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Moderating impacts of antioxidant properties of Annona muricata on carbon tetrachloride induced hepatotoxicity and nephrotoxicity in rabbits

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ABSTRACT: This work demonstrated the defensive impacts of Annona muricata (AM) against carbon tetrachloride (CCl_4) as an initiated hepatotoxicity and nephrotoxicity in rabbits. Carbon tetrachloride (CCl_4)have been proven as hepatotoxic agent. The mechanism of its hepatotoxicity lies in its biotransformation by the cytochrome P450 framework to two free radicals. Annona muricata (AM) a plant that is several studies have confirmed its medicinal activity in different therapeutic benefits. The liver and kidney of dissected rabbits showed scars of depression in response to carbon tetrachloride administration whereas those of the control animals showed normal appearance. Results indicated that treatment with (AM) alone caused significant ($P<0.05$) increase in body weight (BW) and relative weight of liver and kidney compared to control animals. On the other hand, significant ($P<0.05$) decrease in BW and relative weight of liver and kidney in rabbits treated with CCl_4 compared with control. Treatment with (AM) alone decrease urea and creatinine in male rabbits. On other hand, treatment with CCl_4 alone caused a significant increase ($p<0.05$) in plasma urea and creatinine compared to control group. Treatment with AM alone caused a significant ($p<0.05$) decrease in plasma and increase in liver AST, ALT and AlP activities. While, treatment with CCl_4 significantly ($p<0.05$) increased plasma and decreased liver AST, ALT and AlP activities compared to control. The presence of AM with CCl_4 showed significant decrease in the toxicity of CCl_4 on all parameters to reach the control levels.

Keywords: Annona muricata, carbon tetrachloride, liver and rabbits

I. INTRODUCTION

Since the demonstration in 1926 that exposure of CCl_4 has led to liver cirrhosis, the role of this compound in human health and experimental studies has been shown to be enormous in the last 76 year[1]. Hepatic injury through carbon tetrachloride (CCl_4) induced lipid peroxidation is well known and has been extensively used in the experimental models to understand the cellular mechanisms behind oxidative damage and further to evaluate the therapeutic potential of drugs and dietary antioxidants[2].This ultimately causes the body to experience oxidative stress and seems to play a main role in the pathogenesis of both acute and chronic liver damage. During the last decades, application of this compound has been further shown to be an excellent tool for the study of experimental oxidative injury due to its rapid metabolism in the liver to a free radicals and following deleterious effects in the liver[3].

Transaminase and phosphatase are the most sensitive biomarkers directly implicated in the extent of cellular damage and toxicity because these are cytoplasmic in location and are released into the circulation after cellular damage[4]. Transaminases are enzymes that catalyze a type of reaction involve removing the amino group from the amino acid and transferring it to the reactant α-keto acid and converting it into an amino acid [4]. [5] reported that the activities of enzymes like alanine transaminase (ALT) and aspartate transaminase (AST) are found in hepatocytes and striated muscle cells. Increased serum ALT and AST activity has been reported in hepatocellular injury with membrane damage or necrosis of striated muscle. Similarly, phosphatases are enzymes that catalyze the splitting off of phosphoric acids from certain monophosphoric esters, a reaction of considerable importance in several body processes. Alkaline phosphates (AlP) and acid phosphates (AcP) have been directly implicated in the extent of cellular damage and toxicity, particularly of liver and cardiac tissue. The primary importance of measuring AlP is to check the possibility of mainly

International Journal of Pharmaceutical Research and Applications
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liver or bone diseases. The study of AlP activity becomes significant in effectively denoting the alteration of toxicity levels during the period of drug administration. Thus, the changes in activity and concentration of marker enzymes like ALT, AST, AlP and AcP in plasma could reflect the state of hepatotoxicity.

