Studies of anticancer and antipyretic activity of *Bidens pilosa* whole plant

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Abstract

Screening of different extracts and fractions from the plant *Bidens pilosa* Linn. var. (Asteraceae) has been conducted using the in – vitro comet assay for anticancer and the antipyretic action, which was done with in – vivo models. The extract from whole plant was extracted with n – hexane, chloroform and methanol extract (E1 – E3). The extracts were fractioned by column chromatography method and fractioned with ethyl acetate, acetone and water (F1 – F3). All the extracts and fractions were tested for anticancer and antipyretic activity. Among extracts E1 shows remarkable anticancer activity and E3 bears maximum antipyretic activity. In the antipyretic activity, paracetamol was used as the standard test drug. The most promising material (LC50 < 1500 µg / ml) was F1 ethyl acetate fractions of methanolic extract and methanolic crude extract of whole plants. However, little correlation was observed in the degree of antipyretic activity between the test drug and standard drug. In conclusion, the extract obtained from the whole plant of *Bidens pilosa* showed a significant cytotoxic effect to methanolic extract against Hela cells by in vitro method and showed a comparable antipyretic activity effect to paracetamol in rabbit pyrogen test.

Keywords: Bidens pilosa, Comet assay, Antipyretic, Anticancer, MTT assay.

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Introduction

The use of bioassay offers various scientific strategies like screening of extracts, fractions and compounds obtained from plants, which are often used in phytochemical research. In the evaluation of in vitro methods for natural products the biological activity has changed in the past few years, one of the recent developments is comet assay, which gives a ratio between the viable cells in the cell culture to total cells in the culture[1]. These techniques are considered rapid and economical for the evaluation of anticancer[2] compounds. Antipyretic activity[3] of large number of natural products easily allows to guide the isolation and purification of biologically active principles[4]. The genus *Bidens* [Asteraceae] is used in some countries to treat different diseases[5]. Previous studies have confirmed that some of the species produces compounds that exert some pharmacological activities like Phenylheptatriyne, linolic acid and linolenic acid, which have antimicrobial activities, friedelin and friedelan – 3 beta – ol, as well as several of the flavonoids found are anti – inflammatory agents and detection of these compounds in extracts from *Bidens pilosa* may rationalize the use of this plant in traditional medicine in the treatment of wounds, against inflammations and against bacterial infections of the gastrointestinal tract[6].

Bidens pilosa grows rarely in the south of India being known as "Ottrancedi" and is frequently used in traditional medicine as a remedy to treat Glandular sclerosis, wounds, Colds and flu, Acute or chronic hepatitis and Urinary tract infections[7]. Finally, new biological activities of natural products have been just studied[8]. Therefore, one of the objectives of our study was to evaluate the anticancer activity of some species of Asteraceae. Isolated compounds from the plant material have been evaluated for both the activities. The aim of the present study is to evaluate the anticancer activity by in vitro method on Hela[9] (human, black, cervix, carcinoma, epitheloid) and KB (a human nasopharyngeal epidermal carcinoma cell line) [10] carcinoma cell lines in vitro and antipyretic activity by in vivo[11]. The anticancer potency found for some of these compounds may be explained by the capacity that they have for DNA strand breaks and obvious application of the comet assay is the study of apoptosis[12].

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Materials and methods

Materials

Bidens pilosa was collected in Kerala, South India, in October 2003 and identified by a systemic botanist, Dr. B. Sampath Kumar, Department of Botany, Annamalai University. A voucher the specimen was also deposited in Department of Pharmacy, Annamalai University under number DP – XX – F. No.: 0102.

Extraction and Isolation

The shade dried parts of the whole plant were coarsely powdered and extracted with n – hexane, chloroform and methanol respectively for 48 hours in soxhlet apparatus. After evaporation of the solvent under reduced pressure, the respective extracts were obtained. Considering that the methanolic extract suits for better activity, it was successively partitioned with ethyl acetate and acetone affording 0.150 gm and 0.200 gm (residue dry) of each fraction respectively.

