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Assessment of the Anti-Inflammatory and Antioxidant Activities of Adansonia digitata L Combined with doxorubicin

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ABSTRACT
Doxorubicin is a highly effective chemotherapeutic agent; however, its application is limited by its toxic properties in the treatment of several cancers, including acute leukemia and lymphomas, among others. These toxic effects significantly constrain its clinical applications. The baobab (Adansonia digitata L.) is a multi-purpose plant with frequent uses and, importantly, considerable medicinal value. Its seeds serve many purposes, including thickening soups and acting as a flavor enhancer when cooked. The aim of this study was to establish the potential anti-inflammatory and antioxidant activities of Adansonia digitata L. combined with doxorubicin.
Materials and Methods: The study involved forty rats divided into four groups to evaluate the effects of Adansonia digitata extract and doxorubicin. Biochemical examinations assessed total antioxidant status and biomarkers of inflammation, which are important in evaluating the level of oxidative stress and inflammation in the body. Immunohistochemical testing of caspase 3 in rat kidneys and livers revealed numerous pathological changes. The present study aimed to assess the anti-inflammatory and antioxidant activities of Adansonia digitata L. when used in combination with doxorubicin.

Keywords: Doxorubicin, Baobab, Caspase 3.

INTRODUCTION

Doxorubicin is one of the most potent chemotherapeutic agents against several cancers; however, its clinical applicability is limited due to toxic side effects, mainly cardiotoxicity and neurotoxicity. Drug toxicities are dose-dependent and can narrow the therapeutic window of doxorubicin, necessitating the development of safer delivery techniques and formulations. Recently, efforts have been directed at innovative strategies capable of overcoming such toxicities without compromising— or even enhancing— the anti-cancer effect of the drug (G. Zhang et al., 2022).

These conditions are primarily due to the overproduction of reactive oxygen species (ROS), which results in doxorubicin-induced hepato- and nephrotoxicity, leading to cellular damage from oxidative stress affecting DNA, proteins, and lipids. Oxidative stress is considered a critical factor in the pathogenesis of liver and kidney damage caused by doxorubicin. Recent studies have investigated various protective agents and mechanisms that exert their effects against such toxicities through modulation of oxidative stress and related pathways (Hassan and Hasary, 2022; Yarana et al., 2022). The resulting cellular damage leads to the release of inflammatory cytokines and activates further pathways, including the p38 MAPK pathway, which promotes cellular apoptosis and inflammation.

The p38 MAPK pathway is an important mediator in the inflammatory response, as it participates in a wide range of physiological and pathological processes, including cell death and inflammation. This pathway is activated by stress stimuli and involves the regulation of pro-inflammatory cytokines and apoptotic molecules, playing a critical role in the progression of inflammatory diseases (van der Zanden et al., 2021). This inflammation manifests clinically as redness, swelling, and pain, indicative of the body's protective response to cellular injury (Zhu et al., 2016). Additionally, doxorubicin-induced oxidative stress affects non-target tissues, including striated muscles, leading to fatigue and exercise intolerance, significantly impairing the quality of life for cancer patients (Ferreira, 2012).

The baobab tree (Adansonia digitata L.) is a versatile plant with numerous uses and significant medicinal properties. Its leaves are commonly used to make soup, while the seeds serve multiple culinary purposes, such as thickening soups, adding flavor when fermented, and being roasted as snacks(Ofori and Addo, 2023). The resulting cellular damage leads to the release of inflammatory cytokines and activates further pathways, including the p38 MAPK pathway, which in turn promotes cellular apoptosis and inflammation. The p38