The intraperitoneally administration of CCl_4 to experimental animals brought about markedly increased serum aminotransferase and alkaline phosphates activities (used for assessing liver function) in rats treated with CCl_4 0.5 ml/kg which employed for inducing liver damage. In carbon tetrachloride treated livers, exceptional changes were watched. Histopathological examination showed broad greasy alter, expanded hepatocytes, vacuolated cytoplasm, compressed sinusoids, greasy degeneration, region of rot and invasion by incendiary cells[6]. Carbon tetrachloride (CCl_4) has been used drastically to induce liver injury and fibrosis in various experimental models and to elucidate the mechanisms behind hepatotoxicity. The mechanism underlying the CCl_4 hepatoxicity involves oxidative stress induced by CCl_4-derived reactive free radical metabolites[7]. CCl_4 could be a well-established hepatotoxin actuating liver damage by creating free radicals.. Exposure to CCl_4 also induces acute and chronic renal injuries[8]. The liver is the body's most prominent glandular tissue, and it has a significant role in body metabolism. It has a wide range of functions, including the storage of glucose, plasma protein synthesis, and bile production[9]. The liver may be vulnerable to hurt due to harmful substances, since it is included in these broadened capacities. Suitable elective treatments ought to be looked for to extend the treatment's adequacy by recognizing the pathophysiological forms mindful for making hepatic harm. The disparity between the generation of ROS and antioxidants leads to free radical species disruption to oxidative substances in cells, including proteins, lipids, or nucleic acids [10]. Supplementations with common cancer prevention agents have been appeared to make strides the body's proficiency in unpleasant conditions[11].

Annona muricata could be a part of the Annonaceae family and is an evergreen tree. It is cultivated in tropical and subtropical regions and considered as a traditional medicine[12]. Its leaves contain several groups of substances collectively called annonaceous acetogenins that include murihexocin, annocuricin [13] annopentocin A, B and C, (2,4-cis)-annomuricin-D-one, murihexocin A and B, (2,4-trans)- annomuricin-D-one, 4-acetyl gigantetrocin, cis-gigantrionin[14] muricatocin A, B and C[15] and annohexocin [16]. These compounds are highly potent and selective against microbial resistance[17].

They showed anti-tumor effects in vivo and in vitro[18]. The essential oils of A. muricata clears out have parasiticidal, anti-diarrheal, rheumatological, and anti-neuralgic properties[19].The leaves extracts are gastroprotective [20], anti-diabetic, hepatoprotective[21] anti-bacterial [22], anti-arthritic, anti-inflammatory[23] and are modulators of the innate immune system[24].

II. MATERIALS AND METHODS

In this study, the effect of carbon tetra chloride (CCl_4) with or without Annona muricata on liver and kidney of male rabbits were investigated. CCl_4 was got from chemistry department, faculty of science, Omar Al-Mokhtar University 1mg/ml (CCl_4) and Annona muricata leaf (powder) was purchased from maximum international company, Brasil. Each capsule contains 3 g powder and the content of each capsule was dissolved in corn oil just before use. Develop male Modern Zealand White rabbits (6 months old) and starting weight of (1.641 ± 27.2 Kg) were utilized. Animals were individually housed in cages and weighed weekly throughout 4-weeks experimental period. The first group was used as control. While, groups 2,3 and 4 treated with Annona muricata 100 mg/kg BW[25-27]and carbon tetrachloride CCl_4 1 ml/kg BW[28] and the combination of carbon tetrachloride and Annona muricata, respectively. Rabbits were orally administered with 1 ml/kg BW of CCl_4 twice a week. Rabbits were orally administered their respective doses for one month. At the conclusion of the exploratory period body weight of rabbits were recorded. Animals were sacrificed by decapitation and liver and kidney were immediately removed and weighed then the organs weight ratio was calculated. The relative weight of organs (%) was calculated as g/100 g body weight. The other part of heparted blood samples were placed immediately on ice. Plasma was obtained by centrifugation of samples at 860 xg for 20 min, and was stored at -20°C until used for analyses. Stored plasma samples were analyzed for plasma urea and creatinine concentrations were measured by the method of [29-31] , respectively. The activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of [32].

