Pharmacology of *Quercus infectoria*

MOHAMMAD S. DARˣ**, M. IKRAM, and T. FAKOUHI**

Abstract ☐ The galls of *Quercus infectoria* (Fagaceae), a commonly available plant in Iran, were studied pharmacologically. Two fractions were employed, a dried acetone-treated methanol extract dissolved in water (Fraction A) and a subfraction prepared by chloroform–methanol extraction (Fraction B). Fraction A was active as an analgesic in rats and significantly reduced blood sugar levels in rabbits. Fraction B had CNS depressant activity. Data obtained with a treadmill indicated a decreased activity ratio by Fraction B, suggesting a possible interference in motor coordination. It potentiated the barbiturate sleeping time significantly without changing the onset time or the loss of the righting reflex. In addition, Fraction B exhibited a moderate antitremorine activity by causing a delay in the onset and a decrease in the severity of tremorine-induced tremors. The local anesthetic action of Fraction B was evident due to the complete blockade of the isolated frog sciatic nerve conduction.

Keyphrases ☐ *Quercus infectoria*—extracts of galls, analgesic, antidiabetic, and CNS depressant activity screened, mice, rats, and rabbits ☐ Analgesic activity—*Quercus infectoria* extracts of galls screened, mice, rats, and rabbits ☐ Antidiabetic activity—*Quercus infectoria* extracts of galls screened, mice, rats, and rabbits ☐ CNS depressant activity—*Quercus infectoria* extracts of galls screened, mice, rats, and rabbits

Quercus infectoria (Fagaceae) is a commonly available plant in Iran. Its bark is used for treating eczema and impetigo (1). Its galls are reported to contain ellagic acid (1), gallotannins (2), sitosterol (3), methyl betulate (3), and methyl oleanolate (3). Other species of *Quercus* have hypoglycemic, antiviral, and anticholinergic activities (4). Leaves of certain species of *Quercus,* such as *Q. sessiliflora, Q. cerris,* and *Q. pedunculate,* have been reported to cause nephrosonephritis and glomerulonephritis in calves (5). Poisoning by *Q. havardi* also was reported (6). Therefore, it was considered of interest to study *Q. infectoria* pharmacologically, and work was undertaken on the galls of this species.

EXPERIMENTAL

Isolation and Fractionation—The dry, powdered galls of the plant were cold percolated four times with methanol, and the combined extracts were freed of the solvent under reduced pressure at a temperature not exceeding 50°. The semisolid residue thus obtained was dissolved in acetone and filtered to remove a small quantity of insoluble material.

The clear filtrate was distilled in a vacuum rotary evaporator at 50° to remove the solvent. The pale-yellow material thus obtained was again dissolved in acetone and filtered, and the filtrate was freed of the solvent under reduced pressure and temperature. The resulting pale-yellow amorphous powder, which is a partially purified material, is called Fraction A.

Fraction A was subjected to further fractionation by dissolving it in water and extracting four times with chloroform–methanol (2:1). The organic extracts were combined, dried over anhydrous sodium sulfate, treated with charcoal, and filtered. The clear filtrate was freed of the solvent under reduced pressure and temperature, and a pale-yellow amorphous solid (Fraction B) was obtained. The aqueous phase was further fractionated into 1-butanol (Fraction C) and ethyl acetate (Fraction D).

During this study, the isolation of Fraction B was carried out on three different occasions. Each time, Fraction B was subjected to TLC. The R_f values were identical in all cases, confirming the chemical identity and the stability of Fraction B.

During isolation and fractionation, the temperature was never allowed to increase beyond 50° to avoid any decomposition. TLC [chloroform–methanol–petroleum ether (8:1:1)] of the three fractions (B, C, and D) gave three spots with R_f values of 0.09, 0.214, and 0.6, but the intensity of the spot at R_f 0.6 was much higher in Fraction B than in Fractions C and D. Fractions C and D and the aqueous phase were also tested, but the pharmacological activity was negligible.

