

## Study the Anti-Hyperlipidemia Effects of *Ambrosia Maritime* in Albino Female Rats and their Fetus

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**Annotation:** The aim of current study is investigation the anti-hyperlipidemia effects of *Ambrosia maritime* in albino female rats and their fetus. In this experiment 30 male rats (wt 140-160 gm with age 12-16 weeks) at a period April to Augustus 2021. The rats divided to four experimental groups. The present findings show that the concentration of total protein and albumin in high fat diet (HFD) group significant ( $P < 0.05$ ) reduce compared with control group. Otherwise, total cholesterol, triglyceride and malonedialdehydied (MDA) in HFD group show significant ( $P < 0.05$ ) elevate compared with control group. While, catalase show significant ( $P < 0.05$ ) decrease in HFD group compared with control group. After using leaves extract of *A. maritime* in tratmnet, the levels of all above parameters show improvement in their levels. Therefore, it can be concluded that the leaves extract of *A. maritime* show anti-hyperlipidemia activity in current experiment. In the livers of fetal obese rats, a decrease was found in the number of hematopoietic megakaryocytes, along with a decrease in hematopoietic elements. Anucleated nuclei and nucleated nuclei

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were observed in the sinusoids, which appeared in smaller numbers compared to the control group. In the extract group, the central vein and normal hepatocytes were found, as well as the sinusoidal arrangement between the hepatocytes. In the treated group, the normal shape of the central vein, normal hepatocytes, and the sinusoidal arrangement between the hepatocytes were found. An improvement was also observed in the number of hematopoietic megakaryocytes. It can be concluded that the leaves extract of *A. maritime* show anti-hyperlipidemia activity in current experiment. On the other hand, it was found that *A. maritime* dosage is highly effective in improving the liver tissue of fetuses, due to its possession of many active compounds, including phenols and flavonoids.

**Keywords:** *Ambrosia maritime*; hyperlipidemia; lipid profile, liver.

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## 1. Introduction

In local parlance, "Damsissa" refers to *Ambrosia maritima* LHer (Asteraceae). Particularly close to water catchment areas, it is a weed that is widely dispersed (1,2). Rheumatic symptoms, asthma, bilharziasis, diabetes, stomach, and renal issues are all treated with this common folk remedy (3). Following oral ingestion of dried leaves of the plant, Alard et al. (4) found no harmful symptoms. According to phytochemical investigations, some pseudoguaianolide sesquiterpenes, including neoambrosin, chloroambrosin, damsinic acid, and hymenin, have been found in *Ambrosia maritima* extract (5). Two novel sesquiterpene lactones were extracted from *Ambrosia maritima* and identified as 13 dihydrosilostachyin and 1'-noraltarnisin (6). Samyrin, a triterpene that is isolated from the leaves of the damsissa plant, exhibits molluscicidal efficacy against *Shistosoma* spp. intermediate hosts (6). According to certain experts, the *Ambrosia maritima* extract contains coumarins such as scopoletin and isoscopoletin (7). Furthermore, a variety of chemicals were extracted from the extract of *Ambrosia maritima*, including tannin, alkaloids, saponins, resins, and hispidulin (8,9). Additionally, flavonoids produced from the damsissa, such as  $\beta$ -sitosterol and apigenin, have anticancer action (10). Damsissa was used to extract essential (volatile) oils, such as cineole and camphor (11). Triglycerides (TG), fatty acids, cholesterol, and other lipids, such as phospholipids, which are found in the structural cell membrane and also serve as precursors for bile acid, vitamin D, and steroid hormones, are all thought to be necessary for all mammals, including humans (12). Dyslipidemia is the term for the abnormalities in the body's levels of these lipids and/or lipoproteins (13). It is acknowledged that hyperlipidemia poses a substantial risk for the onset of cardiovascular diseases (CVDs) (14). It is characterized by a decrease in high-density

lipoprotein (HDL) levels and an increase in one or more plasma lipids, such as triglycerides, cholesterol, cholesterol esters, and phospholipids, or plasma lipoproteins, such as so-called VLDL and LDL (15). High levels of triglycerides (hypertriglyceridemia) and cholesterol (hypercholesterolemia) are the main causes of atherosclerosis, a disorder that is closely linked to ischemic heart disease (IHD) (16). So, the aim of current study is investigation the anti-hyperlipidemia effects of *Ambrosia maritima* in albino female rats and their fetus.