MAPK pathway represents an important mediator in the inflammatory response, since it takes part in a wide range of physiological and pathological processes such as cell death and inflammation. The pathway, activated by stress stimuli, involves the regulation of pro-inflammatory cytokines and apoptotic molecules and, therefore, plays a critical role in the progress of inflammatory diseases(AKINTAYO, 2023).Adansonia digitata, the baobab tree provides for such a wide range of basic resources-food, shelter, clothing, medicine, and other hunting and fishing accoutrements. Different parts are consumed for their nutritional medicinal and practical values, which include fruit, seeds, leaves, and bark. Its multifunctionality brands it an important portion in the lives of numerousgroups in Africa. The subsequent are the substantialinfluences of the consequence of the baobab tree, the baobab tree provides essential resources for food, shelter, clothing, medicine, and materials for hunting and fishing(Horlu et al., 2023).Therapeutically, frequentparts of the baobab tree, comprisingleaves, bark, roots, bulb, and seeds, offer frequenthealthassistances. These compriseantioxidant possessionsthat protect cells, prebiotic-like consequencesthat encouragegut health, anti-inflammatory properties that reduce swelling, analgesic propertiesthat relieve pain, antipyretic belongingsthat lesseningfever, and anti-diarrhea and anti-dysentery properties that treat stomach issues(Elsayed et al., 2023; Silva et al., 2023). Seeds like those from Adansonia digitata L. and Phoenix dactylifera L. have been processed into coffee substitutes, showing higher contents of ash, protein, fat, fiber, and carbohydrates compared to conventional coffee, but may not have as strong of an aroma or flavor(Garcia et al., 2022; Mwangi et al., 2023).

Caspase-3 is considered a crucial cysteine-aspartic acid protease that acts as an executor molecule in apoptosis-programmed cell death. Its role, however, has recently been extended beyond apoptosis into other processes such as the progression and motility of cells in cancer. Such multifaceted nature of caspase-3 makes it a key focus in research and therapeutic development against cancer(Araya et al., 2021).

Caspase-3 is best known for its roles in apoptosis, where it acts as the final splicing enzyme allowing cellular components to be degraded by programmed cell death.It has also become a biomarker to monitor responses to treatments in cancer therapies, since most treatments rely on the induction of apoptosis in tumor cells(Li et al., 2022).

Apart from causing apoptosis, caspase-3 has also been involved in promoting oncogene-induced malignant transformation. It conveys this through the Src-STAT3 signaling pathway, thus revealing its pro-survival functions in cancer in action(Monteiro et al., 2022).

Caspase 3, a crucial executioner protein in the apoptosis pathway, is activated in response to DOX treatmentOnce activated, Caspase 3 cleaves various cellular substrates, leading to the systematic dismantling of the cell. The interaction between DOX and Caspase 3 is significant because it enhances the drug's ability to kill cancer cells effectively(Li et al., 2022).

Baobab fruit pulp is rich in vitamin C, minerals, and bioactive compounds, contributing to its nutritional and medicinal value(Monteiro et al., 2022).

In experimental models, baobab extracts have demonstrated dose-dependent anti-inflammatory properties, supporting the traditional usage of baobab to treat inflammatory diseases Flavonoids and other phytochemicals that block inflammatory pathways are thought to be responsible for the anti-inflammatory action(Quartey et al., 2021a). Flavonoids and other phytochemicals that block inflammatory pathways are thought to be responsible for the anti-inflammatory action The isAdansonia digitata also known for its antioxidant, antimicrobial, and anti-inflammatory effects, which have been increasingly studied for their health-promoting benefits(Chiacchio et al., 2022).

Objective of study The present study was to assess any potential anti-inflammatory and antioxidant activities of Adansonia digitata L. when combined with doxorubicin. Current study was done in expectation that Adansonia digitata L. could enhance therapeutic effects acrossa decrease in oxidative stress and inflammation accompanying with doxorubicin treatment.

MATERIAL AND METHODS

Experimental animals

The present experiment included a total of forty male albino rats and was carried out under controlled environmental conditions at the Veterinary College, University of Mosul. The rats were individually caged in clean cages, then kept at 24±1°C and a relative humidity of 45-50%, having a 12-hour dark-light phase. They were acclimatized for seven days before starting theexperiment .The animals received tap water and standard pellet feed ad libitum through the experiment. The experimental procedures were approved by the Animal Ethics Committee of the College of Veterinary, Mosul University, and performed under the Guide for the Care and Use of Laboratory Animals NO: UOM/COM/MREC/23-24/DEC2 in 24/12/2023 (Khudair and Al-Okaily, 2022).

