A Study of Ambrein Treatment for the Evaluation of Change in Plasma Biochemical Parameters in Rats

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ABSTRACT—Biochemical effects of acute and subacute treatments with ambrein were investigated in rats by measuring the total proteins, cholesterol, triglycerides, GOT, GPT and alkaline phosphatase in the blood plasma. Also, determinations of prothrombin time (PT), partial thrombin time (PTT), thrombin time (TT) and fibrinogen level were performed. Furthermore, changes in plasma electrolyte concentration were studied. Ambrein administered i.p. did not cause any toxic symptoms in the liver as revealed by the histology of the liver tissue both in acute and subacute treatments. Ambrein itself did not significantly affect the plasma protein, cholesterol, GOT and GPT profiles, but lowered alkaline phosphatase at high doses (50 and 250 mg/kg) after subacute treatment. Thus far, no specific pattern of action of ambrein in electrolyte control has been found. However, it increased PT, PTT and TT and decreased fibrinogen levels in both the acute and subacute studies, pointing towards its potential as an anticoagulant and antifibrinogenic agent.

Keywords: Ambrein, Blood plasma, Elemental analysis

MATERIALS AND METHODS

Drugs and animals
The ambergris was purchased from a local market in Riyadh, Saudi Arabia. Isolation, identification and purification of ambrein from ambergris was done by the method described previously (6). Extra light olive oil was purchased from market, and the drug was dissolved in it by gentle heat. Kits for analysis were purchased from Boehringer Mannheim GmbH, Mannheim, Germany.

Male Wistar albino rats (bred at The Experimental Animal Care Centre, King Saud University, Riyadh), all roughly of the same age (7- to 8- weeks-old) and weighing 180–200 g, were used in this study. All the animals were maintained under controlled conditions of temperature, humidity and light and were provided with Purina chow and water ad libitum except on the day of sacrifice.

Acute treatment
Preliminary experiment revealed that i.p. injection of vehicle had no significant effect on blood plasma contents when compared to the untreated control group. The dose of ambrein used in this study (250 mg/kg) was reported earlier to be pharmacologically active (9, 10).

The animals were randomly assigned to the control and
Groups 1, 2, 3 and 4 were intraperitoneally given ambrein at a dose of 250 mg/kg dissolved in olive oil. Each group had its own control group (consisting of seven animals each) injected with 1 ml/kg of olive oil. At the time of sacrifice (3, 6, 12 and 24 hr post treatment), the animals were killed by cervical dislocation, and blood was taken by cardiac puncture. The blood samples from each animal were collected in two different vials. Each 3-ml aliquot of blood was taken into a heparinized vial and centrifuged. Plasma was isolated and refrigerated at −20°C until used for determinations of proteins, triglycerides, cholesterol, GOT, GPT, alkaline phosphatase and elemental analysis by atomic absorption spectrophotometry for Ca²⁺, Fe²⁺, Mg²⁺, K⁺ and Na⁺ concentrations. Other 3-ml aliquots of blood samples were taken into citrated blood sampling vials and immediately sent to the hospital for prothrombin time (PT), partial thrombin time (PTT), thrombin time (TT) and fibrinogen (θ) level determinations by using a coagulometer (Biomatic Bioasarstedt, Freiburg, Germany).

The liver tissue was excised and fixed in 10% buffered formalin. Hematoxylin and eosin-stained sections (11) were examined under the microscope for histopathological changes in a blind manner.

Table 1. Effect of ambrein at 250 mg/kg on rat plasma contents at various time intervals

| Group No. | Post-treatment time | Treatment | Total proteins g/100 ml | Total cholesterol mg/100 ml | Triglyceride mg/100 ml | GPT U/l | GOT U/l | Alkaline phosphatase U/l |
|-----------|---------------------|-----------|------------------------|----------------------------|-----------------------|---------|--------|-------------------------|
| 1         | 3 hr                | Control   | 4.08±0.08              | 81.38±7.97                 | 52.08±3.98            | 8.76±0.85 | 29.75±2.39 | 31.00±0.27              |
|           |                     | Ambrein   | 5.11±0.10***           | 76.50±3.44                 | 53.71±2.59            | 8.14±0.65 | 31.57±2.00 | 100.28±10.78***         |
| 2         | 6 hr                | Control   | 4.33±0.11              | 79.61±6.00                 | 60.00±3.55            | 10.00±1.10 | 28.00±2.2  | 31.60±0.25              |
|           |                     | Ambrein   | 5.64±0.15***           | 75.71±2.08                 | 60.57±4.22            | 14.42±0.89** | 30.57±3.19 | 92.00±0.643***          |
| 3         | 12 hr               | Control   | 4.45±0.12              | 77.76±3.92                 | 68.30±3.11            | 11.00±1.14 | 25.60±1.93 | 31.80±0.221             |
|           |                     | Ambrein   | 5.44±0.10***           | 81.57±2.73                 | 60.57±4.65            | 15.28±0.72** | 26.42±1.26 | 98.71±0.852***          |
| 4         | 24 hr               | Control   | 4.77±0.24              | 67.58±4.46                 | 72.70±3.65            | 12.50±1.31 | 49.55±4.61 | 47.65±0.360             |
|           |                     | Ambrein   | 5.54±0.10**            | 91.14±3.54**               | 60.57±2.95*           | 17.00±0.97* | 31.57±4.84* | 105.57±0.97***          |

