Biological Effects of Ambrosia Maritima on Rats

ELmuaiz Gasmalbari¹, Hatil Hashim EL-Kamali², Osama Sharafeldin Abbadi³

¹Department of Biochemistry, Orotta College of Medicine and Health Sciences, Asmara, Eritrea.
²Department of Biotechnology, Faculty of Science and Technology, Omdurman Islamic University, Sudan.
³Department of Biochemistry, Faculty of Medicine, Omdurman Islamic University, Sudan.

Corresponding Author: Osama Sharafeldin Abbadi

ABSTRACT

Background and aim: The uses of Ambrosia in herbal medicine are various and still to be explored. This study aimed to evaluate the effect of Ambrosia maritima in the blood glucose, serum lipids, Haemoglobin, hematologic values of blood cells, and the possible organ toxicity in laboratory rats in response to Ambrosia oral ingestion for three weeks.

Materials and methods: Ambrosia maritima leaves were pounded to powder using a pestle. 100mg for each kilogram of bodyweight (mg/kg/bw) were weighted for each rat. Sera for blood glucose, lipid profile, blood parameters, and histology slides for liver and kidneys were prepared and studied after the above dose administration-orally, for three weeks.

Results: There was a significant weight gain; increase in albumin level, mean corpuscular volume of Red blood cells (MCV), also there was a significant reduction in plasma cholesterol, serum Aspartate aminotransferase enzyme (AST), and white blood cells count (WBCs) and Platelets count of the Rats. Other blood and serum changes were insignificant. Histological examination showed hemorrhage and fatty changes, and renal tubular necrosis of epithelial cells and scattered lymphoid nodules in the congested cortex.

Conclusion: Oral ingestion of Ambrosia maritima extract for three weeks reduced Cholesterol and blood glucose, increased the gross body weight, reduced AST, increased Albumin, MCV, MCHC, and reduced WBCs and Platelets in the Albino Rats. The nephrotoxic and hepatotoxic effects of Ambrosia are demonstrable in the histology slides.

Keywords: Ambrosia maritima, Alcohol extract, Albino Rats, liver toxicity, kidney toxicity.

1. INTRODUCTION

Ambrosia maritima (Figure 1) belongs to the family (Asteraceae). It is known as sea wormwood and locally in Sudan as Damssisa. It is widely distributed along the coastal zone of the Mediterranean and throughout Asia, Africa like Madagascar.¹ The major active ingredients of Ambrosia maritima are triterpene groups, the most important of which are sesquiterpene lactones.¹ There are twenty one sesquiterpene lactones identified in Ambrosia maritima, ambrosin and damsin, being the mostly major components. Alard et al., 1991 stated that hyminovin is also isolated from Ambrosia maritima.² Other compounds such as tribromodamsin were synthesized from the naturally occurring sesquiterpene lactones.³ Ambrosia maritima has several medicinal properties.⁴ The whole plant is used but leaves are preferable. Ambrosia maritima is used in folk medicine as appetizer, assisting digestion and tonic when mixed with sugar in a ratio of 1:3. It’s also used for treatment of scurvy, kidney stones, asthma, rheumatic pains as well as febrifuge. Farm animals do not eat the plant but it has been observed that when the plant bound with salt marshes it increased the body weight. This was elucidated by the weight improvement of cattle during Ambrosia maritima pasture. Improvement of the body weight gain was also seen in chickens when their diet was mixed with 2% Ambrosia maritima. A crude 2% aqueous acetic acid extract of A. maritima leaves produced a notable
decrease in blood sugar level in mice and rabbits. [5]

A Dose of Ambrosia maritime Ethanol extract of 500mg/dl ---- 1000mg/kg was lethal to laboratory rats, while 100mg/kg and 200mg/ kg showed No toxicity on Wister rate. [6]

The potential effects of the Ambrosia are still to be explored. This study aimed to evaluate the effect of Ambrosia maritima in the blood glucose, serum lipids, haemoglobin, hematologic values of blood cells, and the possible organ toxicity of this plant by measuring liver and kidney functions and histology.

III. Preparation of the extract:
10 grams of each powdered plant sample were refluxed with 100 ml of 80% of ethanol four 4 hours. The cooled solution was filtered and enough 80% ethanol was passed through the volume of the filtrate to 100 ml. This prepared extract (PE) was used for the various tests.

IV. Collection of blood and serum samples and plasma:
Periodical blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinized bottles for hematological studies. Blood samples collected in clean Non- heparinized bottles were allowed to clot.

V. Histopathology:
The liver and kidney of all rats were fixed to preserve tissues for degradation and to maintain the structure of organelles, in buffered formalin in labeled bottles. Tissues were processed routine and water removed from tissues after dehydrated and cleared. Tissues were then filtered with embedding in paraffin wax. Sections of 5µ thicknesses were cut with microtome, and stained with hematoxylin and eosin (H&E stains) and examined under the light microscope and Photomicrographs were taken in Khartoum hospital.