Fraction B was crystallized from hot water, mp 130–190°, but it was impure and the TLC pattern was the same as before crystallization. Aromatic carboxylic acids like gallic, ellagic, and syringic acids have been obtained from other species of *Quercus* (7) and may be present in this fraction. Fraction B gives a deep-blue color with ferric chloride solution, indicating that it may also contain aromatic carboxylic acids.

The IR spectrum of Fraction B showed peaks for hydroxyl, carbonyl, and aromatic groups, showing the aromatic carboxylic acid nature of the compounds. The IR spectrum of Fraction B was compared with the known constituents of *Quercus* species, but it was not identical to any one of them.

The concentration of Fraction B with respect to the dry plant was 1%; with respect to Fraction A, it was 8%.

Pharmacological Methods—In most of these experiments, mice (white Swiss, Charles River strain) of both sexes were used once; they were previously untreated with any drug and permitted to feed *ad libitum.* However, female rabbits (albino strain) were used in testing the hypoglycemic activity of Fraction A. Adult Sprague–Dawley rats of either sex, 200–225 g, were used for analgesic experiments.

Analgesic Activity of Fraction A in Rats—Fraction A was tested for its possible analgesic activity using the rat tail-flick test. This test was carried out using a modification of the procedure of Dewey *et al.* (8). A 6-sec cutoff time was established for experimental conditions. Reaction time was measured to the nearest 0.10 sec by an electric stopwatch synchronized with the lamp.

Ten rats were used at each dose level and were selected at random. The average of two control readings taken 30 min apart constituted the control reaction time. Any animal for which the average of two controls was greater than 3 sec was discarded. Twenty minutes after the second control reading, Fraction A (30, 100, and 200 mg/kg) was administered intraperitoneally. The concentration of Fraction A was adjusted so that 0.75 ml/100 g was always injected.

Any increase in reaction time was noted at 30, 60, 90, and 120 min after the administration of Fraction A. Results were evaluated by the Wilcoxon Signed Rank Test (9) at each time, using 10% likelihood as the cutoff point.

Hypoglycemic Activity of Fraction A in Rabbits—Female rabbits, 1.5–2.5 kg, were used. The blood sugar determinations were carried out according to the Folin Wu method (10). Fraction A (500 mg/kg) was administered orally by means of a rubber catheter to two groups of four rabbits each. The results were compared with a control group that received normal saline orally and a group that received tolbutamide orally, the standard oral hypoglycemic agent (250 mg/kg).

Blood samples were drawn from the marginal ear vein of animals just before and at 30, 60, 120, 180, 240, and 300 min after Fraction A administration. Blood sugar levels in the control and test animals were then subjected to statistical treatment (Student t test) to determine any significant hypoglycemic activity of Fraction A.

Sedative–Hypnotic Activity of Fraction B in Mice—The central nervous system (CNS) depressant activity of Fraction B was determined by observation of its effect on the spontaneous motor activity and the righting reflex of 20–30-g mice. Fraction B was given intraperitoneally as a suspension in normal saline. The degree of CNS depression, as evidenced by reduction in spontaneous motor activity, was subjectively graded and compared with that of the control animals, who were given normal saline only.

The effect of Fraction B was described in the following terms. When 100% of the normal animals equalled the control animals in spontaneous motor activity, the effect was considered nil. When 50% or more of the test population showed a slight or intermediate decrease in

activity, the effect was considered slight or intermediate. When 50% or more of the test animals showed a loss of the righting reflex, the loss of spontaneous motor activity was considered marked. For each dose (250, 500, and 1000 mg/kg), 10 control and 10 test mice were used.

Experiments Using Treadmill[1] for Mice—Mice, 20–25 g, were used. Fraction B, 100 mg/kg ip, was administered to the test group; the vehicle was administered to the control. Control and test experiments were based on two groups of five mice each. All animals were acclimated to the treadmill by placing them on the mill two or three times just before the start of the experiment. Only five mice (the maximum number that could be placed on the treadmill) were used at one time, and a cutoff time of 180 sec was established for both the control and test groups.

