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HISTOPATHOLOGICAL AND MICROBIOLOGICAL STUDIES ON THE CRUDE EXTRACT OF *PERSICARIA STAGNINA* AND ITS CHIEF CONSTITUENT STAGNINOL

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Abstract: Histopathological studies of stagninol, a sesquiterpene isolated from *Persicaria stagnina* and the crude extract of this plant were carried out on Wistar rats. High dose of the crude extract caused liver necrosis but structural integrity of kidney and spleen remained intact. Low dose of the crude extract and the pure compound stagninol were found to cause no degenerative changes on those organs. Microbiological studies revealed that both the crude extract and stagninol had no inhibitory effect on the growth of a number of bacteria tested.

Keywords: Histopathology; *Persicaria stagnina*; Stagninol; Antimicrobial effects

Introduction

Persicaria stagnina Linn. (Fam. Polygonaceae) locally known as Bishkatali is an erect or ascending annual herb grown mostly in Bangladesh, India, Pakistan, Bhutan and Srilanka in Asia and also in Europe and North America. Extracts of this plant are used in folk medicine. It is considered to be an astringent in Europe and used as a vulnerary and lithontriptic. In Norway, the juice of the plant is introduced into cavities of decayed grinders to relieve pain (Kirtikar and Basu, 1980). The piscicidal and molluscidal activities of this plant have recently been reported (Balza *et al*. 1984). Chemical study on this plant has shown that it contains the hemiacetal type sesquiterpene stagninol, chemically being the 2α, 3β ditigloyloxy derivative of isodrimeninol (Fig. 1) (Ahmed *et al.,* 1991).

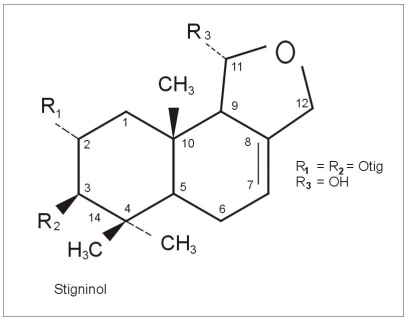

Fig. 1. Structure of stagninol.

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Pharmacological studies on this plant reveals the significant anti inflammatory, analgesic and diuretic activities (Ahmed *et al.,* 1997). The objective of this research work is to reveal the toxic effects on different organs and its antibacterial effects.

Materials and Methods

Isolation of Stagninol: *Persicaria stagnina* was collected from Dhaka and identified by the National Herbarium of Bangladesh. 4 kg powder of dried whole plant was extracted with methanol. The extract was evaporated to dryness and defatted. The resulting extract was separated by column chromatography (silica gel) using petroleum ether, diethyl ether and methanol with increasing polarity. The crude fractions were subsequently separated and purified by repeated TLC to give about 200 mg stagninol. The compound under investigation was characterized and identified by comparing its Rf value and spectral data with those of reference compound (Ahmed *et al.,* 1991).

Histopathology: Wistar rats (65-75gm) of either sex obtained from the animal resources branch of ICDDR,B Dhaka were used. The rats were divided into five groups I, II, III and IV (Experimental) and C (Control) and each group consists of five, all being randomly selected. All the animals were fasted overnight prior to the start of the experiment. Both stagninol and the crude extract were dissolved in propylene glycol to be administered orally to the experimental animals . Stagninol was administered at doses of 10 and 30mg/Kg body weight to group I and II and the extract was administered at doses of 250 and 500mg/Kg body weight to group III and IV. The test materials were applied once regularly early in the morning for 21 days. The animals were observed carefully to see any change in behavior for 2-4 hours. At the end of the experiment, the body weights were taken and subsequently the animals were sacrificed to perform the histopathological investigations according to the methods applied by Precess (Precess 1965).

Microbiology: The antimicrobial effects of stagninol and the crude extract were measured in vitro by the agar disc diffusion technique (Bauer *et al*. 1966). The test organisms in pure culture were collected from the Institute of Nutrition and Food Science, University of Dhaka. With the help of a sterile loop, a small amount of bacterial culture from the supplied nutrient agar culture slant was inoculated into 100ml of sterilized nutrient broth in 250ml conical flask and kept at 37°C in a slow shaking incubator overnight. After 24 hours of growth, the broth culture of bacteria was ready for use as inoculum for antimicrobial sensitivity test.

Paper discs (Diameter 6 mm; Toyo Seisakusho Company, Japan) were sterilized in an autoclave at 120°C for 15 minutes. Both stagninol and the crude extract were dissolved in methanol to get the concentration of 300µgm/0.05ml and 2mg/0.05ml respectively. With the help of micropipette, 0.05ml of the sample solution of stagninol and the crude extract were applied on the discs slowly. The solvent from the discs were evaporated by blowing mild hot air. Standard antibiotic discs (Streptomycin 100µgm/disc, Hi media Lab Pvt. Ltd. India) were used as control. 20ml of sterilized nutrient agar (40-50°C) was poured into each petridish containing 0.1ml of 24 hours broth culture of bacteria (inoculum) under test as prepared previously. Sample discs and standard antibiotic discs were placed on the seeded agar plates. All the plates were kept in a refrigerator for 5-6 hours for complete diffusion. They were then incubated at 37°C for 24 hours. All the determinations were carried out in triplicate, and average zones of inhibition were recorded.

Results and Discussion

Results of histopathological studies indicated major structural changes of liver after administration of the crude extract at a dose of 500mg/Kg body weight but the structural integrity of kidney and

spleen remained normal. Administration of low dose of crude extract and stagninol at two different doses, the structural integrity of liver, kidney and spleen appeared to be normal (Table 3.1). These histopathological findings indicated about the damaging effect of crude extract at high dose on liver cells but the pure compound stagninol and even the crude extract at low dose were found to cause no degenerative changes. Thus it may be concluded that though the crude extract is hepatotoxic, one of its components stagninol is devoid of toxicity.

Table 1. Effect of stagninol and the crude extract of *Persicaria stagnina* at different doses on liver, kidney and spleen.

Animal group	Dose mg/Kg body weight	Effect on Liver	Effect on Kidney	Effect on Spleen
C	0	Normal	Normal	Normal
I	10	Normal	Normal	Normal
II	30	Normal	Normal	Normal
III	250	Normal	Normal	Normal
IV	500	Degeneration in the lobule	Normal	Normal

The *in vitro* antimicrobial study was designed to investigate the antibacterial spectrum of stagninol and the crude extract. Results of this study indicated that both stagninol and the crude extract have no antibacterial activity against any of the pathogenic bacteria tested (Table 3.2).

Table 2. *In vitro* Antibacterial activity of Stagninol and the crude extract of *Persicaria stagnina*.

Bacterial Strain	Zone of Inhibition (Diameter in mm)		
	Streptomycin (100µgm/disc)	Stagninol (300µgm/disc)	Crude Extract (2mg/disc)
Vibrio cholerae	22	00	00
Eschericia coli	20	00	00
Shigella dysentrica	19	00	00
Salmonella typhi	18	00	00
Salmonella paratyphi	17	00	00
Shigella flexneri	19	00	00
Streptococcus pyogenes	18	00	00
Streptococcus faecalis	20	00	00
Staphylococcus aureus	19	00	00
Sarcina lutea	19	00	00
Bacillus cereus	19	00	00
Bacillus subtilis	20	00	00

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