Preparation of extract
In present a study where the dry fruit shells of A. digitata were obtainedfrom Sudan, the fruit pulp was mechanically separated into a fine powder at room temperature. The administered dose of 500 mg/kg/day was calculated based on body surface area and given to rats via oral gavage.

Design of experimental
The study design involving forty rats divided into four groups to assess the effects of Adansonia digitata (A. digitata) extract and doxorubicin. The normal Control group(C1) received 0.5 mL of distilled water, while the second group(C2) was administered 15 mg/kg of DOX intraperitoneally weekly. The third group(C3) received a combination of 15 mg/kg of DOX and 500 mg/kg of A. digitata extract, and the fourth group (C4)was given 500 mg/kg of A. digitata extract alone, with the extract administered orally daily for three weeks**.**

Biochemical Examinations
Biochemical examinations of Total Antioxidant Status (TAS) and inflammation biomarkers are pivotal in assessing the body's oxidative stress and inflammation levels(TNF-α). TAS measures the overall ability of the body to counteract free radicals, which can cause cell damage. Inflammation biomarkers such as TNF-α used in clinical practice to diagnose, prognoses, and monitor inflammatory conditions(Sotak, 2022).

Blood collection
Blood collection from study rats is a critical procedure for biochemical examinations, including the assessment of Total Antioxidant Status (TAS) and inflammation biomarkers(TNF-α), technique was employed to ensure efficient and minimally invasive blood sampling. The lateral tail vein puncture methods, with being suitable for conscious, restrained animals and the latter requiring anesthesia, thus allowing for repeated blood draws with minimal stress and risk to the rats(Charlès et al., 2023). The collected blood samples are then analyzed for various biomarkers, including those related to oxidative stress and inflammation.

Preparation of blood sample
The process described involves collecting blood (5ml) from rats and using EDTA-treated tubes to prevent clotting, followed by centrifugation at 3000 RPM for 15 minutes to separate the serum from other blood components. This method aligns with standard practices for blood sample preparation, as EDTA is a common anticoagulant that helps maintain sample integrity by preventing clot formation(Shperling et al., 2022). Centrifugation at 3000 RPM for 15 minutes is within the recommended range for effective serum separation, ensuring minimal hemolysis and maintaining sample quality(Y. Zhang et al., 2022). The separated serum, which lacks clotting factors and fibrinogen, is preferred for many biochemical assays due to its reduced protein content, which minimizes interference in test results(Azad et al., 2020). Storing the serum in a freezer is a standard practice to preserve its biochemical properties for future analysis, as prolonged storage at low temperatures helps maintain the stability of various analytes(Haque et al., 2022)

Preparation of tissues
The histopathological examination of rat kidneys and livers following sacrifice by cervical dislocation and immediate transfer into formalin solution can reveal various pathological changes.

Histopathological assessment
After the treatment period, rats were sacrificed with chloroform. Samples of kidney, and liver were excised, cleared from connective tissue and fat, and then fixed in 10% formalin. After fixation, the samples were dehydrated in a gradient series of ethyl alcohol concentrations for 2 hours each, with two changes in each concentration. Then, the tissues were treated with xylene for 30 minutes to clear it. The preparation was then infiltrated in melted paraffin wax at 58-60°C and embedded in fresh molten paraffin wax to make paraffin blocks. Sections were cut 5-6 µm thick with a rotary microtome and then deparaffinized, stained with hematoxylin and eosin, and studied under a light microscope. The described protocol for histological analysis of kidney and liver tissues, adapted from Suvarna et al. 2018(Suvarna et al., 2019).

RESULT
The survival rates and corresponding survival percentages for the various groups, designated as C1, C2, C3, and C4, can be seen in a bar chart demonstrated the level of survival rates in control C1healthy group 90%and in theC2 doxorubicin group showed decrease in survival rates to 60%,while when give the treatment with Adansonia digitata increase survival rate to 80 % the C4 showed the 100% survival rate .