The animals were placed on the mill 5 min before and 15, 30, 45, 60, 90, and 120 min after the administration of the drug or vehicle. At the end of the 120-min experimental period, the data were computed to obtain the activity ratio. The computation involved division of time in seconds that the test animals stayed on the mill by the control time. A ratio of one or nearly one was obtained in control experiments. A ratio of less than one indicated possibly a certain degree of precise motor incoordination. The degree of motor incoordination, therefore, was reflected by the extent to which the activity ratio fell below one.

Barbiturate Potentiation in Mice—Mice, 20–30 g, were employed, and Fraction B was administered intraperitoneally as already described. Pentobarbital sodium (50 mg/kg ip) was administered 30 min after Fraction B. For each test and control experiment, 10 mice were used. Animals that went to sleep were placed on their backs until spontaneous righting occurred. They were again placed on their backs until righting was effected within 5 sec, at which time the animals were considered to have regained their righting reflex. The measure of potentiation used was the ratio of (drug plus barbiturate sleep time) divided by (barbiturate sleep time plus drug sleep time).

Antitremorine Activity in Mice—Mice, 20–30 g, were used. Fraction B was administered at a dose of 500 mg/kg ip. Tremorine (20 mg/kg ip) in a 0.25% methylcellulose sterile vehicle was given 30 min after Fraction B. In control mice, the dose produced centrally mediated tremors along with signs of increased cholinergic activity such as salivation, lacrimation, diarrhea, and urination.

Subjective grading was used to establish the degree of protection against tremorine. To evaluate the degree of protection, animals were suspended by the tail. When the tremors were the same as for the controls, protection was rated as nil. For a slight reduction in tremor intensity, the degree of protection was considered as slight; for a slight tremor, protection was moderate. No tremor was rated as complete protection. For each control and test experiment, 10 mice were used.

Local Anesthetic Activity—The procedure employed to test the local anesthetic activity of Fraction B was the one applied by Rud (11) and Jefferson (12). The isolated sciatic nerve of the frog, 44–52 mm in length, was the test object. It was mounted in a wax chamber (153 × 17 × 10 mm internal dimensions) in such a manner that the nerve, while hanging on the two wire electrodes, was fully bathed in Fraction B solution or frog Ringer solution. However, the internal dimensions of the portion of the wax chamber that held Fraction B or frog Ringer solution were 13 × 17 × 14 mm. Fraction B was prepared in two different concentrations, 2 and 4% in frog Ringer solution, and the pH was maintained between 6.5 and 7.0.

Recording of the action potentials was by means of a dual-beam oscilloscope[2], and the nerve was excited by the stimulator[3]. All stimulations were for 0.1 msec, and the voltage was 0.4 mv above that required for the maximal control action potential. The induced action potentials were recorded simultaneously from electrodes placed on each side of that portion of the nerve exposed to the Fraction B solution.

The percent reduction of the action potential at different time intervals after bathing the isolated nerve in Fraction B solution was determined. In addition, the time after which the action potential was completely abolished, indicating a complete nerve blockade, was recorded. At each drug concentration, six isolated frog nerves were employed. Control experiments in which isolated nerves were bathed in frog Ringer solution only were carried out in a similar manner.

[1] Rota Rod treadmill type 7600, Ugo Basile, Milan, Italy.
[2] Type 565, Tektronix, Inc., Portland, Ore.
[3] Type S8, Grass Medical Instruments, Quincy, Mass.

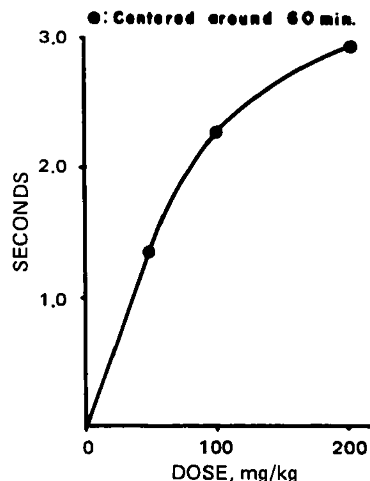

Figure 1—*Analgesic action of Fraction A. Each point represents an average increment in cutoff time in 10 rats after intraperitoneal administration.*

RESULTS AND DISCUSSION

When using Fraction A, data obtained from the analgesic and hypoglycemic experiments were statistically significant.