## 2. Materials and methods:

### Animal model

In this experiment 30 female rats, (wt 140-160 gm with age 12-16 weeks) obtained from Science College/ Kirkuk University at a period April to Augustus 2021.

### Leaves extract

*A. maritima* leaves were gathered from Kirkuk City, Iraq's local market. After the leaves were ground, 5 g of the powdered leaves were boiled for 15 minutes with 200 ml of distillation water to create an aqueous extract of *A. maritima*, which was subsequently filtered through filter paper. The extract from the leaves was kept at 4.0C. The extraction procedure was carried out each time for new extract [17].

### Experimental design

Thirty male rats were used in current experiment and divided as follow (each experimental group contain 5 rats):

- Control group: rats were feeding standard pellet diet and administrated (orally) normal saline.
- Hyperlipidemia group: rats given water containing 0.5% of hydrogen peroxide (Saudi *Hydrogen Peroxide Company* (SHPC)) and feeding on high fat diet (HFD) (contain 1% of cholesterol) for 60 days.
- Aqueous extract group: rats administrated (orally by stomach tube) (25mg/kg/daily) extract for 60 days, and then dissected.
- Treated group: hyperlipidemia rats treated with (25mg/kg/daily) aqueous extract for 30 days, and then dissected.

### Blood samples

The cardiac puncher technique was used to get the blood samples, which were then placed in serum tubes while under ketamine and xylazine anesthesia. A centrifuge was used to collect the serum (4000 ppm and 15 min). Until it was used, the serum was kept in storage.

### Measurements

Total protein, albumin, total cholesterol, triglyceride and HDL were estimate by applying a procedure according to directions of producer firm kits (Randox). Otherwise, a colorimetric reaction with thiobarbituric acid (TBA) was used to determine MDA (malonedialdehyied) (Saleh, 2018). Estimate the catalase level based on the manufacturer's company kit instructions.

### Histological study

Pieces of rat fetus liver were removed, paraffin-processed, fixed with 10% formalin, sliced to a six micrometer thickness using a rotary microtome, and stained with histological stains of Hematoxylin and Eosin (H&E) [18,19]. Sections were examined using an Optica microscope (Italy).

### Statistical analysis

The values of parameters in the current experiment were analyzed using the statistical Minitab application (version 17). One-way analysis of variance (ANOVA) was used to determine a

statistical difference between the group means (Beth et al., 2004). At the  $P \leq 0.05$  level, differences were deemed significant.

### 3. Results:

#### Proteins

The concentration of total protein ( $6.76 \pm 0.14$ ) and albumin ( $2.35 \pm 0.09$ ) in HFD group show high significant changes ( $P \leq 0.05$ ) compared with control group ( $7.15 \pm 0.11$  and  $2.75 \pm 0.07$  respectively). The concentration of total protein ( $7.26 \pm 0.19$ ;  $7.09 \pm 0.15$  respectively) and albumin ( $2.85 \pm 0.04$ ;  $2.62 \pm 0.06$  respectively) in AEG and treated groups show significant ( $P < 0.05$ ) reduce compared with control group as shown in figures (1-2).

