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RESEARCH ARTICLE

BIO ASSAY GUIDED EVALUATION OF HERBAL DRUGS AND MEDICINAL PLANTS

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ABSTRACT

Many important drugs are derived from compounds originally discovered in plants,and there is now increasing interest in searching for new therapeutically useful molecules from natural sources.The naturally occurring compound will, in all likelihood, only provide the starting point for development of analogues,but the most important step in the discovery process is the identification of this source of interesting biological activity. Several approaches to discovery of activities are out lined below.The ethinobotanical route,Bioassay guided serendipity,Straight serendipity,Exploitation of chemotaxonomic knowledge, Investigations based on chemical ecology.The examples are taken from higher plants,but should be remembered that bio activemolecules can be found from many other source of biological material for investigation eg)pteridophytes, fungi, algae, marine organisms and animal venoms .

INTRODUCTION

The resource of biological material for investigation is virtually unlimited. With higher plants alone, current estimates are that the world contains between250,000 and350,000 species ,while in total there may be as many as 30

million species of all types. There fore, many plants may have the capacity for producing many more secondary metabolites than they normally do. The possibilities of stimulating quiescent synthetic pathways could lead to a further expantion of the natural product library.Prospects of herbal research:-There is a world wide "green" resolution, which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. F urthermore, underlying this upsurge of intrest in plants is the fact that many important drugs in use today were derived from plants(or)from starting molecules of plant origin.Digoxine/digitoxin,the vinka alkaloids,reserpine and tubocurarine are some important examples.Plants have also yielded molecules, which are extremely valuable tools in the characterization of enzymes and the classification of receptor systems where physostigmine,morphine,muscarine,atropine, nicotine and are important examples.

Virtually every human society evolved an indigenouns health care system to scope with illness.In western technologically advanced societies, traditional pre-science notions of the causes of disease and how to manage it have given way to modern ideas based on scientific bio medical theories .In the less technologically developed societies, traditional modes of through still dominates the forms of medical practice seen in tho socities.It is imperative that we do not ignore the thought behind these systems for 2 reasons:

1.First, it is the continued use of plants for the treatment of disease in these systems that have invigorated our interest in phytotherapy.

2.Second,the experience from cultural practices of medicine can often be of value in the bio medical scientist's search for understanding of complex aspects of healing.

Bio assay guided evaluation of Dioscorea villosa(DV):-

Dioscorea villosa (DV) has been used in Brazil as an alternative medicine to attenuate menopause symptoms, as well as for the treatment of joint pain and rheumatoid arthritis. In spite of the popular use of DV for the treatment of various disorders, there are limited scientific data regarding safety aspects of this herb. In this regard, we carried out to evaluated both antinociceptive and anti-inflammatory activities in experimental models and assess the toxic effects of the acute (single dose) and subchronic (30 days) oral administration of dry extract of Dioscorea villosa in rodents.

Methodology:-

The LC analyses were performed to assess the presence of the diosgenin in samples of DV.
The antinociceptive study of DV was performed

using models of acetic acid-induced writhing and formalin-induced pain in mice. The anti-inflammatory study was accomplished by leukocyte migration to the peritoneal cavity. A dry extract of DV was tested at doses of 100, 200 and 400 mg/kg (per os or p.o.). The toxicological properties of the dry extract were evaluated by toxicity assays of acute (5 g/kg, single dose) and subchronic (1 g/kg/day, 30 days) treatment. Haematological, biochemical, and histopathological parameters were studied. The results are expressed as mean ± S.D., and statistical analysis of the data were performed with the Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's test. In all cases differences were considered significant if $p < 0.05$.

Results

HPLC-DAD analysis of the extract from DV revealed the presence of diosgenin as the major compound. Doses of 200 and 400 mg⁄kg significantly reduced the amount of acetic acid-induced writhing in relation to the vehicle ($p < 0.0001$). In the first phase, using the formalin-induced neurogenic pain test, only the 400 mg/kg dose of DV showed significant inhibition of neurogenic pain ($p < 0.001$). In the second phase, 200 and 400 mg/kg of DV showed significant inhibition of inflammatory pain ($p < 0.0001$). Significant inhibition of leukocyte migration was observed with doses of 100 ($p < 0.001$), 200 ($p < 0.01$) and 400 mg/kg ($p < 0.01$). Haematological, biochemical and histopathological data obtained in both acute and subchronic toxicological assays revealed only unremarkable changes, which are unlikely to indicate DV toxicity with oral administration.We found that DV possesses antinociceptive and anti-inflammatory properties in rodent models. In addition, no acute or subchronic toxicity was evident when the herbal extract was administered orally. These results supporting the folkloric usage of the plant to treat various inflammatory diseases.

BIOASSAY GUIDED EVALUATION OF MEDICINAL PLANT (PSEUDELEPHANTOPUS SPICATUS)

Material and Methods

Collection of Plant Material: For the purpose of botanicalidentification, small branches or twigs with reproductivestructures, healthy leaves, stipules, bark and wood samples from each plant were collected in duplicate following accuratedocumentation. Mature *P. spicatus* leaves were collected from

.