Analgesic Activity—The time increment in seconds observed due to the administration of Fraction A was plotted against the dose of Fraction A (Fig. 1). It is evident that the test drug is active as an analgesic (administered intraperitoneally) in a subsedative dose range that does not show any effect on the gross behavior of the animal. The onset of the analgesic activity occurred within 30 min and lasted for over 120 min. The peak analgesic action was around 60 min after drug administration. The action started declining after this period but could still be measured up to 120 min after Fraction A administration. The analgesic component of Fraction A has not been isolated.

Hypoglycemic Activity—From the experimental data, it appears that Fraction A possesses hypoglycemic activity. The effect of Fraction A on lowering the blood sugar was significantly greater than that of the control. However, the blood sugar did not fall much below the normal range of 80–120 mg %. The percent reduction in the blood sugar by Fraction A was 25.44, as compared with 47.43 produced by tolbutamide and 3.40 as seen in control animals.

The hypoglycemic action of Fraction A could be seen from the 3rd hr after its administration. The peak hypoglycemic activity was observed between 4 and 5 hr, after which it started returning toward the preadministration level. The reduction in blood sugar produced by Fraction A and tolbutamide was statistically significant ($p < 0.05$ and $p < 0.01$, respectively) (Table I), while the difference between the pre- and posttreatment blood sugar levels was not significant for control

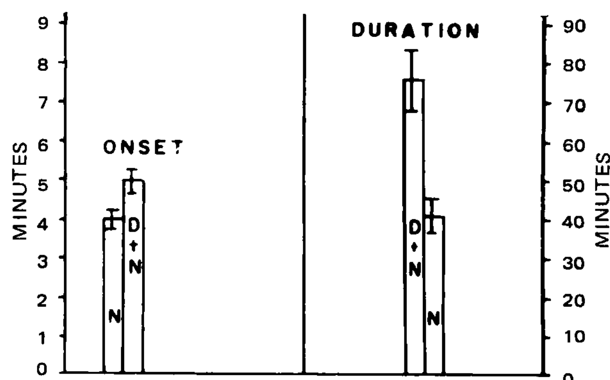

Figure 2—*Effect of Fraction B pretreatment on the hypnosis produced by pentobarbital in mice. Vertical bars represent the standard error of the mean. Key: N, saline (injection volume same as that of Fraction B) followed 30 min later by pentobarbital (50 mg/kg); and N + D, Fraction B (500 mg/kg) followed 30 min later by pentobarbital (50 mg/kg).*

Table I—Effect of Fraction A on the Blood Sugar Levels in Rabbits

Drug	Mean of Initial Blood Sugar, mg %	Mean of Lowest Blood Sugar Levels after Drug Administration, mg %	Percent Reduction	t Value
Tolbutamide, 250 mg/kg po	124.50 ± 3.23[a]	65.45 ± 2.11	47.43	$p < 0.01$
Control	111.50 ± 2.39	107.70 ± 3.86	3.40	NS[b]
Q. infectoria, 500 mg/kg po	119.70 ± 4.34	89.25 ± 3.04	25.44	$p < 0.05$

[a] *SEM*. [b] Not significant.

animals. Peak hypoglycemic action was attained between the 3rd and 4th hr after tolbutamide administration, after which the blood sugar gradually returned to the normal level. However, unless additional studies are carried out, it will be difficult to state the antidiabetic status of Fraction A as well as to explain the mechanism of its hypoglycemic action.

Sedative–Hypnotic Activity—At a dose level of 1000 mg/kg, Fraction B significantly reduced the spontaneous motor activity of animals. The animals lost their righting reflex within the average period of 5 min after Fraction B administration. However, at a dose of 500 mg/kg, Fraction B caused an intermediate loss of spontaneous motor activity; the animals did not lose their righting reflex but exhibited some sedation with a significant reduction in spontaneous activity. Fraction B produced only a slight reduction in spontaneous motor activity at a dose of 250 mg/kg. The data suggest a dose-dependent depressive action of Fraction B on the CNS, perhaps through the inhibition of sensory inputs into the brain.