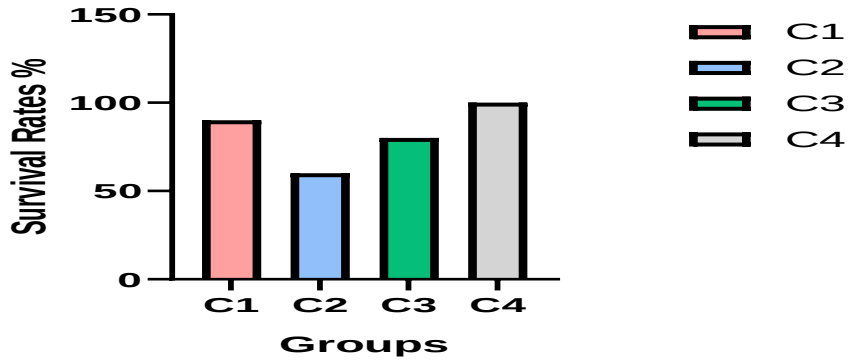

Figure 1: Survival Rates%ofGroups

Group C2 represents a considerably lower TAOS when compared to C1, and C3 is a slight improvement upon C2, but still much lower than that of C1. The level of TAOS in C4 is close to that of C1, suggesting a restoration or no significant decline in the antioxidant status as showed in figure 2 .

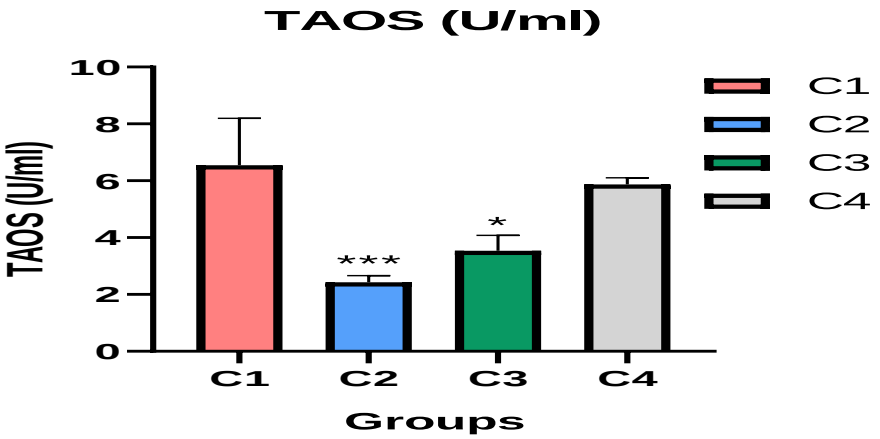

Figure 2: Total Antioxidant Status (TAOS) (U/ml) Levels

While C2 depicts a marked increase in TNF-α, compared to C1 highly significant ***$p < 0.001$, showing a high state of inflammation or an inflammatory condition.
C3 demonstrates the moderate elevation of TNF-α, where *$p < 0.05$ represents less intense but striking inflammation. Moreover, it can be observed that C4 exhibited levels of TNF-α very close to the amount in C1, with no evident changes in inflammation that may point toward normal or regulated conditions. Figure 3 illustrates.

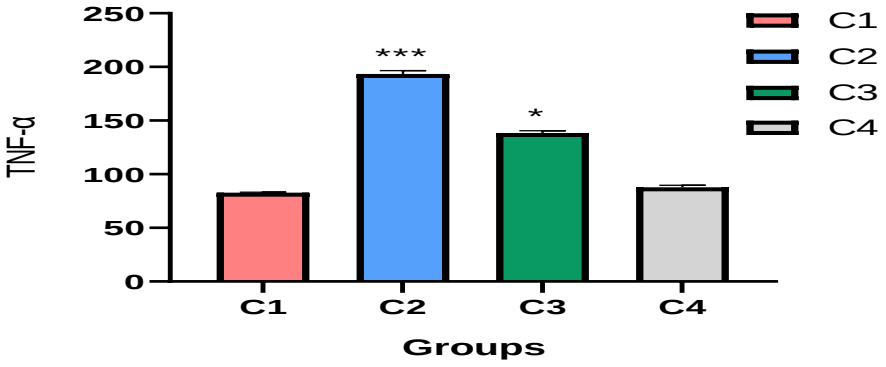

Figure 3: Tumor necrosis factor (TNF-α)levels

Histopathological assessment: Immunohistochemical examination for Caspase 3, the important marker for apoptosis, was performed on liver and kidney tissues of the groups respectively as seen in figure 7 and 8.