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 611-618 www.ijprajournal.com ISSN: 2249-7781

Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma and liver according to the method of [33]. Information were analyzed agreeing to [34].Statistical significance of the difference in values of control and treated animals was calculated by F test with 5% significance level. Data of the present study were statistically analyzed by using Duncan's Multiple Range Test [35].

III. RESULTS

All the rabbits (control and treated) were observed daily after every dosing for 3-5 hrs for clinical symptoms like salivation, hyperactivity, irritability, faecal pellet conditions, diarrhea, weakness, coarse tremor, paralysis of limb, convulsions, wounds, vocalization, aggressive behaviour, ataxia, increased sensitivity to external stimuli, unsteady gait, falling of hair, stress (fur erection and exophthalmia) and changes in non-sexual behaviours (such as cleaning of face, excessive self-grooming, climbing in cages).

Rabbits in the control group did not show any sign of toxicity. However, carbon tetrachloride -fed rabbits showed varying degrees of clinical signs few minutes after dosing. The signs included disorientation, drowsiness, uncoordinated movements, mild tremor and diarrhea. Concerning morphological changes, carbon tetrachloride - treated rabbits showed hair loss whereas control animals did not display such change.

The liver of dissected rabbits also found scars of depression in response to carbon tetrachloride administration (Figure 1) whereas those of the control animals showed normal appearance.

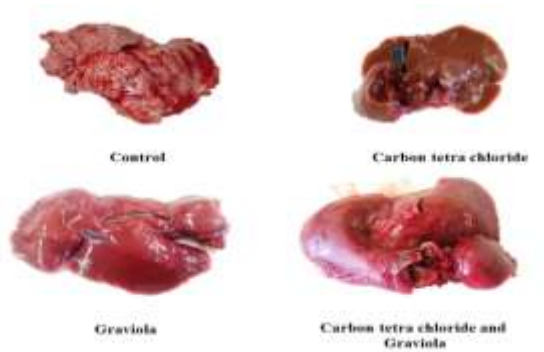

Figure 1: Morphological effect CCl_4, AM, and/or their combinationafter 4 weeks on liver of male rabbit

Table 1 represent body weight and relative weight of liver and kidney of male rabbits treated with Annona muricata, carbon tetrachloride (CCl_4) and their combination. Results indicated that treatment with Annona muricata alone caused significant ($P<0.05$) increase in BW and relative weight of liver and kidney, while decrease urea and creatinine in male rabbits compared to control animals. On the other hand, significant ($P<0.05$) decrease in BW, relative weight of liver and kidney. While, significant increase ($p<0.05$) in plasma urea and creatinine in rabbits treated with CCl_4 compared with control. The presence of Annona muricata with CCl_4 alleviated its effects on the tested parameters. Table2 and 3 summarized the mean values of activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AlP) and gamma glutamyltransferase activity (ɣ-GT) in plasma and liver of male rabbits treated with AM, CCl_4 and their combination. Treatment with Annona muricata alone caused a significant ($p<0.05$) decrease in plasma and increase in liver AST, ALT and AlP activities. While, treatment with CCl4 significantly ($p<0.05$) increased plasma and decreased liver AST, ALT and AlP activities compared to control. The presence of Annona muricata with CCl_4 minimized its toxic effect on plasma and liver enzymes to reach the control levels.

