Alleviating Impact of A-Lipoic Acid and Silver Nanoparticles on 1,2-Dimethylhydrazine Dihydrochloride Induced Hepatic Carcinogenesis

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ABSTRACT

Hepatocellular carcinoma (HCC) is a mainliver malignant tumors with a high rate of death. Nearly one million persons are affected by this hazard diseaseannually worldwide. New chemoprevention strategies are urgently needed to reduce mortality from this disease. The purpose of this study was to investigate the potential chemopreventive impacts of α-lipoic acid (ALA)and/or silver nanoparticles (Ag-NPs) against 1,2-dimethylhydrazine dihydrochloride (DMH) caused liver carcinoma . DMH was administered as a single dose (16mg/kg) intrarectally once weekly for six successive weeks. ALA (8.5 mg/kg) and Ag-NPs (400 µg/kg) were orally administered to DMH injected rats daily for four weeks after DMH administration. The results revealed that administration of ALA and /or Ag-NPsto DMH injected rats, significantly reduced the increases in the levels of the serum hepatic tumor markers, namely α-fetoprotein(AFP), carcinoembryonic antigen(CEA) and α- L-fucosidase(AFU) as well as the alterations in the serum liver function markers,namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin, total protein and albumin compared with DMH untreated animals.

Conclusion: The current study revealed that treatment of HCC induced rats with ALA and /or Ag-NPs was effective in down-modulating the serum liver tumor markers as well as the serum liver function indices. The combination of these two agents was the effective one in ameliorating of the most of studied parameters. Thus,utilizing this combination as a chemopreventive remedy may reduce the liver damage caused by carcinogenesis.

Key Words: Hepatocellular carcinoma, 1,2-dimethylhydrazine dihydrochloride, α-lipoic acid, silver nanoparticles, tumor markers

eIJPPR 2017; 7(6):44-51

HOW TO CITE THIS ARTICLE: Widad M. Al-Bishri. (2017). "Alleviating Impact of A-Lipoic Acid And Silver Nanoparticles on 1,2-Dimethylhydrazine Dihydrochloride Induced Hepatic Carcinogenesis " , *International Journal of Pharmaceutical and Phytopharmacological Research, 7*(6), pp.44-51.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most recurrent malignancy representing the fifth commonest tumor all over the world and the third reason of the death from cancer [1]. Viral hepatitis infection, aflatoxins and chemical carcinogens are considered risk

factors for the incidence of HCC [2].

1,2-Dimethylhydrazine dihydrochloride (DMH) is a very poisonous and tumorigenic compound which influences many tissues , such as liver [3-4]. DMH is present in tobacco [5], mushrooms [6] and other food and food products [7]. DMH undergoes biotransformation by CYP2E1 or alcohol dehydrogenase (ADH) in the liver into the carcinogenic metabolite, methyldiazonium,

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 02 March 2017; **Revised:** 29 September 2017; **Accepted:** 22 November 2017

which causes DNA methylation in the target organ [8].The biotransformation of this compound also produces reactive intermediate such as carbonium ions and alkyl free radicals, which severely causes liver dysfunction by reacting with cellular components causing their degradation and eventually cellular necrosis [9].Chronic exposure to free radicals plays a fundamental role in the initiation, promotion and progression of carcinogenesis phases [10]. These radicals can promote DNA oxidative fragmentation, causing genetic damage which promotes carcinogenicity and tumor development [4]. Exposure to DMH for a long period has been reported to cause liver and colon cancer [3-4].

It is well known that many biomarkers are produced by cancer cells and leaked into the blood stream [11]. Such markers including enzymes, antigens and hormones are used as indicators for tumorigenesis. Sensitive and specific hepatic tumor biomarkers are utilized as indictor of liver damage [12].

New chemoprevention strategies are urgently needed to reduce the mortality of this disease. Searching for chemopreventive compounds, which can delay or curb the incidence of cancer is an important target for combating the risk of cancer development. Many strategies have been utilized for the prevention of HCC such as immunization against viral hepatitis, treatment with antiviral agents, prescribing drugs and using natural agents [13-15].

Alpha-Lipoic acid (ALA, 1, 2-dithiolane-3-pentanoic acid) is used as a natural activator for many enzymes including acetyl- CoA synthesis, acyl CoA synthesis and α-ketoacid dehydrogenases [16]. ALA and its redox couple, dihydrolipoic acid are strong antioxidants [17]. It can pass into the cell and neutralize the reactive ions in different compartments [18]. With its antioxidant impact, it can attenuate the severity of many diseases including hepatitis, diabetes and atherosclerosis [19-20]. ALA also is used in the treatment of hepatotoxicity, hepatic cirrhosis and other hepatic diseases [21-22]. It has found that ALA has anti-inflammatory , anticancer and antiproliferative properties [20,23] .

