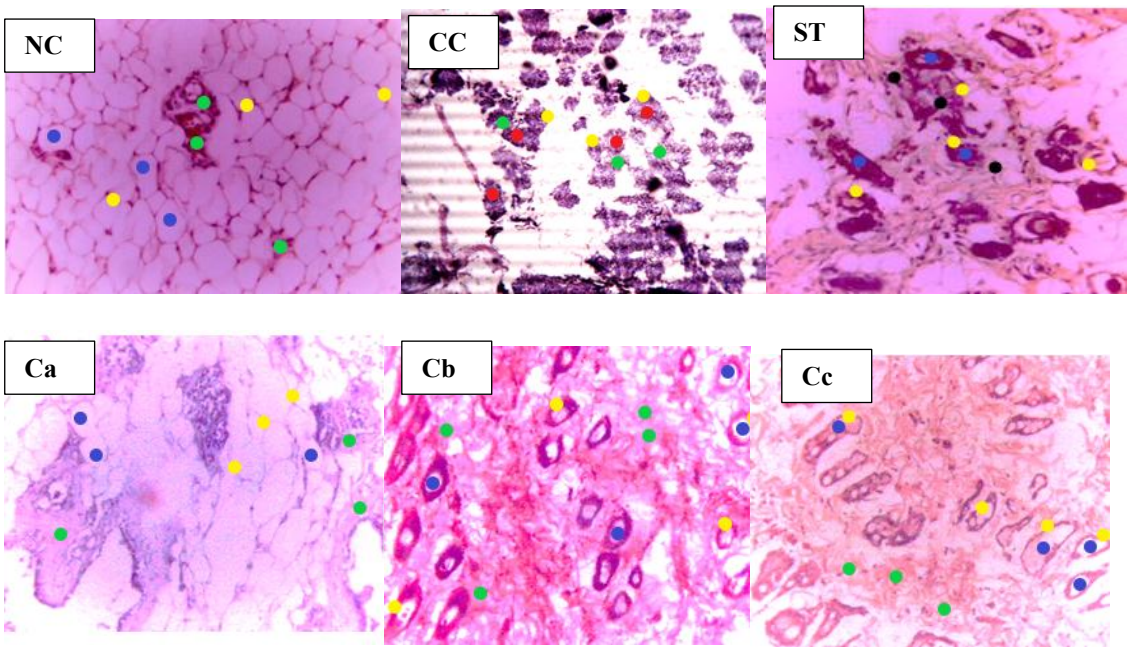


Figure 4: Photomicrograph of a Sections of the Mammary Gland of Female Wistar Rats Showing the Lactiferous Glands of the Rats Treated with C5

NC=normal control, CC=cancer control, ST=Paclitaxel (10mg/kg) C5= compound, a=12.5 mg/kg, b=25 mg/kg and c=50 mg/kg, red dots=cancer cells, blue dots=milk duct, yellow dots=lobules, green dots=connective tissues.

Effects of C5 on Rat's Kidney

There was no significant difference in serum renal parameter levels when compared to the normal control (Figure 5). Histological studies of renal tissue of all rats treated with C5 showed normal sizes of Bowman capsules, glomeruli, and tubules (Figure 6).