Each group contained 7 animals. The control groups were given olive oil at the volume of 0.1 ml/kg, i.p. Each value represents a mean ± S.E.M. Treatment groups were compared with their respective controls, by Student's t-test: *P<0.05, **P<0.01, ***P<0.001.
Each group contained 7 animals. The control groups were given olive oil at a volume of 1 ml/kg, i.p. Each value represents a mean±S.E.M. Treatment groups were compared with their respective controls, by Student's t-test: *P<0.05, **P<0.01, ***P<0.001.

Effect on calcium ions (Table 3) after 3, 6 and 24 hr except at 12 hr when it was slightly higher than that of the control group. However, other ionic concentrations were affected significantly. The concentration of Fe$^{2+}$ and K$^+$ significantly increased throughout the experiment except at 6 hr when the rise in K$^+$ was not significant in comparison with group 1. Of course, hyponatremia was highly significant at all the intervals throughout the study. Histological examination of the liver tissue did not show any pathological symptoms after acute treatment.

Studies on chronic treatment

Tables 4, 5 and 6 show effects of treatment with ambrein at 10, 50 and 250 mg/kg for five weeks on the plasma contents of rats; with a dose of 50 mg/kg, the triglycerides increased significantly, and at a dose of 250 mg/kg, the activity level of alkaline phosphatase decreased significantly from 63.5 to 42.3 U/l. Plasma proteins, cholesterol, GOT and GPT did not change appreciably with any of the doses studied (Table 4).

Ambrein treatment was found to cause a significant increase in prothrombin time at doses of 10 and 250 mg/kg and a highly significant rise at 50 mg/kg. Also, thrombin time showed marked increases at all the tested doses. Ambrein reduced the amount of fibrinogen significantly in a dose-dependent manner, but without any appreciable changes in partial thrombin time at all the tested doses (Table 5).

Treatment with ambrein at 10 mg/kg caused a mild reduction in Fe$^{2+}$ and a highly significant reduction in Na$^+$ when compared to group 1 (Table 6). The treatment with 50 mg/kg increased the Ca$^{2+}$ concentration and decreased Fe$^{2+}$. Ambrein at 250 mg/kg did not cause any noticeable change in concentrations of electrolytes except for K$^+$, which was extensively decreased.

None of the parameters evaluated for histopathology at any dose tested gave any important findings to be reported.

DISCUSSION

Under acute experimental conditions, ambrein seems to
Table 4. Effect of ambrein treatment for five weeks on blood plasma contents in rats

| Group No. | Treatment dose (mg/kg) | Total proteins g/100 ml | Total cholesterol mg/100 ml | Triglycerides mg/100 ml | GPT U/l | GOT U/l | Alkaline phosphate U/l |
|-----------|------------------------|-------------------------|-----------------------------|-------------------------|---------|---------|-----------------------|
| 1         | Control                | 5.1±0.37                | 57.40±5.0                   | 77.00±4.2               | 14.4±1.50 | 73.50±7.30 | 63.50±5.0             |
| 2         | Ambrein, 10           | 5.1±0.20                | 53.85±5.1                   | 79.14±6.5               | 15.3±0.52 | 74.14±5.22 | 67.71±5.2             |
| 3         | Ambrein, 50           | 4.6±0.10                | 47.30±3.1                   | 100.00±7.8*             | 12.1±1.10 | 59.03±5.60 | 62.90±6.1             |
| 4         | Ambrein, 250          | 4.9±0.10                | 54.90±3.7                   | 91.60±5.6               | 14.9±1.30 | 67.90±4.90 | 42.30±2.1**            |

Each group contained 7 animals. The control group was given olive oil, i.p. Each value represents a mean±S.E.M. Groups 2, 3 and 4 were compared with group 1, by Student’s t-test: *P<0.05, **P<0.01.