Nano medicine is a medical field for nanotechnology application that could potentially affect the human health [24]. Nanoparticles with their biological activities have been used in various fields including therapeutics [25]. It has an important role in detection and treatment of cancer. Nano-scale particles can flow into tumor tissue through blood circulation to damage cancer cells [26]. Silver nanoparticles (Ag-NPs) are used in a medical field as potent anticancer [27]. Ag-NPs have a toxic impact on human breast (HTB22) and lung (CCL185) cancer cell lines [28-29]. Ag-NPs also can protect liver damage caused by hepatotoxic agent. The particles are effective in keeping serum liver function enzymes to near normal in animals exposed to paracetamol toxicity [30].

Although few studies have shown the hepatotoxic effect of DMH, its carcinogenic impact on liver is still uncertain. Moreover, the chemotherapeutic impacts of both ALA and Ag-NPs against HCC have not been studied so far. Thus, the purpose of this work was to investigate the carcinogenic effect of DMH on rat livers and the potential therapeutic use of ALA and /or Ag-NPs as anti-tumorigenic agents against hepatic carcinogenesis induced by DMH in rats.

MATERIALS AND METHODS

Chemicals

1,2-Dimethylhydrazine dihydrochloride ,Ag-NPs and other chemicals were bought from Sigma chemical company (St. Louis, USA) . Ag-NPs was bought as an aqueous solution.

Animals

Fifty male Wistaralbino rats (120-130 g) were utilized for this investigation. Rats were bought from the animal care center, King Fahd Medical Research Center, Jeddah, KSA. Rats. Rats were adapted for the environmental conditions (24°C ± 2 , 12h light/dark cycle and 50% humidity) for one week before the experiment. The animals were provided with unlimited supply food and water. The experiment was carried out in accordance to the Ethical Committee of King Fahad Medical Research Center. Jeddah, KSA.

Experimental Design

Rats were categorized into five groups of ten animals each as follows:

Group 1: Control animals were given orally 1 ml of saline daily during the experiment.

Group 2: Rats were intra-rectally injected with a single dose weekly (16mg/kg) of 1,2-dimethylhydrazine dihydrochloride (DMH) for six weeks [31] .

Group 3: DMH-injected rats were orally administered with ALA (8.5 mg/kg) daily for 4 weeks after the administration period (six weeks) of DMH [32] .

Group 4: DMH-injected rats were orally treated with Ag-NPs (400 µ g/kg) daily for 4 weeks after the administration period of DMH [33].

Group 5: DMH-injected rats were orally treated with the combination of ALA (8.5 mg/kg) and Ag-NPs (400 µ g/kg) for 4 weeks after the administration period of DMH.

DMH was prepared in a saline solution and ALA was prepared as a suspension in 1% between 80 before administration. After the experimental period (10 weeks), the animals were starved for 12-14 hours and blood specimen were gathered and clotted for serum isolation and utilization for estimation of different biochemical analysis.

2.1. Biochemical Analysis

Serum CEA was measured utilizing rat CEA sandwich enzyme-linked immunosorbent assay (ELISA) kit (Fine Biotech, China) according to the instruction provided by the manufacture. AFP was determined utilizing rat AFP ELISA kit (Cat. No. KT-59172, Kamiya Biomedical Company, USA) in accordance to instruction provided by the manufacture. AFU activity was estimated by the method of Wang and Cao [34]. Serum ALT, AST. ALP, total bilirubin, direct bilirubin, total protein and albumin were determined utilizing an automatic analyzer(Hitachi Model 917 multichannel analyzer)

Statistical Analysis

Results are presented as mean ± standard error (SE). The significant variations among data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's test post-ANOVA. The variations among data were significant at P≤0.05.

RESULTS

The impacts of ALA and/or Ag-NPs on the levels of serum liver tumor markers, AFP, CEA and AFU in DMH injected rats are depicted in Figures 1, 2 & 3, respectively. The results demonstrated that increase in these tumor markers in HCC induced rats (group 2) versus control ones (group 1, $P \leq 0.001$). Oral ingestion of ALA and/or Ag-NPs to DMH treated rats (groups 3, 4 & 5, respectively), markedly depleted the increases in AFP, CEA and AFU in comparison to HCC untreated rats ($P \leq 0.001$). Treatment with the combination of ALA and Ag-NPs. was markedly the effective one in down-modulating AFP and α- fucosidase versus each agent lonely .