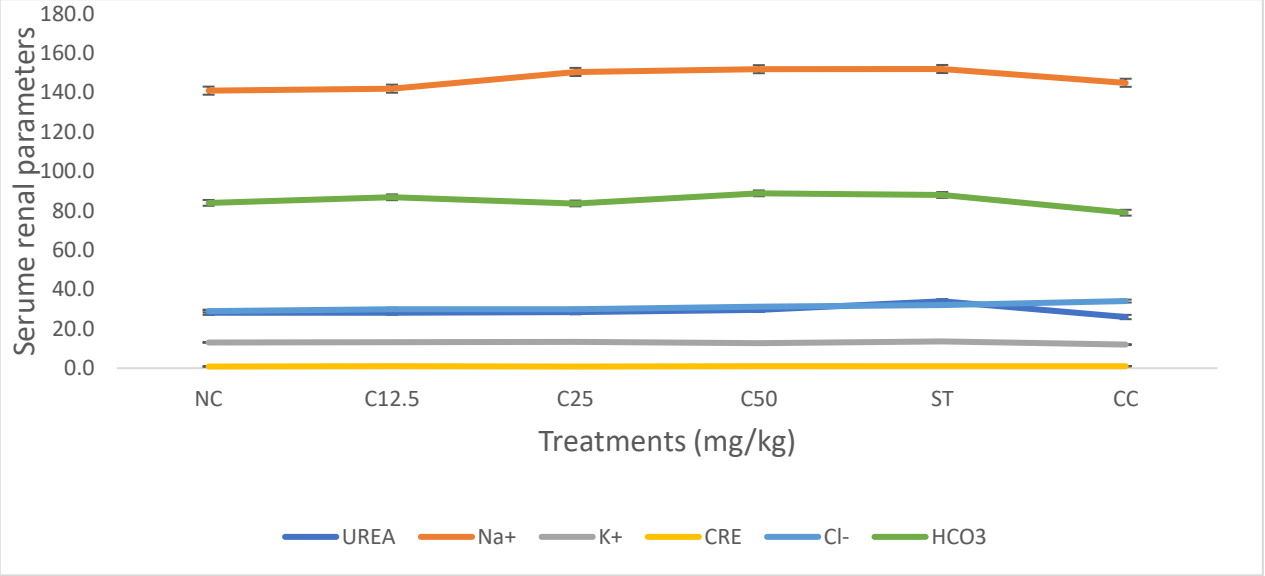


Figure 5. Effect of C5 on Renal Parameters of Wistar Rats
Values are expressed as mean ± SEM. One-way ANOVA followed by Dunnet post hoc test. n = 6, NC= Normal control, C = C5, ST = Paclitaxel CC = Cancer control, 12.5, 25 and 50 = graded doses of C5 (mg/kg)

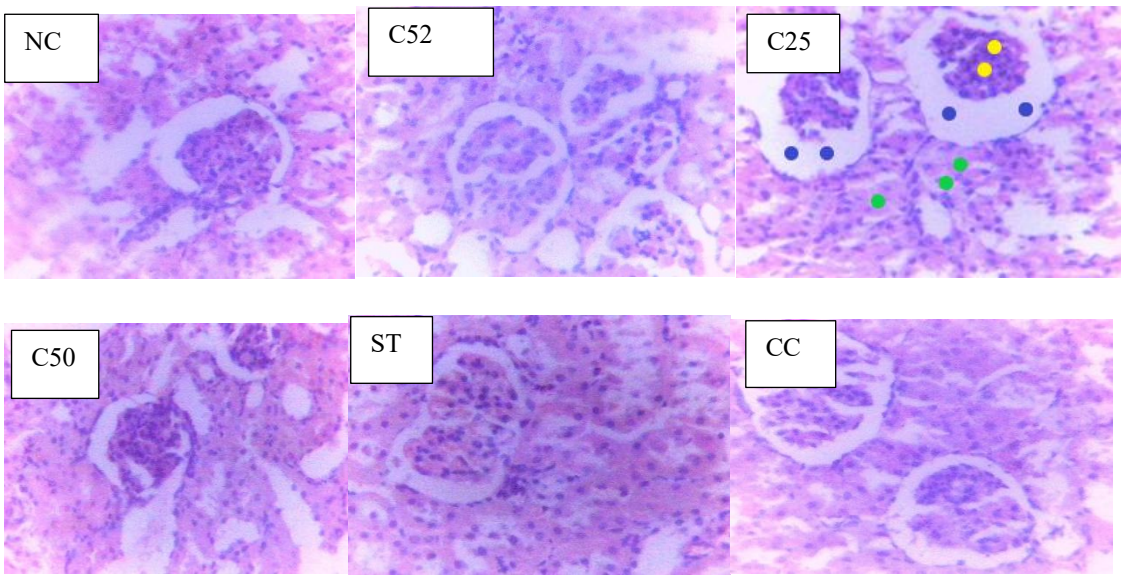


Figure 6: Photomicrograph of a section of the kidney of female Wistar rats treated with normal saline, MNU a**nd C5**(H and E X 250)

Effects of the Compounds on Liver

There was no significant difference in serum liver enzyme levels between the two groups (Figure 7).

Histopathological assessment of the liver showed mitotic bodies, clear sinusoids, cords of hepatocytes, a well-outlined central vein and portal triad, and conspicuous nucleoli across the group (Figure 8).