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 611-618 www.ijprajournal.com ISSN: 2249-7781

Table 1. Body weight (BW) and relative weight of organs, urea and creatinine of male rabbits treated with Annona muricata, carbon tetrachloride (CCl_4) and their combination

Parameter	Experimental groups			
	C	**AM**	**CCl_4**	**AM + CCl_4**
BW (gm)	263 ± 14^{a}	258 ± 5^{a}	182 ± 5.1^{c}	226 ± 3.6^{b}
Liver (g/100gm)	43.7 ± 3.7^{ab}	50.8 ± 0.9^{a}	37.1 ± 0.1^{b}	44.8 ± 1.4^{ab}
Kidney (g/100gm)	11.4 ± 1.2^{b}	16.8 ± 0.02^{a}	9.8 ± 0.5^{b}	11.9 ± 0.8^{b}
Urea (mg/dl)	37.8 ± 0.7^{b}	18.3 ± 1.5^{c}	55.2 ± 4.3^{a}	36.1 ± 1.7^{b}
Creatinine (g/dl)	0.6 ± 0.02^{bc}	0.6 ± 0.1^{c}	1.2 ± 0.1^{a}	0.7 ± 0.1^{b}

Values are expressed as means ± SE; n = 10 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.

Table 2. The enzyme activities of plasma enzymes of male rabbits treated with Annona muricata, carbon tetrachloride (CCl_4) and/or their combination.

Enzyme	Experimental groups			
	C	**AM**	**CCl4**	**AM + CCl_4**
AST (U/L)	43.1 ± 1.1^{b}	21.3 ± 2.9^{c}	85.4 ± 13.4^{a}	49.8 ± 4.3^{b}
ALT (U/L)	45.6 ± 2.3^{b}	30.5 ± 2.5^{c}	75.5 ± 4.9^{a}	43.9 ± 2.6^{b}
ALP (U/L)	334.0 ± 8.8^{a}	365.5 ± 26.1^{a}	318.0 ± 10.9^{a}	335.6 ± 16.7^{a}
ɣ-GT (U/L)	7.1 ± 0.2^{a}	6.6 ± 0.03^{b}	7.6 ± 0.4^{a}	7.3 ± 0.1^{a}

Values are expressed as means ± SE; n = 10 for each treatment group.Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.
Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AlP), gamma glutamyltransferase activity (ɣ-GT).

Table 3: The enzyme activities in liver of male rabbits treated with Annona muricata (AM), carbon tetrachloride (CCl_4) and/or their combination.

Enzyme	Experimental groups			
	C	**AM**	**CCl_4**	**AM + CCl_4**
AST (U/mg protein)	34.7 ± 3.6^{b}	50.8 ± 0.9^{a}	37.1 ± 0.04^{b}	44.8 ± 1.4^{ab}

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 611-618 www.ijprajournal.com ISSN: 2249-7781

ALT (U/mg protein)	141.1 ± 3.9[a]	135.8 ± 3.50[a]	107.6± 5.5[ab]	114.0 ± 8.2[b]
ALP (U/mg protein)	344 ± 8.8[a]	365.5 ±26.1[a]	318 ±10.9[a]	335.5 ± 16.7[a]

Values are expressed as means ± SE; n = 10 for each treatment group.Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$
Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AlP)

IV. DISCUSSION

Table 1 appeared a critical diminish of body weight in carbon tetra chloride compared with the control along time of the test periods.. Carbon tetrachloride is readily absorbed from the gastrointestinal tract causes vomiting and gastrointestinal pain apparent following acute exposure [36,37] but in chronic exposure causes irritation to the gastrointestinal tract with cell injury and necrosis followed by degeneration growth, decrease body weight gradually[38,39]. Data in (Table 1) demonstrated that there was noteworthy diminish in liver and kidney weight with carbon tetrachloride when compared with control gather. The decrease in body weight of rabbits treated with CCl_4 (Table 1) is in agreement with the result of [40] who found that feeding CCl_4 to rats for two months resulted in a significant loss of body weight. Also[41] found that the reduction in body weight may be related to the toxicity of CCl_4, because CCl_4 impairs the activation and utilization of nutrients due to maldigestion and malabsorption caused by gastrointestinal disturbances.