The effectiveness of ALA and/or Ag-NPs on the levels of serum hepatic function markers, namely ALT,AST,ALP, total and direct bilirubin, total protein and albumin, in DMH injected rats are shown in Table 1. The data revealed that significant increases in the levels of ALT, AST, ALP, total and direct bilirubin and decreases in total protein and albumin in DMH injected rats with relation to control animals ($P \leq 0.001$). Intake of ALA and/or Ag-NPs to DMH treated rats significantly modulated the disorders in these markers. Treatment with the combination of ALA and Ag-NPs was the successful one in ameliorating the serum levels of these markers.

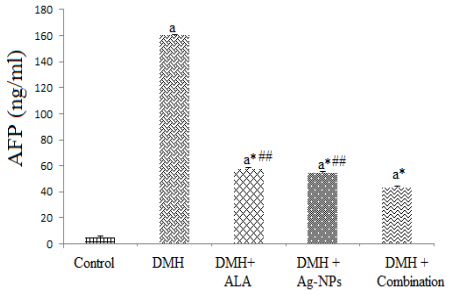

Fig 1: Effect of ALA and Ag-NPs and their combination (ALA + Ag-NPs) on the levels of serum tumor marker (AFP) in DMH treated rat groups. Values are calculated as mean ± S.E. (n=10), $^{a}P \leq 0.001$ in comparison with the control group, $^{*}P \leq 0.001$ with respect to DMH treated rats, $^{\#\#}P \leq 0.05$ with respect to DMH rats treated with the combination

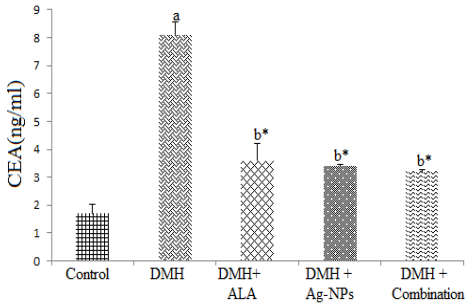

Fig 2 : Effect of ALA and Ag-NPs and their combination (ALA + Ag-NPs) on the levels of serum tumor marker (CEA) in DMH treated rat groups. Values are calculated as mean ± S.E. (n=10), $^{a}P \leq 0.001$, $^{b}P \leq 0.01$ in comparison with the control group, $^{*}P \leq 0.001$ with respect to DMH treated rats.

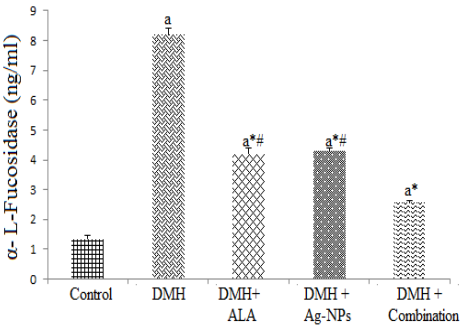

Fig 3: Effect of ALA and Ag-NPs and their combination (ALA + Ag-NPs) on the levels of serum tumor marker (α-L-fucosidase) in DMH treated rat groups. Values are calculated as mean ± S.E. (n=10), $^{a}P \leq 0.001$ in comparison with the control group, $^{*}P \leq 0.001$ with respect to DMH treated rats, $^{\#}P \leq 0.01$ with respect to DMH rats treated with the combination

Table 1 Effect of ALA and /or Ag-NPs on thelevels of theserum liver injury indices in DMH treated rat groups

Parameters	Control	DMH	DMH+ ALA	DMH+ Ag-NPs	DMH+ Combination
ALT (U/L)	38.33±2.02	143.66±.88^{a}	102±5.56$^{a*\#\#}$	88.33±4.37$^{a*\#}$	70.0±2.52^{a*}
AST(U/L)	34.33±.88	151±2.08^{a}	91.33±3.5$^{a*\#\#}$	94.33±1.2$^{a*\#}$	74.66±1.45^{a*}
ALP(U/L)	68±4.58	207.33±2.9^{a}	129.33±2.33$^{a*\#\#}$	111.66±.88$^{a*\#}$	84.33±1.45^{c*}
T bilirubin (mg/dl)	0.5±0.04	1.3±.057^{a}	0.58±0.045*	0.56±.027*	0.46±.023*
D bilirubin(mg/dl)	0.243±0.021	0.63±.054^{a}	0.233±.006*	0.25±.017*	0.203±.012*
T protein (g/dl)	8.1±0.208	3.5±.17^{a}	5.46±.26$^{b*\#\#}$	4.46±.033$^{b*\#\#}$	6.96±.12^{c*}
Albumin (g/dl)	5.9±0.08	2.43±0.12^{a}	3.96±0.39$^{b*\#}$	4.1±0.17$^{b*\#\#}$	5.35±0.088^{c*}