Preparation of Plant Extract: For the preparation of the crude extract, about 2-3kg of plant or plant parts were cleaned bywashing with tap water followed by distilled water. The sample

was air-dried for about 2-3 weeks, and the dried samples werepulverized using a sterile electric

blender. The half portion ofthe pulverized leaves was soaked in pure absolute ethanol, and the other was soaked in 50:50 ethanol-water mixtures for threedays. Each solution was filtered with Whatman No. 1 filterpaper and concentrated at 40°-50°C under reduced pressure using the rotary evaporator21.For the preparation of the plant decoction, about 1 kg fresh and clean samples of the plant were cut into pieces and boiled insufficient amount of distilled water (1:2 ratio) for 5 minutes.The mixture was filtered, cooled and stored in glass containersand freeze-dried until all the water was removed to giveconcentrated decoction. It was then kept until required.Stock solutions were prepared. Thirty milligrams of driedsamples from decoction, crude ethanol extract and crudeethanol-water extract, were dissolved with 3000 ppm ethanol and distilled water respectively and then sonicated to dissolvethe dried samples. From the stock solution, 10, 100, 500, and1000 ppm concentrations were prepared by the addition of 5 ppm, 50 ppm, 250 ppm and 500 ppm of solution, respectively ina 20 mL test tube. Addition of a minimal amount of dimethylsulfoxide (DMSO) was done to completely dissolve the solution in each test tube.

Brine Shrimp Lethality Bioassay: Hatching of Brine

Shrimp: Brine shrimp (*Artemia salina*) lethality bioassay wascarried out to investigate the cytotoxicity of extracts ofmedicinal plants. Artificial seawater was prepared by dissolving 40 grams of natural table salt in every liter of distilled water.Sea water was kept in a small tank, and *A. salina* eggs wereadded to the divided tank21. Constant temperature (around 37° C) was maintained and constant supply of oxygen was carriedout. Brine shrimps were allowed to mature and hatch as naupliifor two days. The newly hatched shrimp was collected using a dropper.

Assay Proper: Ten brine shrimp larvae were introduced intoeach sample vials containing different concentrations of theextracts. Seawater was added to make a total volume of 5 ml. The vials were maintained under illumination. Survivors werecounted after 6, and 24 hours and the deaths at control and eachdose level were determined.

Lethal concentration Determination: After 6h and 24h, thelethal concentrations of the *P. spicatus* extract resulting to 50%mortality of the brine shrimp (LC50) were determined. Then, by

means of a trendline fit linear regression analysis (MS Excelversion 7) the dose-response data were transformed into astraight line. From the best-fit line obtained the LC50 wasderived.

Statistical Analysis: Reed-Muench statistical method was used to determine the relative toxicity of the *P. spicatus* extracts to living organisms. It was done by testing the response of *A.*

salina under various concentrations of the extract. LC50 represents the dose lethal to the half members of the A. salina.This was determined by plotting the mortality (y-axis) versus

log of concentration (x-axis). The concentration that rendered 50% mortality was the LC50.

Results and Discussion

Table 1 shows the toxicity of the *P. spicatus* extracts on the brine shrimp after 6 and 24-hour exposure. The extractsobtained from decoction and ethanol-water extract exhibited no

lethality on the brine shrimps at any of the concentrations at 6hand 24h. The brine shrimps were still actively moving, and nosigns of behavioral changes were observed. Crude plant extract

with LC50 value of less than 1000 ppm is toxic while non-toxic(inactive) if it is higher than 1000 ppm20. Since the LC50 in theboth of this extract taken from decoction and ethanol-water mixture was higher than 1000 ppm, it was considered inactive.It may be because the active components present in the *P.spicatus* were not extracted through the two methods mentioned above. Even though decoction process is economical due to itslow cost in terms of instrumentation and reagents21 it may be aninefficient process given that ingredients may be damagedduring the prolonged heating of substances, and otheringredients may be oxidized and lose activity 22. During thedecoction process, many aromatic herbs with high levels ofvolatile oils are easily lost through evaporation 23. Also, ethanolwatermixture extraction process was stillineffective and it is inaccordance with the previous study in which the alcohol/water

mixture (typically 20–40% alcohol) is actually a poor mediumfor extraction. It is because it causes the desired components tocondense out of the liquid therefore none is left in the finished product24. The ethanol extract of *P. spicatus* showed a toxicityeffect at 6h and 24h, with LC50 value at 944.07 and 266.07 ppm,respectively. This suggests that the extract could have

compounds that are cytotoxic as the LC50 value was lower than1000 ppm (table 1). The brine

shrimp mortality rate at differentconcentrations in the ethanol extract was found to increase with increasing concentration of the sample, and it clearly shows thatthe extraction with ethanol was a better way of obtaining *P.spicatus* extract bioactive components. The previous studies show that ethanolic extract of *P. spicatus* demonstrated strongbiological activity toward *Leishmania amazonensis*8. Ursolicacid and the two hirsutinolides (the 8-acetyl-13-O-ethylpiptocarpholand 8, 13-diacetyl-piptocarphol 8-acetyl-13-Oethyl-piptocarphol) isolated through phytochemical screeningmight be responsible for its pharmacological activities thus giving support to its use in Peru.