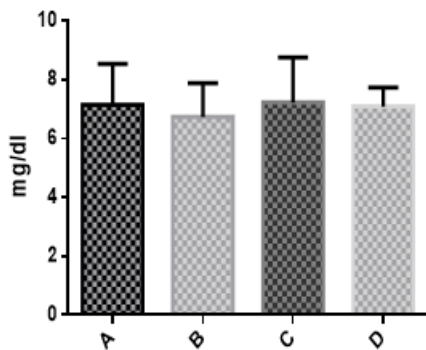


Figure (1): Concentration of total protein in all groups

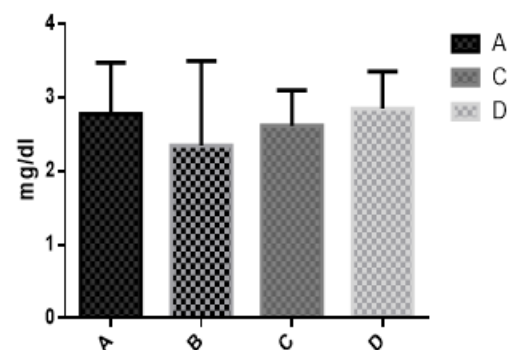


Figure (2): concentration of albumin in all groups

#### Lipid profile

Total cholesterol ( $207.25 \pm 6.3$ ), triglyceride ( $244.83 \pm 9.52$ ) and HDL ( $23.18 \pm 1.66$ ) in HFD group show high significant ( $P \leq 0.05$ ) elevate compared with control group ( $81.64 \pm 5.02$ ;  $72.93 \pm 8.62$  and  $32.24 \pm 1.27$  respectively). Total cholesterol ( $75.37 \pm 6.82$  and  $106.12 \pm 9.04$  respectively), triglyceride ( $65.11 \pm 4.59$  and  $95.17 \pm 5.64$  respectively) and HDL ( $39.12 \pm 2.84$  and  $28.24 \pm 1.53$  respectively) in AEG and treated groups show significant ( $P \leq 0.05$ ) changes compared with control group as shown in figures (3-5).

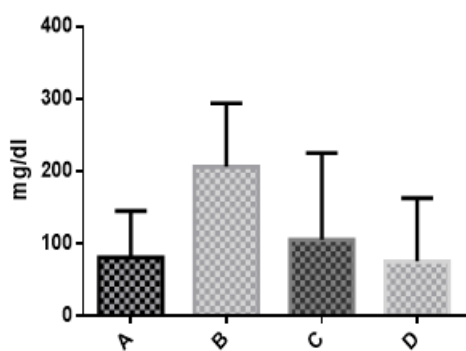


Figure (3): Total cholesterol in all groups

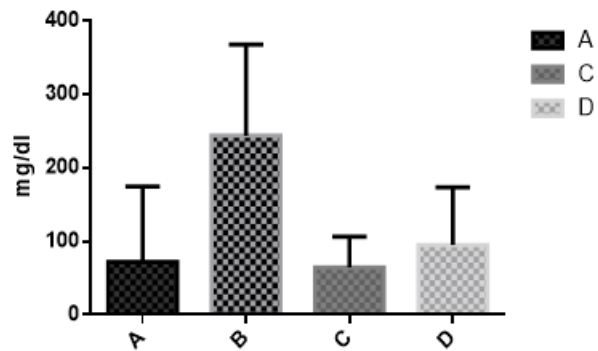


Figure (4): Triglyceride in all groups

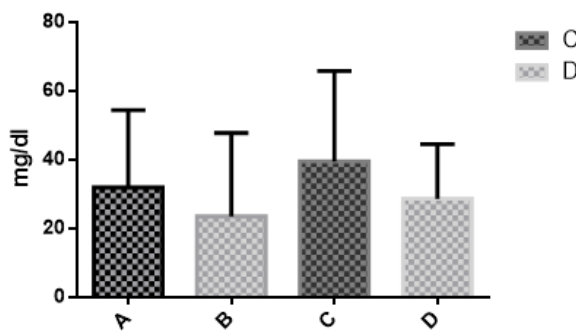


Figure (5): HDL concentration in all groups

### MDA and catalase

MDA levels ( $1.94 \pm 0.15$ ) and catalase ( $0.94 \pm 0.03$ ) in HFD group show high significant changes ( $P \leq 0.05$ ) compared with control group ( $1.39 \pm 0.08$  and  $1.42 \pm 0.11$  respectively). MDA levels ( $1.21 \pm 0.06$ ;  $1.58 \pm 0.07$  respectively) and catalase ( $1.56 \pm 0.08$ ;  $1.28 \pm 0.13$  respectively) in AEG and treated groups show significant ( $P \leq 0.05$ ) changes compared with control group as shown in figures (6-7).