Values are calculated as mean ± S.E. (n=10), $^{a}P \le 0.001$, $^{b}P \le 0.01$, $^{c}P \le 0.05$ in comparison to the control group,$^{*}P \le 0.001$ versus DMH treated rats, $^{\#}P \le 0.01$, $^{\#\#}P \le 0.05$versus DMH rat group treated with the combination (ALA + Ag-NPs).

DISCUSSION

HCC is a common tumor with a poor prognosis. Using natural products and / or nano-medicine with low or no side effects as chemoprevention strategies are required to reduce the mortality rate due to this hazard disease.

It is well known that DMH can promote cancer particularly in the colon; however, its tumorigenic effect on the liver is still doubtful. Thus, the current study was design to examine the carcinogenicity of DMH on rat livers. Also, the chemopreventive effectiveness of ALA and /or Ag-NPs was investigated in a trial to alleviate the carcinogenic efficacy of this hazard compound on rat liver.

Tumor markers are potential screening tools, which are widely used for early diagnosis of cancer diseases [11]. Different tumor markers including antigens, enzymes, hormones, and proteins are considered as indicators for tumorigenesis of many organs including liver [12].

The current study showed that administration of DMH to rats caused a pronounced increases in the serum contents of tumor markers, AFP, CEA, and AFU of DMH treated rats in comparison to the control ones. Our result may indicate the hepatic carcinogenic impact of DMH suggesting that the liver is another target organ of DMH carcinogenicity. Our result is supported by clinical studies revealed that increases in the serum AFP, CEA, and AFU are used as markers of HCC [35-37].

The concentration of AFP in blood can be utilized for the early diagnosis and monitoring HCC [38-40]. An increased AFP level has been also found in certain other benign hepatic disorders including viral hepatitis and liver cirrhosis [41].CEA is a glycoprotein tumor-antigen utilized as a particular index for the diagnosis of persons with colon tumor. Also, CEA has been broadly utilized as a tumor index in detection and monitoring of some other malignancies including lung cancer, gastrointestinal adenocarcinoma and HCC [35, 42-43].It has reported that serum CEA concentration is associated with differentiated tumor type and considered as an indicator in detecting tumor stage [44-45].High level of CEA has been found in patients with advanced cancer diseases [46]. AFU is a liposomal enzyme broadly found in blood, cells, and body secretions [47]. However, increased serum level of this enzyme is considered as a sensitive tumor marker for detection of an early HCC [37, 48]. Sivaramakrishnan et al. [36] have indicated that the serum activity of AFU is higher in HCC patients than hepatic fibrosis patients, suggest that an increase in this enzyme activity in the sera of patients with hepatic fibrosis is a primary indicative element for the development of HCC.

The carcinogenicity of DMH may be ascribed to heritable modifications of DNA resulted from its carcinogenic metabolite, methyldiazonium that is responsible for DNA methylation in the target organ [8]. Some authors demonstrated a direct causal link between DNA methylation and development of cancer [49]. DNA methylation has a major impact on genes causing their mutations, which results in organ tumorogenesis [50]. DNA methylation can affect tumor suppressor genes, which involved in the molecular mechanisms of carcinogenesis e.g., regulation of cell cycle, apoptosis and DNA repair [50]. It has reported that methylation of DNA is common in different tumors and can be used potentially as an indicator in the early detection of tumors [49]. Besides, some investigators declared that DMH could induce the production of free radicals, which have the fundamental role in the carcinogenic effect of DMH by stimulating cell division, survival and migration. In addition, these reactive free ions can cause DNA oxidative fragmentation, promoting carcinogenicity and tumor development [4]. Administration of ALA and /or Ag-NPs to DMH treated rats successfully reduced the increases in the serum tumor markers (AFP, CEA and AFU) compared with DMH untreated animals. Treatment with the combination of ALA and Ag-NPs was the effective one in modulating the tumor markers. The modulating effect of both ALA and Ag-NPs on the serum liver tumor markers may attribute to their cytotoxic effects on hepatic cancer cells along