Further insight into the action of Fraction B on the motor functions of the animals was obtained by experiments using the treadmill for mice. The data obtained indicate that Fraction B at a dose level of 100 mg/kg significantly reduced the cutoff time of animals after about 60 min until 120 min postadministration. The decrease in cutoff time, as compared to the control group, possibly suggests that Fraction B causes motor incoordination. A dose of 200 mg/kg or above caused so much motor incoordination that the animals could hardly stay on the treadmill.

Thus, a dose much smaller than the sedative–hypnotic dose significantly disturbed the motor functions of the animals, as measured by the treadmill, without any visible sedative–hypnotic action. The motor incoordination produced by Fraction B probably is due to its action on the higher areas of the brain and seems to suggest that, at a lower concentration, Fraction B is more specific to higher CNS areas only.

Barbiturate Potentiation—The locus for barbiturate potentiation appears to be functionally different from the sites involved in the directly measured depressant effect (13). Fraction B was tested for possible pentobarbital potentiation activity at a dose of 500 mg/kg; a sleep prolongation factor, R, was used as the criterion for effect (14). The significance of these data was determined by the use of Duncan's multiple range test (15).

As is evident from Fig. 2, Fraction B at the employed dose level acted both as a significant potentiator ($p < 0.005$) of pentobarbital as well as a CNS depressant. The value of R was 3.4. Nevertheless, the possibility that Fraction B causes potentiation of barbiturate sleeping time *via* the inhibition of the liver microsomal enzyme system cannot be excluded. Consequently, a definite conclusion regarding the mechanism of barbiturate potentiation cannot be made.

Local Anesthetic Activity—The data obtained (Fig. 3) indicate that Fraction B is potent as a local anesthetic. The action potential was completely abolished within an average of 7 min when the isolated nerve was placed in a 4% solution of Fraction B. However, in a 2% concentration, there was a significant reduction of the action potential but not complete nerve blockade. With the available data, it is difficult to explain the mechanism of complete nerve blockade, except that Fraction B perhaps interferes with nerve membrane permeability.

Antitremorine Activity—Fraction B also was tested for its ability to antagonize tremorine-induced peripheral parasympathetic stimulation and parkinsonian-like tremors of central origin (16, 17). At a dose of 500 mg/kg, Fraction B exhibited moderate antitremorine activity, shown by 60% of the animals, but none of the animals was completely protected. There was a slight degree of protection in 40% of the animals. Fraction B, therefore, could be classified as moderately active, since a highly active compound should be able to protect 100% of the test animals completely at a dose much lower than used to test this compound. Standard agents such as atropine, scopolamine, and trihexyphenidyl block tremorine effects in doses of 5–10 mg/kg in mice. Fraction B showed very little protection against tremorine-induced peripheral parasympathetic stimulation.

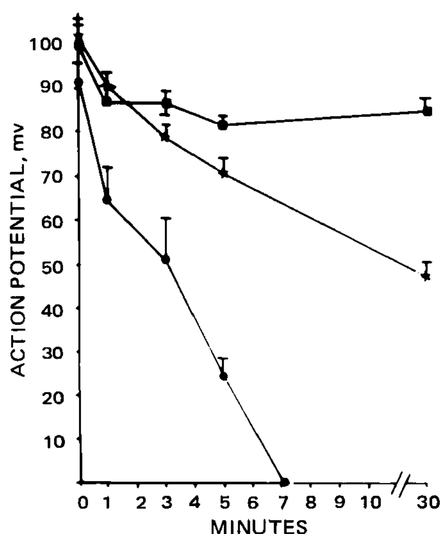

Figure 3—*Local anesthetic activity of Fraction B. Each point represents an average of six frog nerves. Vertical bars represent the standard error of the mean. Key: ■, control (frog Ringer); ★, drug (2% solution); and ●, drug (4% solution).*

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Potentiometric and Spectral Investigations of Anhydrotetracycline and Its Metal-Ion Complexes