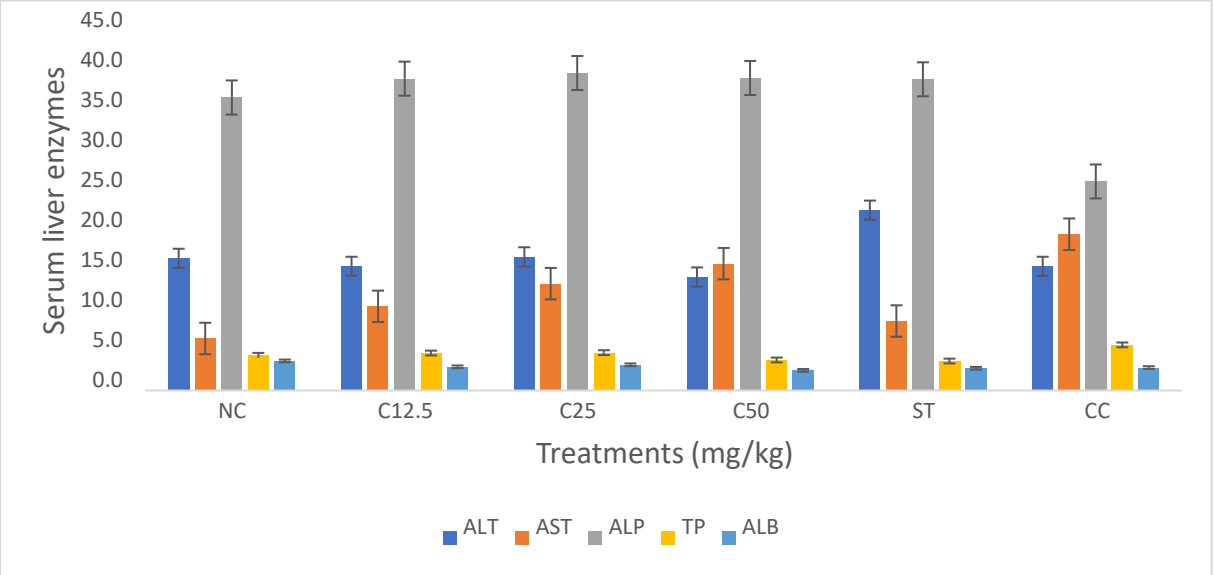


Figure 7. Effect of C5 on Serum Liver Enzymes of Wistar Rats
Values are expressed as mean ± SEM. One-way ANOVA followed by Dunnet post hoc test. n = 6, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, TP= Total protein; ALB = Albumin, NC= Normal control, C = C5, ST = Paclitaxel CC = Cancer control, 12.5, 25 and 50 = graded doses of C5 (mg/kg

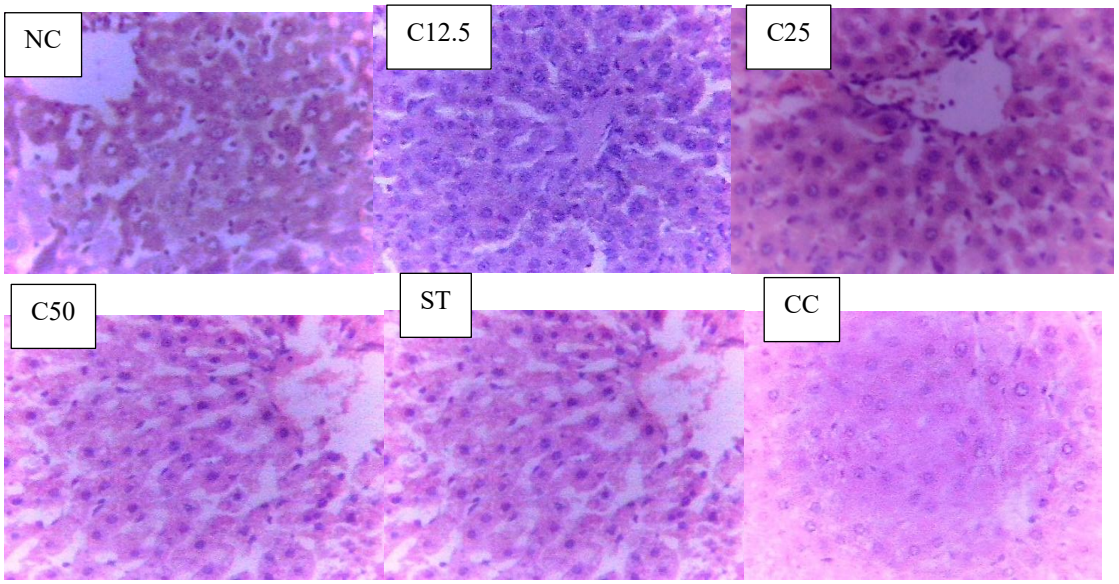


Figure 8: Photomicrograph of a section of the liver of female Wistar rats treated with normal saline, MNU and C5(H and E X 250)

Discussion

The LD_{50} of a compound provides an insight into the potential toxicity of that compound and serves as a guide for the doses to be used for its pharmacological activity (Gbolo *et al.,* 2020). The synthesized compound (C5) was found to be relatively safe, with an LD_{50} above 2000 mg/kg orally. This provides initial evidence of the potential safety of the compound.

