

HEPATOPROTECTIVE EFFECT OF *ANVILLEA RADIATA* AQUEOUS EXTRACT AGAINST CARBON TETRACHLORIDE-INDUCED INJURY IN RATS

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Received on September 17, 2023

Presented by I. Ivanov, Member of BAS, on December 19, 2023

Abstract

The present study aimed to assess the hepatoprotective property of *Anvillea radiata* aqueous extract (AAE) against carbon tetrachloride (CCl₄)-induced liver injury in rats. Twenty rats were divided into four groups: the control group received no treatment; the second group received a 1 ml dose of CCl₄ (30%) orally; the third received a 1 ml dose of CCl₄ (30%) orally and AAE at a dosage of 40 mg per kilogram of body weight; the fourth group received AAE alone at the same dose. The results indicated that co-exposure to CCl₄ and AAE significantly improved hepatic enzyme markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), compared to the CCl₄-exposed group. Histological examinations demonstrated that AAE preserved the normal architecture of hepatocytes. In conclusion, *Anvillea radiata* exhibits notable hepatoprotective property against CCl₄-induced toxicity in rats.

Key words: *Anvillea radiata*, hepatoprotection, nephroprotection, carbon tetrachloride, histopathology

Introduction. The liver plays an important role in regulating many vital physiological processes such as storage, secretion, and metabolism. The detoxification of numerous xenobiotics and drugs can also occur in the liver. Bile acid from the liver, along with other elements, directs the digestion process. Liver

diseases are among the most serious illnesses. They can be classified as chronic or acute (inflammatory infection), cirrhosis (hepatic fibrosis), and hepatosis (non-inflammatory infection) [1]. They are primarily caused by various risk factors that damage liver cells by inducing lipid peroxidation and other oxidative damage due to the generation of oxidative stress in the liver [2]. Hepatic lesions can be induced by various factors and hepatotoxins, such as carbon tetrachloride (CCl₄), ethanol, lead, and acetaminophen, which are metabolized by cytochrome P450 [3]. CCl₄, a highly potent hepatotoxin, is widely used to induce liver lesions in animal models and to study the role of lipid peroxidation as a mediator of liver injury [4]. CCl₄ is metabolized into a highly reactive trichloromethyl radical (CCl₃) by cytochrome P450 in the liver and can induce lipid peroxidation, leading to damage to the hepatocellular membrane [5]. Several studies have reported that many constituents and extracts of medicinal plants exhibit strong antioxidant activity that could potentially counteract CCl₄-induced liver lesions, as they contain a high level of free radical scavengers such as phenolic acids and flavonoid compounds [6]. In recent years, there has been a growing interest in traditional medicine and the use of natural plant extracts as a source of potential therapeutic agents [7]. Native medicinal plants have been used for centuries in various cultures to treat a wide range of ailments [8]. One region that stands out for its rich botanical diversity is Algeria, known for its numerous endemic plant species with unique therapeutic properties [9]. Among the promising Algerian endemic plants are *Anvillea radiata* (Coss and Dur), commonly known as “Nogd”, which has been traditionally used by the local Berber population for its medicinal properties [10]. Studies have shown that this plant has powerful medical benefits thanks to its rich phytochemical composition, including flavonoids, terpenoids, and phenolic compounds [11]. However, to our knowledge the hepatoprotective effect of the aqueous extract of *Anvillea radiata* has not been investigated. Consequently, the aim of this study was devoted to assessing the potentially hepatoprotective property of *A. radiata* against CCl₄-induced damage in rats.

Materials and methods. Preparation of the aqueous *Anvillea radiata* extract (AAE). The aerial part of *Anvillea radiata* was carefully washed under running water, then dried and pulverized. A 10 g quantity of the powder obtained was subjected to a 15-minute boiling process in 100 ml of water in a refluxing water bath at a temperature of 100 °C. The resulting mixture was then filtered using Whatman Millipore filter paper, in particular type No. 1. The filtrate was amalgamated and centrifuged at 4000 rpm for 20 min. After centrifugation, the supernatant was concentrated to dryness using a rotary evaporator, and the residue obtained was stored at 4 °C until further use. This procedure was repeated every week for the duration of the study [12].

Animals and experimental design. The experiment was conducted using twenty-four mature albino male rats, all aged 3 months. These rats were kept in a room with a 12-hour light and 12-hour dark cycle, maintained at a temperature of

22 ± 2 °C, and were provided unrestricted access to both water and a specialized diet designed for rodents. The rats were randomly divided into four groups, each containing six rats. These groups were as follows: The CCL₄ group, which received a 1 ml dose of CCL₄ (30%). The CCL₄+AAE group, which received both CCL₄ at the same dose and AAE at a dosage of 40 mg per kilogram of body weight, which was administered orally. The third group was given AAE alone, at the same dose, and via the same route as the second group. The control group (CONT) received no treatment.