Organization of watery extricate of Annona muricata natural product mash or watery extricate of DS of Annona muricata capsules initiated critical increment within the last body weight comparing to control group.The defensive antioxidant instruments keep up the cellular oxidation-reduction possibilities required for ordinary digestion system and to avoid free radical assault of amino acids, proteins, and the lipid components of cell films fundamental for useful and auxiliary keenness of cells and tissues [42]. Too, body weight changes serve as a touchy sign of the common wellbeing status of creature [43], and utilized as an pointer of antagonistic impact of drugs and chemicals[44]. The diminishment in body weight picks up may be due to oxidative push[45] and[46] and/or due to the expanded corruption of lipids and proteins as coordinate impacts of harmful compound exposure [47] The defensive activity against CCl_4 in rabbits on body weight may be credited to the antioxidant impact of Annona muricata and its bioactive compounds The kidney assistances in keeping homeostasis of the body by reabsorbing imperative fabric and emptying squander yields. Creatinine could be a commonly utilized as degree of kidney function [48].The increase within the level of creatinine within the blood considered an marker to the kidney harm [49]. In this try, verbal organization of CCl_4 brought about in a noteworthy increment in creatinine and blood urea nitrogen concentrations. The watched increment is characteristic of cellular spillage and misfortune of utilitarian judgment of cell film in renal tissue [50]. [51] reported that Annona muricata extricate contains the tall add up to antioxidant, which made strides the full antioxidant status of Sprague-Dawley rats as the measurement expanded, which is sweet in advancing wellbeing.

The decrease in plasma AST, ALT and AlP activities (Table 2) is in agreement with the results of[53].It has been shown that Annona muricata extract contains high total antioxidant which is good in promoting health. The results from this study have shown that the plant Annona muricata has moderate anti-hepatotoxic ability. Generation of free radicals in the body beyond antioxidants capacity leads to oxidative stress which has been implicated in diseases such as cancer, cardiovascular disease, aging and several other chronic diseases because of their ability to induce oxidative damage to biomolecules such as lipid, DNA and protein [53]. With regards to serum biochemistry, a significant decrease in liver function test parameters such as serum ALT, AST, LDH, GGT and ALP were noted. [52]. The present results showed that AST and ALT increased in plasma and decreased in liver of rabbits treated with CCl_4 (Table 2 and 3). Also, [53] reported that CCl_4 is known to cause hepatic damage with a marked elevation in serum levels of transaminase enzymes (AST and ALT), because these enzymes are cytoplasmic in location and are released into the blood after cellular damage. Additionally, AST is

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 611-618 www.ijprajournal.com ISSN: 2249-7781

found in every tissue of the body, and the amount of AST is particularly high in the cardiac muscle. In contrast, ALT is present in moderately high concentration in liver and low in cardiac. After a single infusion of CCl_4, serum exercises of ALT and AST chemicals within the hepatotoxic demonstrate bunch were essentially expanded ($P < 0.005$).The hepatotoxicity induced by CCl_4 is due to its activation by cytochrome P-450 to form a trichloromethyl radical, CCl^{3-}. The trichloromethyl radical leads to liver damage by alkylating cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids to produce lipid peroxides. This radical can tie to cellular particles (nucleic corrosive, protein, lipid), disabling significant cellular forms such as lipid digestion system, with the potential result of greasy degeneration. The covalent binding of trichloromethyl radicals to cellular macromolecules is considered to function as the initiator of membrane lipid peroxidation and cell necrosis[54]. Hepatocellular corruption leads to rises of serum AST and ALT exercises and an expanded frequency and seriousness of histopathological hepatic injuries in rabbits. It has been conjectured that one of the foremost causes of CCl_4 induced liver harm is arrangement of lipid peroxides by free radical subordinates of CCl_4[55].

In conclusion, the results of the present study convincingly indicated that CCl_4 exposure resulted in varying degree of changes in liver and kidney parameters in plasma of rabbits. Annona muricata
is broadly utilized in conventional medication to treat diseases. Using Annona muricata capability to alleviate the harmful effect of CCl_4.

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