VI. Hematology:
Lewis (1991) manual Methods were followed to determine Hemoglobin concentration (Hb%), Packed cell volume (PCV %), Red blood cells count (RBCs), White blood cells count (WBCs), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH), and Red cell distribution (RDW).

VI. Sero-biochemical analysis:
The tests involved were: Total protein; Total urea and creatinine; Albumin; Total bilirubin; Determination of triglycerides, cholesterol, and Glucose;
measurement of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase.

VIII. Statistical analysis:

Results were expressed as mean±SD. The data were subjected to one-way analysis of variance (ANOVA) test and differences between samples were determined by Dunnett’s multiple comparison test, using the Graph pad prism (statistical) software. Results were considered to be significant at P<0.05.

RESULTS

Table (1): List of the results of Ambrosia maritima administration to albino rats for 21 days.

<table>
<thead>
<tr>
<th>Test</th>
<th>Value before Ambrosia maritima (control)</th>
<th>Value after Ambrosia maritima (test)</th>
<th>P-value of the difference.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerols (mg/dl)</td>
<td>82.8</td>
<td>82.72±0.71</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>55</td>
<td>31.01±0.81</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Blood sugar (mg/dl)</td>
<td>74</td>
<td>63.1±0.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body Weight (gm)</td>
<td>97.2</td>
<td>103.5±1.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AST (iu)</td>
<td>135</td>
<td>113.0±6.87</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ALT (iu)</td>
<td>42</td>
<td>42.42±11.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ALP (iu)</td>
<td>152.5</td>
<td>133.6±14.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Protein (g/ dl)</td>
<td>7.2</td>
<td>7.36±0.144</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ALB (g/ dl)</td>
<td>4.2</td>
<td>4.50±0.10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Bilirubin (mg/ dl)</td>
<td>0.1</td>
<td>0.14±0.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RBC (x10⁶/mm³)</td>
<td>6</td>
<td>6.35±0.32</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MCV (micro m³)</td>
<td>55</td>
<td>58.40±1.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21</td>
<td>20.50±0.20</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>33</td>
<td>36.13±0.50</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>WBC (x10⁶/mm³)</td>
<td>9.3</td>
<td>8.42±0.71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RDW/SD (%)</td>
<td>30</td>
<td>30.08±1.64</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>13</td>
<td>14.37±0.71</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PLT (10³/ml)</td>
<td>275</td>
<td>20.18±2.48</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>41.8</td>
<td>41.67±0.81</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Urea (mg /dl)</td>
<td>30</td>
<td>24.42±2.62</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Creatinine (mg/ dl)</td>
<td>1</td>
<td>0.90±0.03</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Administration of aqueous Ambrosia leaves100mg/kg/bw to rats for 21 days caused:
- Insignificant reduction in plasma TAG; mean of 82.72±0.71mg/dl, while the control 82.8 mg/dl)
- Significant reduction in plasma cholesterol; 31.01±0.81mg/dl while the control was 55 mg/dl.
- Significant reduction at on plasma glucose; mean value of 63.1±0.75 mg/dl, compared to control group (74 mg/dl).
- Significant weight gain: mean 103.5±0.071gm, compared to control (mean 97.2 gm).
- Significant reduction on AST 113.0±6.87 iu while the control group mean was 135 iu.
- No significant change on ALT (mean of 42.42±11.4 iu, while the control 42 iu) or ALP (mean of 133.6±14.7 iu, while the control 152.5iu).
- Significant increase in albumin level (mean of 4.50±0.10 g/ dl, while the control 4.2 g/ dl)
- Insignificant protein levels change (mean of 7.36±0.144 g/ dl, while the control was 7.2g/ dl).
- Significant increase on RBCs (mean of 6.752±0.32 x106mm3, while the control 6 x106mm3).
- Insignificant increase in Hemoglobin (mean of 14.37±0.71 g/dl, while the control 13 g/ dl).
- Significant reduction in WBCs (mean of 8.42±0.71 x106mm3, while the control 9.3 x106mm3).
- Insignificant reduction in both plasma creatinine (mean of 0.900±0.03mg /dl, while the control 1mg /dl) and blood urea (mean of 24.42±2.62mg /dl, while the control 30mg /dl).
Insignificant reduction in PCV (mean of 41.67±0.81%, while the control 41.8%)

Significant increase in MCV (mean: 58.40±1.02 micro m3, while the control 55 micro m3)

Insignificant decrease in MCH (mean of 20.50±0.20 pg, while the control 21 pg)

Significant raise on MCHC (mean 36.13±0.50%, while the control 33%)

Insignificant increase in RDWSD (mean was 30.08±1.64 %, while the control 30%)

Significant reduction in PLT count (mean of 20.18±2.48×103/ml, while the control 275×103/ml).

All results are demonstrated in table (1). Changes in the liver texture are shown in figure (2), and changes in kidneys histology are shown in figure (3).