Collection of biological samples and analytical methods. Upon concluding the experiment, the animals were euthanized in the morning following a 12-hour fasting period. An incision was made in the abdominal area, and blood was extracted from the inferior vena cava and placed into heparin tubes for subsequent biochemical analysis. Measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels were conducted using colourimetric methods with a Chronolab kit. Additionally, the livers were carefully extracted, separated from any adipose tissue, rinsed with saline solution, and then weighed to calculate their respective organ indices using the following formula: Organ index = (Organ weight (measured in grams))/(Body weight (measured in grams)). Finally, the organs were subsequently employed in a histological study following standard techniques, including formalin fixation, paraffin embedding, and hematoxylin-eosin staining.

Statistical analysis. The results were expressed as the mean ± standard error (SE). Data analysis was carried out using one-way analysis of variance (ANOVA), followed by the Bonferroni *t*-test, utilizing SigmaPlot version 11.0. Statistical significance was defined as *p*-values less than 0.05.

Results and discussion. A statistically significant change in body weight and organ weight of experimental animals was shown. However, the results in Table 1 show a significant decrease in body weight and organ weight in the CCL₄-exposed group compared with the control group of rats and with the AAE-treated group of rats. Whereas, serum ALT, AST, and ALP activities increased significantly compared to the control groups and the group treated only with the plant extract (AAE). The combination of both experimental treatments (CCL₄+ AAE)

T a b l e 1
Bodyweight evolution and Liver index

Groups	Initial weight (g)	Final weight (g)	Body weight gain (g)	Liver index (g)
CONT	244±2.60	285±2.92	41±4.05 ^a	5.58±3.02 ^a
CCL ₄	191±4.10	207±4.98	16±5.03 ^b	3.24±1.17 ^b
CCL ₄ + AAE	191±2.85	211±7.09	20±4.33 ^c	4.16±1.61 ^b
AAE	193±5.89	213±4.11	20±7.11 ^c	5.38±1.52 ^a

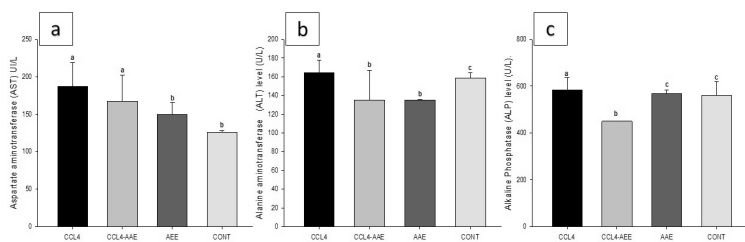


Fig. 1. Biochemical parameters of renal function tests. a: Aspartate aminotransferase (AST); b: Alanine aminotransferase (ALT) level (U/L); c: Alkaline phosphatase (ALP) level (U/L)

did not show any significant differences in ALT and AST levels compared to the group exposed only to CCl₄. However, the ALP level significantly decreased after AAE treatment in rats previously exposed to CCl₄ (Fig. 1). Our results indicate that *A. radiata* extract partially protects against the increased ALT and AST activities induced by CCl₄. In the current context, the elevation of Amino-transaminase in rats treated with CCl₄ is attributed to damage to the structural integrity of the liver. Indeed, the estimation of activities of serum marker enzymes such as AST and ALP allows for the evaluation of liver function. When the plasma membrane of liver cells is damaged, various enzymes normally located in the cytosol are released into the bloodstream. Their estimation in the serum serves as a useful quantitative marker for the extent and type of hepatocellular damage [13]. On the other hand, as demonstrated in our results, *A. radiata* did not induce any effects on hepatic enzymatic markers (AAE groups), which is a clear manifestation of the hepatoprotective effects of the extract. The ability of *A. radiata* to reduce the level of amino-transaminase could be attributed to the inhibition of cytochrome P450-dependent oxygenase activity and the reduction of the accumulation of toxic metabolites derived from CCl₄, as well as the prevention of lipid peroxidation [14]. The hepatoprotective activity of plants may be due to the presence of polyphenols such as gallic acid, vanillic acid, 3,4-hydroxybenzoic acid, catechin, rutin, and quercetin. Indeed, the High-performance liquid chromatography profile of the aqueous extract of *Anvillea radiata* revealed the presence of catechin, syringic acid, protocatechuic acid, vanillic acid, quercetin-3- β -D-glucoside, and rutin. In general, phenolic compounds can directly contribute to antioxidant action due to their antioxidant redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [15]. The protective effect of *Anvillea radiata* extract against oxidative stress induced by CCl₄ in the livers of rats was also demonstrated through histopathological observations. The study of histological sections of the livers of rats treated only with AAE revealed a normal architecture with hepatocytes and normal Kupffer cells. In contrast, the observation of histological sections from groups exposed to CCl₄ showed several anomalies, including congestion of central-lobular veins, along with sinusoidal