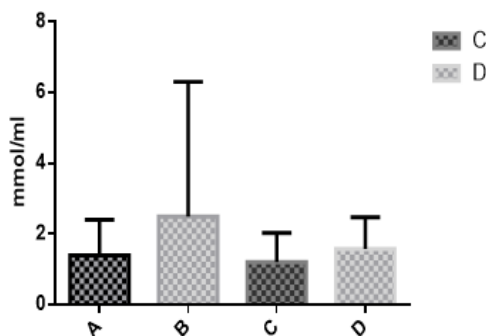


Figure (6): MDA levels in all groups

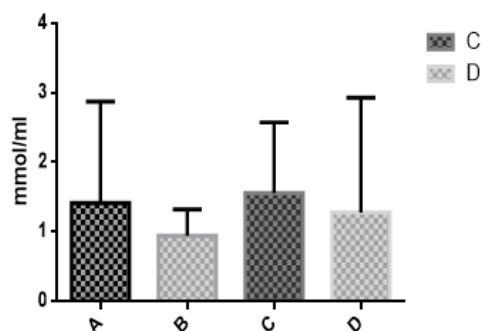


Figure (7): Catalase levels in all groups

### Histological Study

#### Negative Control Group

Fetal liver showing the normal shape of the central vein and blood vessels, as well as the normal shape of hepatocytes, the arrangement of sinusoids between hepatocytes, and the presence of hematopoietic elements, including lymphocytes and pluripotential stem cells (fig: 8).

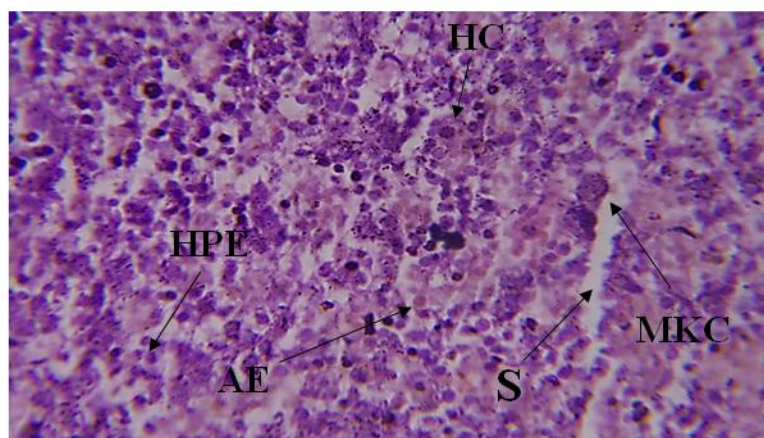
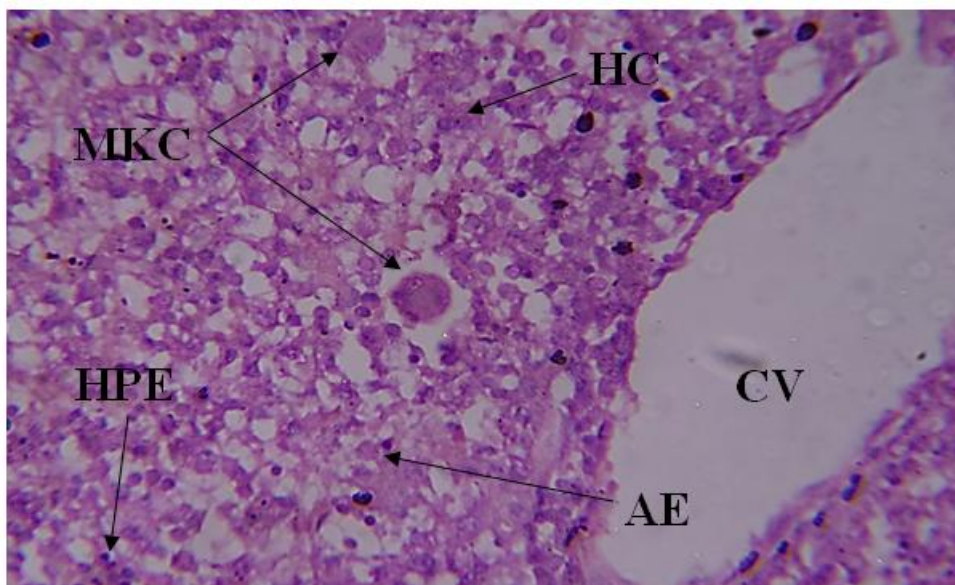


Figure (8): Liver of the control fetus showing the normal shape of the central vein (CV), hepatocytes (HC), and sinusoids (S) with the presence of Megakaryocyte (MKC), hematopoietic elements (HPE), and anuclear red blood cells (AE) H&E X400.

