**Modulatory Role of Gallic Acid and Vitamin C on Amoxicillin/Clavulanic Acid Combination Induced**

**Hepatotoxicity in Adult Albino Rats**

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**Abstract**

Amoxicillin/ Clavulanate (AC) combination has become one of the antibiotics most widely prescribed used in the treatment of several bacterial infections, associated with liver injury. The purpose of this study was to examine the modulatory effects of gallic acid (GA) and vitamin C (VTC), both separately and together, on oxidative stress-related liver damage. 64 male albino rats were divided into eight groups at random; negative control; GA group; VTC group; GA +VTC group; AC- treated group; AC + GA- treated group; AC + VTC treated group and AC+ GA + VTC treated group. A twice daily dose of AC (31.83 mg/kg) and a single daily dose of both GA (60 mg/kg) and VTC (200 mg/kg/day) were introduced to rats orally for 7 consecutive days. After sacrificed, blood was collected for biochemical analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum tumor necrosis factor alpha (TNF-α), caspase-3, RNA of heme oxygenase-1 gene (HMOX-1) and liver sample for lipid peroxidation and histopathological study. In rats given AC, the protein caspase-3 was upregulated together with the serum levels of AST, ALT, and TNF. Following AC delivery, hepatic levels of malondialdehyde (MDA) significantly increased, although reduced glutathione (GSH), glutathione-S-transferase (GST), and HMOX-1 expression levels were decreased. These results were consistent with the histopathology results. Rats receiving GA and/or VTC in addition to AC experienced less liver damage, oxidative stress, apoptosis, and histological changes. We therefore came to the conclusion that GA and VTC had a favourable modulatory impact against AC-induced hepatotoxicity.

**Keywords:** Amoxicillin, Clavulanate, Gallic acid, Vitamin C, hemoxygenase gene.

**Introduction**

 Drug-induced liver injury is becoming popular around the world. Antibiotics are known as one of the causes of liver injury, due to its high exposure rate (Leitner et al., 2010; Devarbhavi and Andrade, 2014).

 Amoxicillin/clavulanic acid (AC) is an oral Broad-spectrum antibacterial compound composite of an antibiotic semi-synthetic penicillin (amoxicillin) and an inhibitor of β-lactamase (potassium clavulanate) (Fig. 1). For more than 20 years, it has been used successfully to treat a variety of bacterial illnesses (White et al., 2004; Olayinka and Olukowade, 2010).



**Fig. (1):** Chemical structure of amoxicillin/clavulanic acid (Olayinka et al., 2012).

Despite being one of the most frequently given antibiotics, AC may be linked to cholestatic and hepatocellular liver damage, which appears to be predominantly caused by the clavulanate component (Stine and Chalasani, 2015; Yu et al., 2017a).

 Hemeoxygenase (HO) in the heme degradation pathway is a rate-limiting enzyme. Many compounds such as metal ions and heme compounds are produced in animal tissues, especially the liver. Oxidant species have a vital role in the production of heme oxygenase gene by direct action or by consumption of GSH. Increased activity of heme oxygenase will enhance unconjugated bilirubin formation which is recognised as a biological protector against oxidative stress in the liver and an efficient scavenger of reactive oxygen species (ROS) (Guillermo et al., 2000).

 Gallic acid (3,4,5-trihydroxybenzoic acid, GA) is a polyphenolic substance extracted naturally from plants in particular green tea, grapes, various berries and popularly used in food, drugs and cosmetic products. It has been received great attention because of it has been proven to possess powerful antioxidant properties of the diminution reactive oxygen species (ROS), including hypochlorous acid, hydrogen peroxide, hydroxyl radicals, and superoxide anions (Priscilla and Prince, 2009; Chaphalkar et al., 2017).