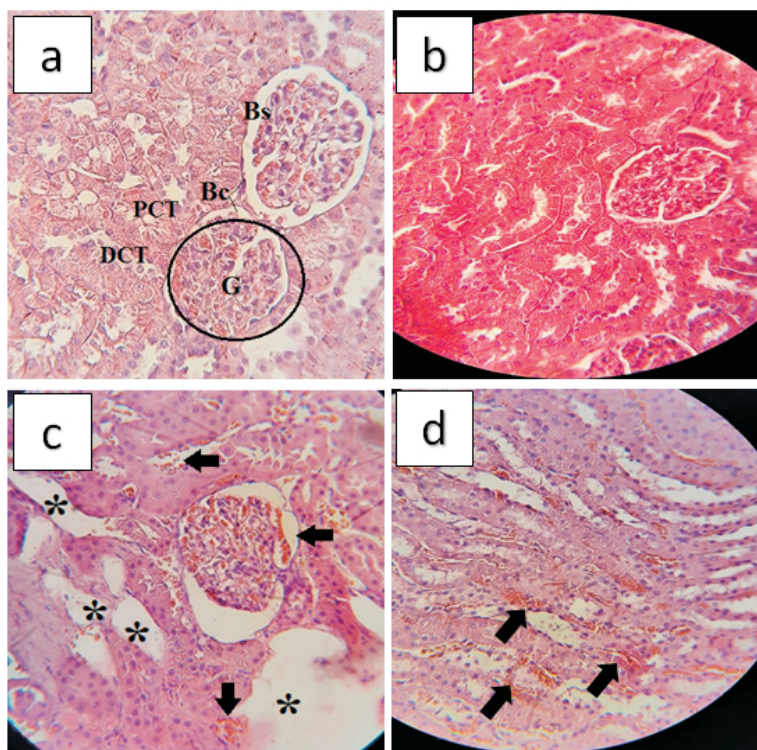


Fig. 2. Histological morphology of rat livers. a-b: Control and AAE groups, respectively, showing the normal architecture of hepatocytes with normal Kupffer cells. c-d: CCl_4 and CCl_4 +AAE groups, respectively, display several anomalies, including congestion of centro-lobular veins along with sinusoidal damage (circles). Hepatocytes also contain abundant bile pigment granules (arrows), indicating cholestasis, appearing brownish-green. Hematoxylin and eosin staining ($\times 40$)

damage characterized by the presence of significant sinusoidal spaces. Additionally, hepatocytes contained abundant bile pigment granules, indicating cholestasis, which is histologically defined as a visible accumulation of bile in liver tissue, manifested by clusters of bile in inter-hepatocyte canaliculi, appearing brownish-green in H&E staining (Fig. 2). These results align with those obtained by HSOUNA et al. [16] and LAOUAR et al. [17] who indicated that CCl_4 -induced histopathological changes in the liver and kidneys of rats. CCl_4 is indeed known for its hepatotoxic properties, and one of its main effects is to cause liver inflammation. When CCl_4 is metabolized in the liver, it generates highly reactive free radicals and toxic intermediates that can cause oxidative stress and damage liver cells [18]. A variety of strategies, including antioxidant activation, anti-inflammatory agents, detoxification pathways and tissue regeneration, are employed to counter CCl_4 -induced liver toxicity by addressing oxidative stress, inflammation and cellular

damage [19]. In fact, studies have demonstrated the anti-inflammatory effects of secondary herbal metabolites, highlighting their role in suppressing inflammation and mitigating CCl₄-induced liver damage [20].

Conclusion. This study demonstrates that *Anvillea radiata* aqueous extract exhibits significant hepatoprotective property against carbon tetrachloride (CCl₄)-induced liver injury in rats. AAE administration effectively ameliorated CCl₄-induced hepatic enzyme imbalances and preserved the normal architecture of hepatocytes. These findings suggest that AAE may hold promise as a natural therapeutic agent for safeguarding liver function against toxic insults. Further research is warranted to elucidate the underlying mechanisms and clinical applications of AAE in liver protection.

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