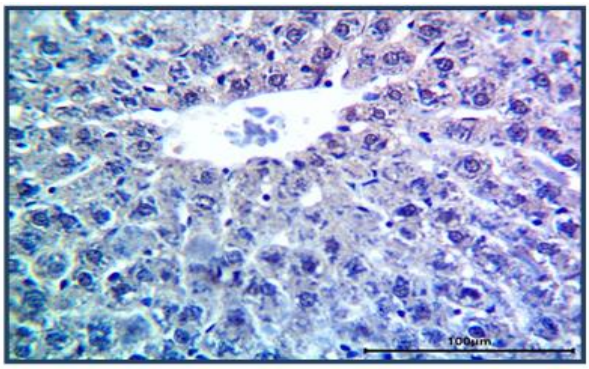

Figure7 A: Immunohistochemical expression of the Caspase 3 in the liver of the control group showing negative expression (score 0-); hematoxylin; 400X.

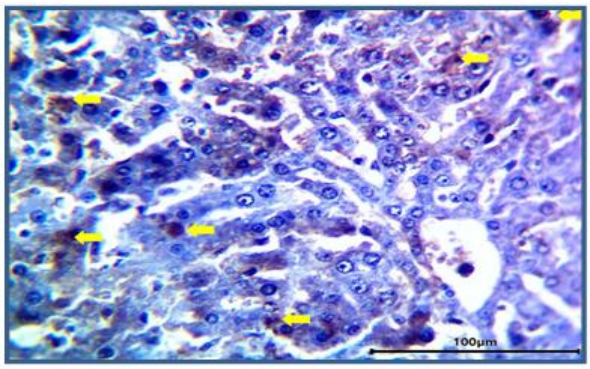

Figure 7B: Immunohistochemical expression of the Caspase 3 in the liver of the G2 Doxorubucine 1.2 group showing intense expression (score 3++++) as a brown color (arrows); hematoxylin; 400X.

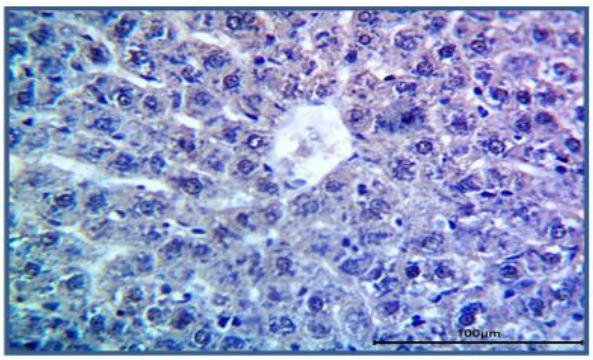

Figure 7C: Immunohistochemical expression of the Caspase 3 in the liver of the G3 Babao 92 group showing negative expression (score 0-); hematoxylin; 400X.

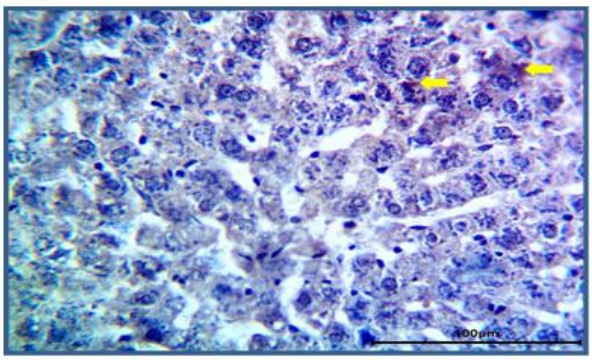

Figure 4D: Immunohistochemical expression of the Caspase 3 in the liver of the G4 Doxo 1.4+boabo 97 group showing weak expression (score 1+) as a brown color (arrows); hematoxylin; 400X.