 Vitamin C (ascorbic acid, VTC) is an antioxidant vitamin soluble in water present in the diet which representing essential cofactor for many enzymes serves as a reducing agent or an electron donor and is involved in a variety of metabolic processes. Reactive oxygen and nitrogen species have a much reduced negative impact, which can induce oxidative damage to macromolecules like those of lipids, proteins and DNA. It is also capable of scavenging/neutralizing singlet oxygen, hydroxyl, superoxide and water soluble peroxyl radical (Jacob and Sotoudeh, 2002; Seghrouchni et al., 2002).

 The current work was planned to assess the scavenging antioxidative bioactivities of gallic acid/ vitamin C and synergistic is involved in several metabolic processes and acts as an electron donor or reducing agent. The detrimental effects of reactive oxygen and nitrogen species are greatly diminished.

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***الدور المعدل لحمض الجاليك وفيتامين سي لمجموعة الاموكسيسيلين/ حمض الكالفولنيك المسبب لتسمم الكبد في الجرذان البيضاء البالغة***

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أصبح الاموكسيسيلين/ حمض الكالفولنيك واحدا من المضادات الحيوية الأكثر وصفا على نطاق واسع حيث يستخدم في علاج الكثير من العدوى البكتيرية ويرتبط ايضا بالاصابة بالكبد. هدفت هذه الدراسة إلى البحث في تسمم الكبد الناجم عن الأموكسيسيلين / حمض الكالفولنيك و التأثير التعديلي لحمض الجاليك / فيتامين سي بشكل فردي ومجتمعيين على تلف الكبد الناتج عن الإكسدة. تم فصل أربعة وستين ذكور من الجرذان البيضاء بشكل عشوائي إلى ثماني مجموعات: مجموعة سلبية ضابطة, مجموعة حمض الجاليك , مجموعة فيتامين سي, مجموعة حمض الجاليك و فيتامين سي , مجموعة الاموكسيسيلين/ حمض الكالفولنيك , مجموعة الاموكسيسيلين/ حمض الكالفولنيك و حمض الجاليك , مجموعة الاموكسيسيلين/ حمض الكالفولنيك و فيتامين سي , مجموعة الاموكسيسيلين/ حمض الكالفولنيك و حمض الجاليك و فيتامين سي. تم إعطاء الجرذان جرعة مرتين يوميًا من الاموكسيسيلين/ حمض الكالفولنيك ( 31.83 ملجم / كجم) وجرعة يومية واحدة لكل من حمض الجاليك (60 ملجم / كجم) و فيتامين سي (200 ملجم / كجم / يوم) عن طريق الفم لمدة 7 أيام متتالية. وبعد الذبح تم أخذ عينات دم لتحليل أمينوترفراز (AST) ، ألانين أمينوترفراز (ALT) وعامل نخر الورم ألفا (TNF-α) ، كاسباس -3 و الهيمواوكسيجيناز الجيني (HMOX-1) وعينات من الكبد للدراسة بيروكسيد الدهون والهستوباثولوجية. يتضح وجود زيادة ذو دلالة احصائية عالية في مستويات AST ،ALT،TNF-α بشكل ملحوظ وزيادة تنظيم بروتين كاسباس -3 في الجرذان التي تم اعطاءها الاموكسيسيلين/ حمض الكالفولنيك. تمت زيادة المحتويات الكبديّة للمالونديالديهايد (MDA) بشكل ملحوظ بعد تناول الاموكسيسيلين/ حمض الكالفولنيك ، ولكنها أنتجت انخفاضًا كبيرًا في المستويات الكبدية من الجلوتاثيون (GSH) و الجلوتاثيون اس ترانسفيريز (GST) وكذلك جنبًا إلى جنب مع التعبير المنتظم عن.(HMOX-1) وكانت هذه النتائج وفقا لنتائج الهستوباثولوجي. ولذلك فان التناول المشترك لـحمض الجاليك و فيتامين سي مع الاموكسيسيلين/ حمض الكالفولنيك للجرذان يؤدي إلى تقليل إصابة الكبد والإجهاد التأكسدي وموت الخلايا المبرمج والتغيرات الهستوباثولوجية.