Table 5. Effects on clotting time and fibrinogen levels in rat blood after five weeks treatment with i.p. injection of ambrein every alternate day

| Group No. | Treatment dose (mg/kg) | Prothrombin time (PT) sec | Partial thrombin time (PTT) sec | Thrombin time (TT) sec | Fibrinogen (g) mg/dl |
|-----------|------------------------|---------------------------|-------------------------------|-----------------------|---------------------|
| 1         | Control                | 14.11±0.35*              | 27.41±0.55                   | 15.00±0.50            | 228±14.6            |
| 2         | Ambrein, 10           | 16.00±0.28**             | 27.70±2.80                   | 18.50±0.42***         | 172±13.0*           |
| 3         | Ambrein, 50           | 19.60±0.80***            | 28.40±2.29                   | 20.90±1.05***         | 175±0.80**          |
| 4         | Ambrein, 250          | 17.60±0.93**             | 29.00±2.08                   | 25.40±1.25***         | 151±14.1**          |

Each group contained 7 animals. The control group was given olive oil, i.p. Each value represents mean±S.E.M. Groups 2, 3 and 4 were compared with group 1, by Student’s t-test: *P<0.05, **P<0.01, ***P<0.001.

Table 6. Effects of ambrein at 10, 50 and 250 mg/kg on plasma ionic concentrations (ug/ml) post chronic treatment for five weeks

| Group No. | Treatment dose (mg/kg) | Calcium (Ca²⁺) | Ferrous (Fe⁺⁺) | Magnesium (Mg⁺⁺) | Potassium (K⁺) | Sodium (Na⁺) |
|-----------|------------------------|----------------|--------------|-----------------|---------------|--------------|
| 1         | Control                | 10.17±0.45     | 0.205±0.01   | 2.40±0.17       | 16.00±1.25    | 364.5±10.00  |
| 2         | Ambrein, 10           | 10.41±0.60     | 0.153±0.01** | 2.14±0.20       | 16.30±0.96    | 312.6±0.67***|
| 3         | Ambrein, 50           | 11.90±0.60*    | 0.174±0.01** | 2.42±0.16       | 14.74±0.70    | 388.2±27.65  |
| 4         | Ambrein, 250          | 10.53±0.60     | 0.227±0.02   | 2.05±0.13       | 09.93±0.46*** | 338.8±07.71  |

Each group contained 7 animals. The control group was given olive oil, i.p. Each value represents a mean±S.E.M. Groups 2, 3 and 4 were compared with group 1, by Student’s t-test: *P<0.05, **P<0.01, ***P<0.001.

be effective at 3 and 6 hr post administration, causing highly significant elevation in prothrombin, partial thrombin and thrombin times. However, a highly significant decrease in fibrinogen level was observed at 6 hr which recovered to the normal level at 24 hr when compared to their respective controls. Similarly, after chronic treatment, increases in PT and TT were observed at any dose tested, and there was a dose-dependent significant decrease in fibrinogen. Prothrombin is converted to thrombin in the clotting process. The sequence of reactions involved in this process requires Ca²⁺, thromboplastin, the Stuart-Prower factor, a labile factor and a stable factor. Deficiencies in any of the components result in a prolonged prothrombin time (12). Fibrinogen changes into fibrin by the action of thrombin. Therefore a prolongation of thrombin time reflects the effects of ambrein on fibrinogen. Hypofibrinogenemia is generally caused not by deficient production but by increased consumption or breakdown by the action of fibrinolytic enzymes (12). Several reports suggested that terpenes possess the ability to stabilize lysosomal membranes and cause significant anti-coagulant and anti-fibrinogenic effects (13). Most likely, this effect of ambrein is attributable to its terpene structure.

Ambrein treatment is found to elevate testosterone levels in plasma (S.A. Taha, unpublished data). In the present study, after acute treatment with ambrein, a highly significant hyperproteinemia was observed at 3, 6 and 12 hr and a moderately significant increase seen at 24 hr. Androgens and growth hormones are reported to increase protein synthesis and serum protein levels (12, 14). However, there is no significant change in protein level in chronic treatment. This discrepancy might be due to the hormonal balance achieved during the chronic treatment. The activity profile of ambrein in the time course study showed a markedly significant rise in alkaline phos-
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Phosphate, while in chronic studies, only the highest dose decreased its activity. Certainly, we can not exclude the possibility that this discrepancy is related to its distribution, presence in tissue and activity in blood plasma. Since this enzyme is widely distributed in tissues and muscles so a rise in its activity might be attributed to some scars, abdominal masses or muscle damage due to injection (15, 16). After the chronic treatment, the aminotransferases GGT and GPT showed no significant change at any of the tested doses compared to the control group. The liver is a rich source of aminotransferases, and the serum level of both these aminotransferases usually rise and fall together indicating hepatic damages (17). Our results are further in agreement with the histology of liver tissue that revealed no liver damage, consistent with no significant change in these enzymes.