with protection of cellular components from free radicals. ALA has been demonstrated to suppress tumor cell growth and to induce cell death [51]. Several studies has shown the beneficial effect of ALA in cancer chemoprevention. It has found that ALA could stop the cell cycle of tumor cells and induce apoptosis in Ki-v-Ras-transformed Balb/c-3T3 murine tumor mesenchymal cell line [52]. It has found that ALA could promote apoptosis in HT-29 human colon cancer cells and in leukemia cells via increasing caspase-3-like activity coupled with DNA disintegration [53-54]. ALA has the capability to induce the generation of reactive free ions and apoptosis in H460 human lung tumor cells via the activation of caspase-9 and the reduction of Bcl-2 protein in mitochondria [51]. Furthermore, derivative of ALA was reported to stimulate lysosomal degradation pathway (autophagy) of colon cancer cells [55]. In addition, ALA has reported to induce DNA damage in HCT116 colorectal cancer cells by inhibiting the DNA repairing enzyme, O_6-methylguanine-DNA methyl transferase (MGMT) [56]. The anticancer benefit of Ag-NPs has also been documented [57]. Some authors have reported that Ag-NPs have a cytotoxic effect on HEpG-2 cell line suggesting that Ag-NPs can penetrate into cells and may attack the functional proteins inside the cells resulting in unfolding and aggregation of cellular proteins; thus, interfere with the proper function of protein [58]. Other study has confirmed that Ag-NPs have a cytotoxic suppressing impact on the development of human liver HepG2 cell line [59], explaining that Ag-NPs can build up in the cytoplasm and nuclei of hepatoma cells and induce intracellular oxidative stress. In addition, another finding has suggested that the cytotoxic impact of Ag-NPs is due to the oxidative stress and the toxicity of Ag+ ions [60]. Also, it has reported that Ag-NPs could arrest cell cycle, damage the mitochondria and reduce the concentration of ATP of tumor cells [61]. In vitro study has shown that Ag- NPs have anti-proliferative impact on human monocytes [62].The anticancer therapeutic ability of Ag-NPs may be due to their nanoparticle characterization such as composition , shape, size, charge functionalization, or surface modification , directing the Nano particles to the target organs [63].

The activities of blood liver enzymes (ALT, AST and ALP), total and direct bilirubin as well as total protein and albumin are utilized as diagnostic markers of cellular liver damage [3]. In DMH treated rats, significant increases in the serum hepatic function enzymes were found (AST and ALT) compared with control rats. These results are coped with Shrama and Sharma [3] who found that the activities of these enzymes are significantly increased following administration of DMH in rats. The elevation of such enzymes is a sign of hepatic cellular leakage due to loss of functional integrity of hepatocyte membranes in response to DMH hepatotoxicity. Administration of ALA and /or Ag-NPs to DMH treated rats; markedly reduced the increases in the serum liver function enzymes compared with DMH untreated rats, indicating to their hepatoprotective potential impact. These results may suggest that both ALA and Ag-NPs could repair and stabilize the damaged hepatic plasma membranes in response to DMH. The hepatoprotective impact of both ALA and Ag-NPs has been documented [22, 30].

Serum ALP and total and direct bilirubin were also elevated in rats under the effect of DMH toxicity. ALP is secreted with bile by the liver. Elevation in ALP and bilirubin is an indicator of inability of liver to excrete bilirubin [3]. Reduction of elevated serum ALP and bilirubin in DMH rats treated with ALA and /or Ag-NPs may indicate that these agents could attenuate biliary dysfunction in rats under the effect of DMH.

Lowering in the serum total protein and albumin is another indicator of liver disorder in rats injected with DMH presented in the current study. These data may give a clue of protein metabolic disorder and / or modification induced by DMH. Previous investigation has reported that tissue proteins may be influenced by the accumulation of free radicals causing the generation of carbonyl protein derivatives. These free radicals can cause peptide cleavage and oxidative alteration of amino acid side chains [64].Treatment with ALA and /or Ag-NPs beneficially increased the serum levels of total protein and albumin. This result may suggest the ability of both agents with their antioxidant properties to neutralize free radicals induced by DMH, which have the fundamental role in liver damage and oxidative modification of protein [20, 65]

CONCLUSION

The present study demonstrates that treatment of rats with DMH can induce HCC as indicated by elevation in the serum tumor markers. The study also revealed the therapeutic beneficial impact of both ALA and /or Ag-NPs against DMH induced HCC. These agents could reduce the serum tumor markers indicating their potential anticancer abilities. The used agent can also ameliorate DMH -induced liver injury. Treatment with the combination of two agents was the successful one in modulating most of the studied markers. Therefore, this study may support the use of this combination as a promising chemo-preventive drug against liver carcinogenesis induced by carcinogenic agents.

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