Figure 7: Immunohistochemical examination for Caspase 3 achieved on liver

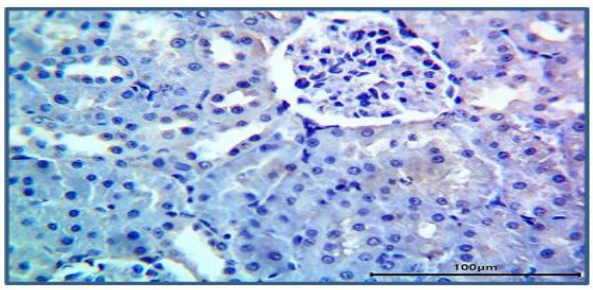

Figure 8A :Immunohistochemical expression of the Caspase 3 in the kidney of the control group showing negative expression (score 0-); hematoxylin; 400X.

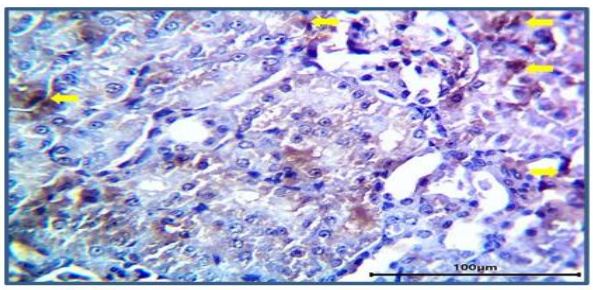

Figure 8B: Immunohistochemical expression of the Caspase 3 in the kidney of the G4 Doxo 1.4+boabo 97 group showing intense expression (score 3++++) as a brown color (arrows); hematoxylin; 400X.

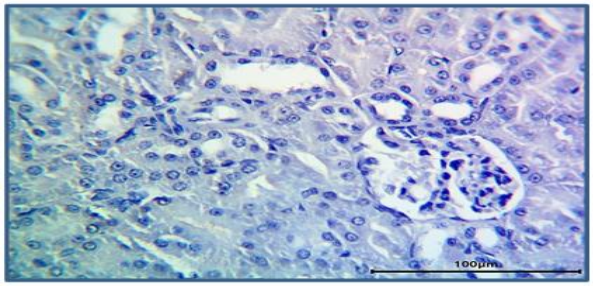

Figure 8C :Immunohistochemical expression of the Caspase 3 in the kidney of the G3 Babao 92 group showing negative expression (score 0-); hematoxylin; 400X.

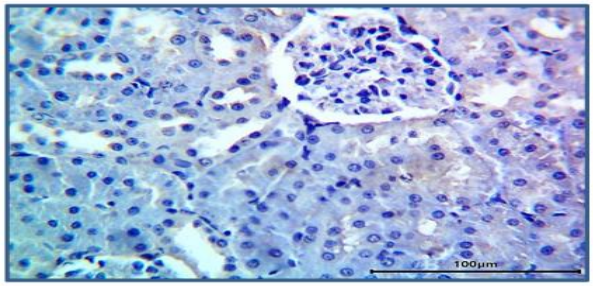

Figure 8D: Immunohistochemical expression of the Caspase 3 in the kidney of the G4 Doxo 1.4+boabo 97 group showing weak expression (score 1+) as a brown color (arrows); hematoxylin; 400X.

Figure 8: Immunohistochemical examination for Caspase 3 achieved on kidney

DISCUSSION

The study showed that treatments influenced the survival rate differently. The survival rate for the control group C1 stood high at 90%, while for doxorubicin in the C2 group, it dropped to half at 60%. The inclusion of Adansonia digitata treatment in C3 improved survival to 80%, while C4 attained 100% survival, which should denote the best treatment approach.(Oktaviani et al., 2019).

Decreased survival rates may be the consequence of doxorubicin's adverse reactions, including higher toxicity and challenges (Cavalcanti Balint et al., 2024).

Following getting Adansonia digitata treatment, the C3 group's survival rate increased to 80%.This implies that Adansonia digitata may improve overall survival byreducing some of the side effects linked to doxorubicin.These findings identify the need to investigate adjunct therapies that could improve conventional therapies, such as doxorubicin, for which traditional medicine may provide a source. Increased survival observed with Adansonia digitata would suggest that phytochemicals in this plant may mop up some of the toxic effects of doxorubicin; thus, mechanisms and potential applications in cancer therapy merit further investigation(Oktaviani et al., 2019; Voon et al., 2013).