### Positive Control Group

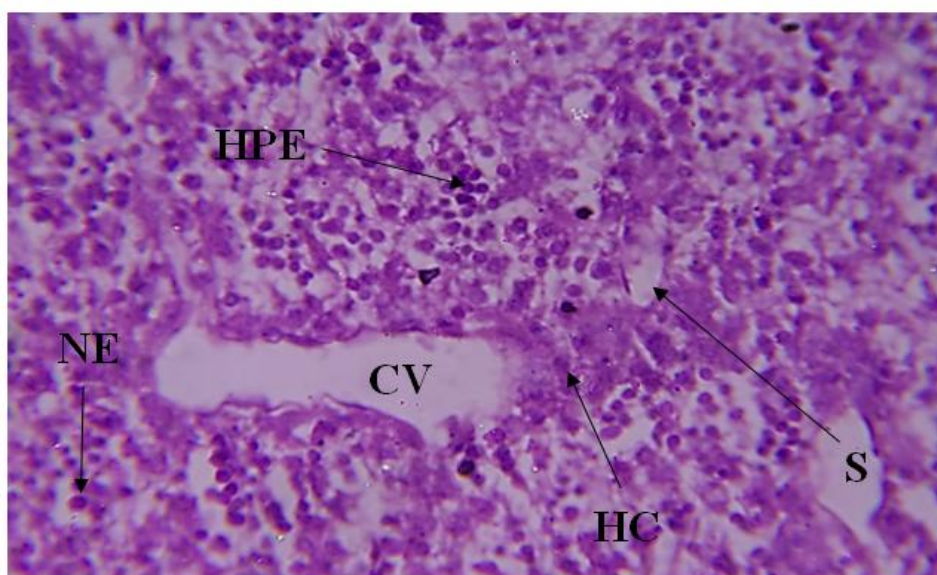
A decrease in the number of hematopoietic megakaryocytes and a decrease in hematopoietic elements, including lymphocytes and pluripotential stem cells, was observed compared to the control groups. Anucleated blood cells and nucleated nuclei were observed in the sinusoids, appearing in smaller numbers compared to the control group (fig: 9).



**Figure (9):** Liver of the control group fetus showing the central vein (CV), hepatocytes (HC), with the presence of Megakaryocyte (MKC), hematopoietic elements (HPE), anuclear red blood cells (AE) H&E X400.

### Extract Group

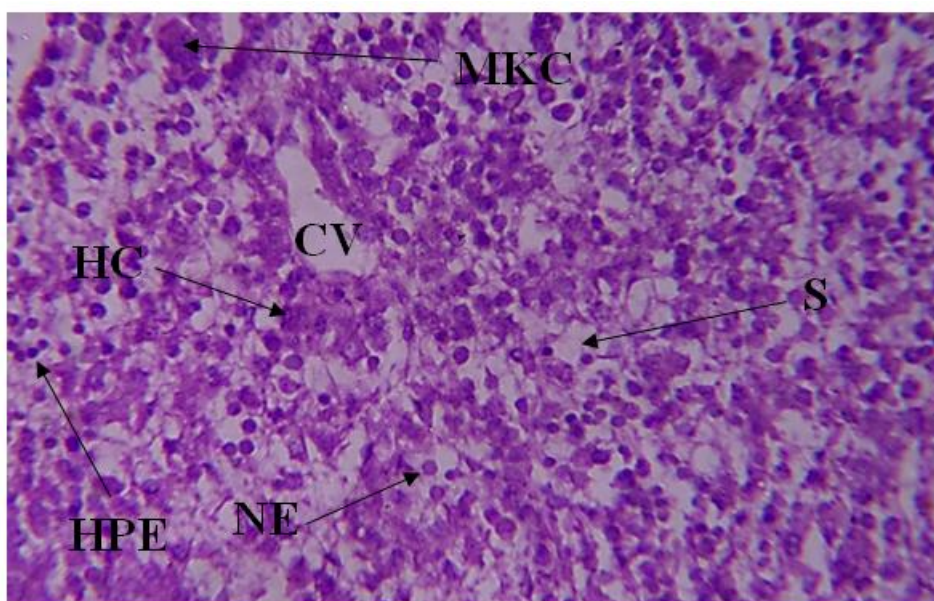
Transverse sections from this group show the central vein. On the other hand, the tissue slides showed normal hepatocytes and a sinusoidal arrangement between the hepatocytes. Anucleated and nucleated blood cells were observed in the sinusoids, appearing in smaller numbers compared to the control group (fig: 10).