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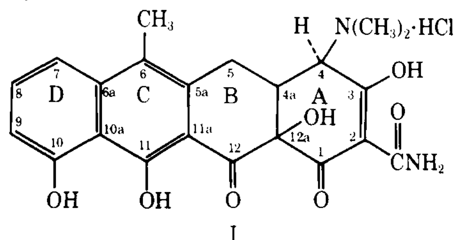
Abstract □ The interaction of various metal ions with anhydrotetracycline was investigated. A comparison of the UV–visible and fluorescence spectral characteristics of anhydrotetracycline and its metal-ion complexes with a number of modified anhydrotetracyclines in the presence of metal ions suggested that the C-11 oxygen was involved in metal-ion binding. Secondary binding was observed in the A-ring by circular dichroism when the primary site was blocked.

Keyphrases □ Anhydrotetracycline—and various metal-ion complexes, potentiometric and spectral investigation of binding properties □ Metal-ion complexes, various—with anhydrotetracycline, potentiometric and spectral investigation of binding properties □ Complexes, metal ion, various—with anhydrotetracycline, potentiometric and spectral investigation of binding properties □ Spectrometry, UV–visible, fluorescence, and circular dichroism—investigation of binding properties of anhydrotetracycline with various metal ions □ Potentiometry—investigation of complexation of anhydrotetracycline with various metal ions □ Antibiotics—anhydrotetracycline, complexation with various metal ions, potentiometric and spectral investigation

As a result of the extensive use of tetracyclines, several adverse side effects have been reported (1). Many of these side effects have been traced to impurities in the tetracycline preparation, as in the case of the "reversible Fanconi-type syndrome," which is thought to be induced by anhydrotetracycline (I) and 4-epianhydrotetracycline (1, 2). Tetracycline degradation in an acidic medium was investigated previously, and the principal degradation products were 5a,6-anhydrotetracycline and 4-epitetracycline (3).

Treatment of tetracycline with warm mineral acid results in the loss of a molecule of water from the C-5a and C-6 positions, forming anhydrotetracycline (4). X-ray diffraction studies revealed that the bond lengths of anhydrotetracycline agree with those of tetracycline, except for the C-ring and adjacent bonds. The anhydrotetracycline molecule was more planar in the C–D-ring region than its tetracycline parent (5). A slightly different conformation of the A-ring of anhydrotetracycline also was revealed. The nitrogen of the C-4 position was reported to be closer to the C-12a oxygen than to the C-3 oxygen in anhydrotetracycline (5). All active tetracyclines were reported to have the same conformation of the A-ring, with the C-4 nitrogen closer to the C-3 oxygen than to the C-12a oxygen (6, 7).



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This investigation concerned the complexing properties of anhydrotetracycline, with particular emphasis on the determination of the complexation sites in this molecule. The functional groups proposed as binding sites in tetracycline remain in anhydrotetracycline and are considered possible binding sites of this compound. On the A-ring, these sites include a carbonyl and hydroxyl group at the C-1 or C-3 position, the amide group at the C-2 position, and the dimethylamine group at the C-4 position. The C-10–C-12 position carbonyl and hydroxyl groups of the B-, C-, and D-rings are also considered possible complexation sites.

While the UV region of both tetracycline and anhydrotetracycline contains multiple chromophore absorptions, the visible region of anhydrotetracycline exhibits a single absorption at 430 nm. This absorption is assigned to the $\pi \rightarrow \pi^*$ transition of the B–C–D-ring chromophore of anhydrotetracycline. In the presence of certain metal ions, this absorption band undergoes a bathochromic shift, strongly suggesting participation of this region of the molecule in complexation. However, the degree of intramolecular transfer of complexation effects is not known.

To establish the primary binding site(s) of metal ions to anhydrotetracycline, some modified anhydrotetracyclines were prepared. The effects of complexation within the A-ring were observed by comparison of the spectral properties of anhydrotetracycline with those of 4-dedimethylaminoanhydrotetracycline and 2-cyanoanhydrotetracycline. Possible complexation involving the C-10 hydroxyl was removed when 10-benzenesulfonyl-2-cyanoanhydrotetracycline was used; finally, any complexation through the C-11 hydroxyl was blocked using 4-dedimethylamino-11-methoxyanhydrotetracycline.