The administration of A. digitata extract in DOX-treated rats has the potential for plant-based bioactive compounds to mitigate oxidative stress and, hence improve antioxidant status. In Group C2, those treated with only DOX, there was a significant decrease in the TAOS level as compared to the normal control group, indicating the oxidative stress caused by DOX. However, TAOS in group C3 was slightly improved with C2, but still lower than C1. Notably, group C4, receiving only A. digitata extract, had a TAOS level close to C1, hence showing the potential of the extract to restore or maintain antioxidant status.These compounds help in scavenging free radicals and reducing oxidative stress, which is crucial in counteracting the cardiotoxic effects of DOX(Uhuo et al., 2022).

This improvement in TAOS was slightly enhanced by a combination between DOX and A. digitata in group C3, an indication of some sort of protection by the extract against DOX-induced oxidative stress, as reported in Uhuo et al. (2022).

This also agrees with the results from some studies that requested plant extracts improve the efficacy of chemotherapeutic drugs and reduce their side effects.Whereas the study demonstrates the potential of A. digitata in mitigating oxidative stress, it thus points toward the potential areas of future research needed for complete elucidation of mechanisms and optimization of plant-based therapies in concert with conventional chemotherapy. This may provide a better treatment outcome with minimal side effects in cancer therapy by the incorporation of such natural compounds(Lee et al., 2020). Adansonia digitata which shows several useful effects, at least concerning the stimulus of extracts on tumor necrosis factor-alpha.TNF-α is a cytokine facilitating systemic inflammation and involved in immune response(Hussein and Ali, 2022). Extracts from various parts of Adansonia digitata have demonstrated a potential for modulation of TNF-α levels at least and are believed to be useful in the treatment of inflammatory and infectious diseases. Specific effects and mechanisms through which Adansonia digitata acts on TNF-α are detailed in sections below, together with the associated health benefits. The extracts from Adansonia digitata have been reported to contain noteworthy anti-inflammatory activity. Crucialeffect , as reduction in levels of TNF-α and symptoms associated with inflammation depended vastly on it(Quartey et al., 2021b).\

Caspase-3 immunohistochemical strong expression in the liver of the G2 Doxorubicin 1.2 group indicated an intense degree of apoptosis. These findings agreed with one of the anticipated general responses to Doxorubicin, being that it is a well-documented chemotherapeutic agent capable of irritating cell death through the pathway of apoptosis. Caspase-3 has been observed as one of the most important effectors of the apoptotic pathway and has been utilized as a marker for assessing apoptosis in several tissues, including the liver. In fact, Caspase-3 expression can vary according to the pathological context, as it has been reported by several studies. attention is focused on sympathetic the denotation of its strong expression in the liver underneath the action of Doxorubicin(Silva et al., 2022).

Caspase-3 is one of the main enzymes of the execution phase of apoptosis, responsible for chromatin condensation and DNA fragmentation(Silva et al., 2022).Though the high expression level of Caspase-3 in the liver indicates the drug Doxorubicin's apoptotic effect, one should not forget that the very process of apoptosis is dependent on the interaction of a lot of factors, including other liver disorders or treatments being taken. Such an understanding might provide a broader look into the pathology and treatment outcomes of the liver(Tokin et al., 2013).

CONCLUSION

Current study was done to evaluate the anti-inflammatory and antioxidant activities of Adansonia digitata L. when used in combination with doxorubicin. From the results represented, one can observe a minimum level of inflammatory markers such as TNF-α, along with enhanced antioxidant status in the combination therapy groups compared to the groups treated with doxorubicin alone. Indeed, Adansonia digitata L. Finally, Adansonia digitata L. effectively attenuated the oxidative stress and inflammatory response induced by doxorubicin,

showing a lower TNF-α level and higher TAOS level in the groups treated with the extract. Such findings suggest that Adansonia digitata L. might enhance the therapeutic efficiency of doxorubicin and could act to reduce its associated toxicity through antioxidant and anti-inflammatory activity. Further studies will be necessitated to inspect the mechanisms of these protective effects and to find out whether this combination is clinically applicable.

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