**Figure (10):** Fetal liver of the group treated with the extract showing the central vein (CV), hepatocytes (HC), and sinusoids (S) with the presence of nucleated RBC (NE) and blood-forming elements (HPE) H&E X400.

### The treated group

In this group, the normal shape of the central vein, normal hepatocytes, and the sinusoidal arrangement between the hepatocytes were found. An improvement in the number of hematopoietic megakaryocytes was also observed (fig: 11).



**Figure 11: Liver of the fetus of the fattened and extract-treated group showing the central vein (CV), hepatocytes (HC), sinusoids (S) with the presence of hematopoietic elements (HPE), with the presence of Megakaryocyte (MKC) and nucleated section (NE) H&E X400.**

### 4. Discussion:

The current study showed that feeding male rats a high-fat diet causes hyperlipidemia and oxidative stress, which alters the amounts of total protein. However, upon treatment with *A. maritime*, the leaves extract showed anti-hyperlipidemia action. Rats given *Ambrosia maritime* extract on a daily basis had serum protein profile characteristics that were close to normal. This could be explained by the flavonoids found in *Ambrosia maritime*, which raise hepatic glucokinase activity and reduce glucose levels, most likely by increasing the release of insulin from pancreatic islets (20). Additionally, the leaf extract raised HDL levels while lowering triglyceride and cholesterol levels. The saponins in *A. maritime* extract, which have direct antioxidant activity and can reduce atherosclerosis, may be the cause of the current findings. However, flavonoids' antioxidant qualities, as demonstrated by the free radical scavenging assay, can lower total cholesterol and triglycerides (21). The present investigation also supports the findings of Elmuaiz et al. (22) that rats given an extract of *ambrosia* leaves (100 mg/kg/bw) for 21 days saw a significant reduction in cholesterol and a non-significant change in triglycerides. The present findings concur with Bakhiet and Adam's (23) findings about the hypocholesterolemic and lipid-lowering effects of flavonoids and phenols. If not, the leaf extract's ability to lower MDA and raise catalase levels may result in flavonoids with strong antioxidant properties that lessen the oxidative stress brought on by free radicals (24). Mouse liver tissue is harmed by hyperlipidemia (25), which impairs regular metabolism and exacerbates hyperlipidemia (26, 27). In this study, mice fed a high-fat diet experienced liver injury as a result of inflammation and fat buildup. According to the findings, administering *A. maritime* enhanced the fetal liver. The active ingredients in the *Ambrosia maritime* extract, flavonoids, may be the cause of the effects of the plant. Free radical-induced oxidative stress is lessened by flavonoids' strong antioxidant, hypoglycemic, and hypolipidemic effects (9, 10). Therefore, through hepatocyte protection and the suppression of pro-inflammatory mediators, *Ambrosia maritime* can improve liver function and lower body weight (10). These findings are consistent with those of Ahmed and Khater (28)

who found that *Ambrosia maritime* functions as an antioxidant and hepato-protective agent against the biochemical changes brought on by the alloxan effect by preventing liver damage, enhancing liver function, and promoting hepatocyte regeneration.

## 5. Conclusion:

According to the above results of current study, it can be concluded that the leaves extract of *A. maritime* show anti-hyperlipidemia activity in current experiment. On the other hand, it was found that *A. maritime* dosage is highly effective in improving the liver tissue of fetuses, due to its possession of many active compounds, including phenols and flavonoids.

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