![Figure 2](image1.png)

**Figure (2): Photomicrograph section through the liver of albino rat, left slide is a control, right side is a liver treated with Ambrosia maritima (0.4% w/v) showing hemorrhage and fatty cytoplasmic vaculation.**

![Figure 3](image2.png)

**Figure (3): Photomicrograph section through the kidney of albino rat left slide is a control, right side is a kidney treated with Ambrosia maritime where necrosis of epithelial cells of proximal and distal convoluted tubules and scattered lymphoid nodules in congested cortex are seen.**

4. DISCUSSION

This research was performed to measure the effects of alcohol extract Ambrosia maritima in the biochemical, histological, and hematological parameters of Laboratory Rats.

Administration of aqueous Ambrosia leaves100mg/kg/bw to rats for 21 days was caused significant reduction in plasma cholesterol and non-significant changes in triglycerides compared to control group. These results are in agreement with the study of Bahkiet and Adam of (1996) which reported a hypocholesterolemic and lipid lowering effect of Ambrosia maritime, but no alteration on plasma triglyceride. The possibility that Ambrosia contains compounds which affect cholesterol biosynthesis needs further analysis and study.
Administration of aqueous Ambrosia leaves extracts for three weeks shown to cause significant reduction on plasma glucose compared to control group. These results in agreement with a previous similar report of Ammar, 1993. [8] This hypoglycemic effect of Ambrosia may be due to the presence of compounds that act in a similar fashion as anti-diabetic medication by simulating β. cells or the action of some agents that delays glucose absorption. [9] Ambrosia may also be capable of inhibiting glycogenolysis and/or gluconeogenesis process, that are very effective in glucose homoeostasis. [10] Ambrosia also contains sesquiterpene lactone which acts as a hypoglycemic agent. The hypoglycemic effects might be exerted through the inhibition of glucose absorption, the increase in sensitivity of receptors to insulin, or stimulation of peripheral tissues uptake of glucose. [11]

Aqueous Ambrosia maritima showed significant weight gaining effect compared to control group. This result is in agreement with Bakhiet and Adam study of 1996.[11] This increase in weight gain is most probably due to the high protein content in Ambrosia maritima.

Administration of aqueous Ambrosia maritima leaves 100mg/kg/bw to rats for 21 days caused significant reduction on AST but no significant change on ALT and ALP, and significant increase in albumin level while total protein level was normal. In this prospect, Pavetta indica and Osbeckia octandra possesses more potent damage-reversing effect on liver cells. [12]

In this current study there was no elevation of liver enzymes, instead, the ratio ALT/AST changed due to the reduction in AST this does not necessarily indicate liver damage. Decrease of the whole enzymes may be a sign of liver atrophy. [13] Ambrosia maritima-induced reduction of the enzyme AST indicates that Ambrosia has compounds with hepato-protective abilities which lead to improvement of the hepatocytes, and preserve the structural integrity of the hepato-cellular membrane.

No doubt that biochemical changes reflect pathological changes. The histology revealed that there was fatty cytoplasmic vacuolation and individual cell necrosis in the hepatitis centri-lobular zone. Phytochemical analyses on Ambrosia extract have identified the presence of some phenol compounds that may have a role in hepatic lipid anti-oxidation. [13] Protein level was normal but albumin increased significantly (P<0.05).This elevation may be due to high crude protein in Ambrosia. [14]

Ambrosia maritima caused a significant increase in MCV and MCHC levels, a non significant increase in RBCs count, RDWSD, and Hemoglobin, but there was a significant reduction in WBCs and Platelets at (P<0.05). These results partially agree with other reports that Ambrosia has shown significant change on MCV, RDWSD, and bilirubin, [15] however, significant reduction in WBCs and MCV and platelets was seen (P<0.05) which disagrees with the 1996 Bakhiet study. [11]

RDWSD non significant increase definitely excludes iron deficiency anemia. Reduction in WBCs and platelets suggest thrombocytopenia; may be the abundancy of coumarins in Ambrosia had lead to this reduction in platelets and WBCs. [16]

Administration of aqueous Ambrosia maritima leaves100mg/kg/bw to rats for 21 days did not significantly changed plasma creatinine and blood urea; a similar result was previously reported by Ghazanfer. [17] Nonetheless, there were degeneration and necrosis of epithelial cells of proximal convoluted tubules, scattered lymphoid nodules in the congested cortex. Administration of Ambrosia maritima for long time may affect Kidney and leads to congestion. Macromolecules secondary metabolites such as alkaloids or coumarins may affect Kidney. On the other hand, Ambrosia leaves are widely used in kidney stones as it acts as antispasmodic and diuretic, which helps stone release.
CONCLUSION
This was an experimental study performed to measure the effects of ethanol extract of Ambrosia maritime on the Hematology, Liver and kidneys serology and histology, and Lipid and glucose profile of the laboratory breeds of Rats. It had been found that Ambrosia reduced Cholesterol and blood glucose, increased the gross bodyweight, reduced AST, increased Albumin, MCV, MCHC, and reduced WBCs and Platelets of the Albino Rats. These results agreed partially with previous published literature on Ambrosia.

Conflicts of interest: No conflicts to be declared by authors.

REFERENCES


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