Investigation of the Anti-inflammatory Activity of Acacia nilotica and Hibiscus sabdariffa

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Abstract: The aqueous extracts of Acacia nilotica and Hibiscus sabdariffa were tested for anti-inflammatory, analgesic and antipyretic activities in animal models. Acacia nilotica extract had an inhibitory effect on carrageenan induced paw edema and yeast-induced pyrexia in rats. It also produced a significant increase in the hot plate reaction time in mice. Hibiscus sabdariffa extract had no effect on paw edema but had an inhibitory effect on yeast induced pyrexia and a significant effect on the hot plate reaction time. Among the phytoconstituents found in both plants, flavanoids, polysaccharides and organic acids may be mainly responsible for their pharmacological activities.

Acacia nilotica Delile (Leguminosae) is a spiny tree about 3 meters high growing in the lowland plains of western and central Sudan and some parts of Africa and Arabia. Its flat brown pods locally known in the Sudan as “Garad” are widely used in folk medicine for treatment of many conditions. Aqueous extracts of powdered pods are taken orally for treatment of diarrhea, cough and fever (Al-Gazali et al., 1987). Fumes evolving from heated pods are inhaled for treatment of common cold and influenza. Powdered pods are applied topically to various parts of the body to decrease body temperature and to bleeding sites to stop hemorrhage. Chemical analysis of the pods revealed the presence of tannins, the most important of which are gallic acid (3,4,-5-tetrahydrobenzoic acid, m-digallic acid; catechin and some galloylated flavans (Goodwin and Nurstem, 1973; El-Sayyad, 1983; Gupta et al., 1981; Gupta and Bokadia 1975). In vitro studies using the pods’ tannin have shown that it has an antiviral and antifungal activity against Piricularia oxyzae (Fischer et al., 1954; Baruah et al., 1963).
Hibiscus sabdariffa Linne (Malvaceae), an attractive plant believed to be native to Africa, is cultivated in central and western Sudan and other tropical countries. The plant is about 1 meter high and is characterized by purple stems, leaves and branches. The calyx is fleshy and reddish-purple in color. The seeds are black brown (Blatter et al., 1975). In the Sudan, the dried calyces locally known as ‘’Karkade’’ are used for treatment of common cold, cough and throat infections. The chemical composition of the calyx has been extensively studied. It has been reported to contain a number of organic acids including citric acid and ascorbic acid (Reaubourg and Monceause 1940; Kerharo, 1971); sugars, mainly galactose, glucose and fructose, (El-Afry et al., 1980; El-Hamidi et al., 1966); pectin and flavonoids (Koepfli, 1932). \textit{In vitro} experimental studies have shown that administration of the aqueous extract of the calyces produces antispasmodic (Ali et al., 1991), hypocholesterolaemic (El Saadany et al., 1991), hypotensive (Esselene and Sammg, 1973), diuretic and antibacterial effects (Leclere, 1938). In spite of the wide use of \textit{A. nilotica} pods and \textit{H. sabdariffa} calyces in folk medicine for treatment of inflammatory conditions, to the best of the authors’ knowledge there are no reports in the literature on their anti-inflammatory activity. The purpose of this study is to establish if they possess any anti-inflammatory analgesic, or antipyretic activities in experimental animals and rationalize their therapeutic use.

\textbf{Materials and Methods}

\textit{Animals}

Albino Wistar rats and Albino Swiss mice of either sex fed on standard chow diet and water were obtained from the Animal House, College of Medicine, King Faisal University and used for the study.

\textit{Preparation of extracts}

Dry \textit{A. nilotica} pods and \textit{H. sabdariffa} calyces were purchased from a local dealer and ground to a fine powder. 12.5 g of each powder was extracted overnight in 100 ml distilled water at room temperature. The crude extracts were centrifuged at 3000 g for 10 minutes. The supernatant was collected and kept in stoppered glass tubes at 8° C.

\textit{Anti-inflammatory activity in rats}

Inflammation was produced in the rats according to the method of Winter et al. (1962). 0.05 ml of 1% carrageenan sodium salt (BDH) suspension was injected in the right hind foot of each rat under the plantar aponeurosis. 2 test groups of 6 rats each were treated orally with 1 ml (500 mg/kg body weight) of the aqueous extract of \textit{A. nilotica} and \textit{H. sabdariffa} respectively 1 hour before the carrageenan injection. The control group was given 1 ml normal saline and a standard reference group was treated with 1 ml aqueous solution of aspirin (100 mg/kg body weight). Measurements of foot volume were performed by the displacement technique using a calibrated glass tube immediately before and 3 hours after the injection of carrageenan. Inhibitory activity was calculated according to the following
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formula:

\[
\text{Percentage Inhibition} = 100 \left( 1 - \frac{a-x}{b-y} \right)
\]

where \(a\) is the mean paw volume of treated rats after carrageenan injection, \(x\) is the mean paw volume of treated rats before carrageenan injection, \(b\) is the mean paw volume of control rats after carrageenan injection and \(y\) is the mean paw volume of control rats before carrageenan injection.