Tumor size is one of the indices used for assessing the progress of cancer in patients. The mean tumor diameter is one of the tools used to assess the treatment in this study, the ability of C5 to significantly decrease the tumor size of the rats, indicating that C5 possesses antitumor activity in MNU-induced mammary tumor rats. This is because an increase in the size of the tumor signifies disease progression; any substance that is capable of significantly decreasing the tumor diameter implies that it possesses cytotoxic activity. Histological evaluation is a crucial step in the assessment of breast cancer and helps determine the type, grade, and stage of the disease, which are important factors in guiding treatment decisions and determining prognosis. Histological features of breast cancer (ductal carcinoma) include inflammation and dilation of the duct filled with tumor cells, hyperplasia of the lobules, and decreased number of connective tissues (Jagsi et *al.,* 2019). All features were present in the MNU-treated group of rats, thus confirming the induction of cancer. Induction of tumors was performed using MNU, which is a chemical carcinogen that is known to destroy the p53 gene. This gene is responsible for inhibition of cell proliferation and induction of apoptosis (Mantovani, 2018) Treatments with the C5 was found to have a remarkable antitumor activity at all the tested doses in MNU-induced mammary tumor. The ability of C5 to reduce tumor diameter is a result of its ability to kill cancer cells and control hyperplasia seen in histological slides, leading to tumor shrinkage. An increase in tumor diameter indicates an increase in cell proliferation, which leads to hyperplasia and formation of cancer cells. The highest activity was observed in the group of rats treated with 25 mg/kg of C5, which implies that C5 at this dose was able to almost completely kill the cancer cells and prevent further abnormal proliferation of the cells of the lactiferous gland. Chalcones have been shown to exert cytotoxic activity against many cancer cell lines through multiple mechanisms, such as inhibition of angiogenesis, inhibition of cell proliferation, and induction of apoptosis (Muhammad *et al*., 2023b; Banerjee*et et al.,* 2018). The ability of C5 to inhibit or kill cancer cells induced by MNU could be suggestive of the activity of the compound via either or all of these mechanisms.

Evaluation of the hematological parameters of the rats that were treated with different doses of C5 showed no significant differences compared with those of rats in the normal control group, indicating no abnormalities in metabolic processes, injury, deprivation, or drug-related stress (Smith *et al.,* 2012). Anticancer agents, such as alkylating agents, antimetabolites, and microtubule inhibitors, are known to cause hematological disorders, such as neutropenia, leukopenia, myelosuppression, and myeloblastic anemia. These disorders often lead to discontinuation or delay in chemotherapy and can sometimes be lethal (Henry *et al.*, 2020). Based on the results obtained, it can be assumed that the tested doses of C5 did not cause any alterations in the biological importance of hematological parameters. The synthesized compound (C5) might have an advantage over the available anticancer agents in terms of hematological parameters.

Evaluation of the liver enzymes and renal parameters of the rats that were treated with the different doses of C5 showed no significant differences compared with those of rats in the normal control group, indicating no abnormalities in metabolic processes, injury, deprivation, or drug- related stress. The liver and kidney are two vital organs affected by drugs because of their roles in the metabolism and excretion of drugs. Anticancer agents are known to cause liver and renal damage (Henry *et al.,* 2020).

Histopathological evaluation of the liver and kidney did not reveal gross morphological abnormalities, which could be attributed to the administration of C5 to the rats. It could be assumed that C5 was relatively safe at all tested doses in rats.

Conclusion

This study showed that 2,3,4– trimethoxy benzaldehyde chalcone is relatively safe and possesses anticancer activity against MNU- induced mammary tumors in rats.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

This work was supported by the Tertiary Education Trust Fund (TETFUND) under grant number TETF/DR&D/UNI/ZARIA/IBR/2024/BATCH 8/20

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CITATION: Muhammad Z, Ya'u J, Zezi AU, Magaji MG, Hamza AN (2025) Evaluation of Antitumor Activities of Trimethoxy Benzaldehyde Substituted Chalcone (C5)
Trend Nat Prod Res Vol 6(2). 60-69. https://doi.org/10.61594/tnpr.v6(2).2025.122