A slight increase in serum Ca²⁺ is attributable to the overall increase of total proteins in time course studies at 12 hr. It is known that protein bound non-diffusible calcium constitutes 40–50% of the total serum calcium (12), and hypercalcemia due to hyperproteinemia is generally associated with normal ionized calcium values (18). The increase in Fe²⁺ in acute treatment is also related to the increase in proteins or an increase in the iron binding protein fraction. Little is known about the factors regulating Mg²⁺ levels in the plasma. It is believed, however, that parathyroid hormones (parathyrin) and aldosterone play a role, and the latter is known to regulate the excretion of Mg²⁺ in a way similar to K⁺. As it has been suggested in a previous report (19), ambrein probably exerts its analgesic activity by preventing synthesis and/or release of prostaglandins. So this inhibition of prostaglandins may result in retention of K⁺ in the serum (12).

From our present data, it appears likely that ambrein is devoid of any serious adverse effects on biochemical contents of the plasma including proteins, cholesterol, triglycerides and aminotransferases. However, ambrein was found to be a potent anticoagulant and antifibrinogenic agent. Further studies are warranted to explore its mode of action to determine its safety for medicinal use and to provide support for its folkloric claims.

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REFERENCES

1 Dannenfeldt KH: Ambergris: The search for its origin. Isis 73, 382–397 (1982)
2 Jegou E, Polonsky J, Lederer E, Schute-Elte K, Egger B and Ohloff G: Ambergris revisited. Isolation of volatile constituents; Identification and synthesis of ambra-aldehyde C₁₄H₂₂O. Nouv J Chim 1, 529–531 (1977)
3 Al-Gozech K: Prophet Medicine, 2nd ed, p 340, Syria Al-Resala Publishers, Damascus (1981)
4 Epstein WL: Report to RIFM Fenarolis Handbook of Flavor Ingredients, Edited by Furia TE and Bailleau N, 2nd ed, Vol 1, p 273, CRC Press, Cleaveland (1975)
5 Merck Index: An Encyclopedia of Chemicals and Drugs, 8th ed, p 49, Merck & Co Inc, Rahway (1968)
6 Taha SA: General pharmacological screening of ambergris extract. Pakistan J Pharmacol 6, 75–88 (1989)
7 Taha SA and Rashid S: Preliminary investigations on cardiovascular profile of ambrein. Pakistan J Pharmacol 7, 95–100 (1990)
8 Taha SA: Effect of ambrein on blood glucose levels of rats. J Ethnopharmacol 35, 145–148 (1991)
9 Taha SA and Ginawi OT: Ambrein, the major constituent of ambergris inhibits oedema responses to carrageenin and serotonin in the rat paw. Bull Fac Pharm Cairo Univ 31, 113–114 (1993)
10 Taha SA: Studies on the mode of action of ambrein as a new antinociceptive compound. Jpn J Pharmacol 60, 67–71 (1992)
11 Culling CFA: Handbook of Histopathological and Histochemical Techniques, 3rd ed, p 73, 126 and 159, Butlter Worth, London (1974)
12 Tietz NW: Fundamentals of Clinical Chemistry, 2nd ed, p 300, 877, 901 and 1053, WB Saunders Company, Philadelphia (1982)
13 Thomas OO: Anticoagulant and antifibrinogenic properties of Tanacetum corymbosum. Fitoterapia 60, 231–233 (1989)
14 Gilman AG, Rall TW, Nies AS and Taylor P: The Pharmacological Bases of Therapeutics, 8th ed, p 741 and 1419, Pergamon Press, Inc, New York (1990)
15 Posen S: Alkaline phosphatase in abnormal laboratory results. Australian Prescriber, Supp 10, 6–7 (1987)
16 Posen S and Doherty E: The measurement of serum alkaline phosphatase in clinical medicine. Adv Clin Chem 22, 161–245 (1981)
17 Powell LW: Liver function tests in abnormal laboratory results. Australian Prescriber, Supp 10, 30–33 (1987)
18 Low JC, Schaft M, Earl JM, Pichochocki JT and Li TK: Ionic calcium determination in primary hyperthyroidism. JAMA 232, 152–155 (1981)
19 Taha SA: The role of prostaglandin E₂ in the anti-inflammatory and antinociceptive activities of ambrein. Med Sci Res 22, 97–98 (1994)