Analgesic activity in mice

The hot plate method described by Turner (1965) was used. The animals were dropped gently on a hot plate maintained at 55 ± 0.5°C. Reaction time was taken as the interval extending from the instant the animal reaches the hot plate till the moment the animal licks its forepaws or jumps out. Measurements were made 10 and 5 minutes before oral administration of the extracts and at 30, 90 and 150 minutes thereafter. 2 test groups of 12 mice each were treated orally with 1 ml (500 mg/kg body weight) of \(A.\) nilotica and \(H.\) sabdariffa extracts respectively. The control group was given 1 ml normal saline, and a standard reference group was treated with 1 ml (100 mg/kg) aqueous solution of aspirin. Each result was calculated as a mean of three readings.

Antipyretic activity in rats

Pyrexia was induced in the rats by subcutaneous injection of 10 ml/kg body weight 15% yeast solution. The rectal temperature of each animal was recorded before and 24 hours after the yeast injection. 2 test groups of 5 rats each were treated orally with 1 ml (500 mg/Kg body weight) of \(A.\) nilotica and \(H.\) sabdariffa extracts respectively. The control group was given 1 ml normal saline and a standard reference group was treated with 1 ml aqueous solution of aspirin (100 mg/kg body weight). The measurements of rectal temperatures of each animal was repeated 45, 90 and 150 minutes after treatment. Each result was calculated as a mean of three readings.

Statistical Methods

Statistical analysis of the results of each study was performed by Student’s t-test for independent values.

Results

The study of the anti-inflammatory activity showed that \(A.\) nilotica produced a reduction in carrageenan induced paw edema in rats that was comparatively less than that produced by aspirin. The calculated mean percentage inhibition of paw edema produced by \(Acacia\) was 20% compared to 47% for aspirin. A similar dose of \(H.\) sabdariffa aqueous extract had no inhibitory effect on paw edema (Figure 1).
Results of the hot plate experiment showed that *A. nilotica* produced a significant increase in reaction time, which was maximum 90 minutes post treatment. Aspirin produced its maximum effect on reaction time 30 minutes post treatment. *H. sabcarijfa* caused prolongation of reaction time but was less than that produced by *A. nilotica*. (Figure 2).

The study of antipyretic activity revealed that aqueous extracts of both *A. nilotica* and *H. sabcarijfa* produced a reduction in rectal temperature of the tested rats that was comparatively less than that produced by aspirin, but *Hibiscus* had a greater effect than that produced by *Acacia* (Figure 3). However, this reduction in rectal temperature did not reach statistical significance for both extracts.

**Discussion**

The results of these studies indicate that the aqueous extract of *Acacia nilotica* possesses significant analgesic activity as well as some antipyretic and anti-inflammatory action, which lends support to the wide use of this plant in traditional medicine for treatment of inflammatory conditions. Moreover, the results show that in the hot plate experiment, *Acacia* had a more pronounced effect on reaction time than either aspirin or *Hibiscus*. These activities can be due to the presence of flavonoids and other phenolic groups reported in the literature to possess such activities (Gabor, 1972; Parmar and Ghosch, 1980). The apparent delayed
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Figure 2. Effect of Acacia (open triangle), 500 mg/kg, Hibiscus (open square) 500 mg/kg and aspirin 100 mg/kg (open circle) on hot plate reaction time in mice compared with the control group (closed circle).

Figure 3. Effect of Acacia (open triangle) 500 mg/kg Hibiscus (open square) 500 mg/Kg on yeast-induced pyrexia in rats compared to the controls (closed circle).
effect of *Acacia* on reaction time and rectal temperature may be due to the fact that it was given as a crude extract, which had a slower rate of absorption and/or may have required metabolic activation. Further studies are needed to isolate individual chemical components and pinpoint their pharmacological activities. However, the results of this study substantiate the reported use of this plant in folk medicine.

The aqueous extract of *Hibiscus sabdariffa* was devoid of any anti-inflammatory activity as shown by the carrageenan induced paw edema experiment, but its effect on reaction time was similar to that observed with aspirin suggesting the presence of analgesic activity. In addition, *Hibiscus* produced a reduction in the rectal temperature of febrile animals. These activities could be attributed to flavanoids (Gabor 1972, Parmar and Ghosch 1980) and organic acids like ascorbic acid and citric acid as well as polysaccharides reported to be present in this plant. Muller and Franz (1992) reported the isolation of three polysaccharides from *H. sabdariffa* that showed immune-modulating effects. The exact role of each one of these components in the pharmacological activity of *Hibiscus sabdariffa* requires further studies.

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**References**

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