Table 1: Anticancer activity of *Bidens pilosa* extracts and fractions on Hela and KB cell lines by Comet Assay Method

Test Sample	*CTC_{50} ± SEM (µg / ml)**	
	Hela Cell	KB Cell
n – Hexane Extract	509.2 ± 6.3	385.2 ± 4.7
Chloroform extract	849.4 ± 7.7	088.6 ± 2.1
Methanol extract	875.3 ± 9.9	423.1 ± 1.4
Ethyl acetate extract fraction	965. 2 ± 8.9	586.2 ± 6.9
Acetone fraction	472.3 ± 4.8	311.5 ± 7.9
Water fraction	372.4 ± 5.1	200.1 ± 3.4

[** Average of three independent determinations three replicates values are mean ± SD.
* CTC_{50} = concentration of the sample tolerated by 50% of the cultures exposed.

The ethyl acetate fraction exhibited anticancer activity (Table 1) and was selected for phytochemical studies.

Thus it was fractioned on chromatoragphic column over silica gel 60 by elution with n – hexane – ethyl acetate (80:20), given 50 mg of compound A, 75 mg of compound B and 100 mg of compound C which were identified by spectroscopic data IR only. 1H, CNMR and MS have to be taken and analyzed for their structures.

Comet assay (partially modified procedure of Singh et al 1988) [12, 13]

Unpolished microscopic slides were used. 1% solution of normal melting point agarose (NMP) was brought and pipette on the slide, covered by coverslip, cover slip removed and the agarose gel let to dry at normal room temperature. At the following step 0.6% NMP agarose was brought on top of the first layer, covered by coverslip and let to solidify in ice plate for 10 minutes. The third layer was a mixture of prepared cell in 0.5% LMP at 37%. About 50,000 cells were allowed to solidify for 10 minutes on ice and covered by the last layer of 0.5% LMP agarose. The coverslip was removed and the slides brought into lysis buffer for at least 1 hr at 4°C (0.03 M sodium hydroxide, 1.2 M sodium chloride, 0.5% lauryl sacrose, pH > 13; for spermatozoa lysing solution contained 1 % triton – X 100, too). The prepared agarose gels were brought into electrophoresis apparatus. Denaturation (40 minutes) and electrophoresis (20 minutes) occurred in the same buffer (0.03 M sodium hydroxide, 2 mM EDTA). The electrophoretic conditions were as following: 30V and 350 mA. Following electrophoresis the studies were washed three times for 5 minutes with the neutralization buffer (0.4 M Tris, pH 7.4), then the gels were stained with ethidium bromide (2 mcg/ml of dH_2O) for 20 minutes, reused in distilled water and kept in humid environment and dark at 4°C. Epifluorescent microscope at 400 X magnifications was used for the examination of studies. Photographs were taken.

Rabbit Pyrogen Test [14, 15, 16, 17, 18]

A group of 5 experimental rabbits having average weight of 2.5 kg were taken on the day of the study, food was withheld and rabbit was acclimatized to the test condition for 2 hrs, the base line temperature was recorded initially half an hour prior to injection. Temperature was monitored by means of thermometer inserted at least 10 cm in to the rectum and recorded by the calibrated thermometers. An animal was excluded from the study if the baseline temperature was not within the range of 39.2° C to 39.8° C. 15 mg/kg of brewer's yeast solution (1ml/kg) was injected into the marginal ear vein of rabbit. Each dose was administered to the group of 5 rabbits. Temperature measurements were taken over an hour period at 50 minutes interval. The observations are tabulated in table 2.

Table 2: The effect of BPEA and paracetamol on yeast – induced pyrexia in rabbits.

Treatment	Rectal temperature (°C) [a]					
	After yeast injection at		After drug administration			
	0h	24h	1h	2h	3h	4h
Control	37.6±0.02	39.9±0.03	39.8±0.02	38.2±0.05	38.9±0.05	39.9±0.04
BPEA 50 mg/kg	38.7±0.04[b]	37.8±0.03[b]	37.5±0.02	38.7±0.02[b]	39.4±0.04[b]	38.1±0.06[b]
BPEA 100 mg/kg	38.6±0.02[b]	37.7±0.04	37.8±0.05[b]	38.3±0.05	39.1±0.03[b]	37.9±0.06[b]
BPEA 200 mg/kg	37.7±0.07	39.9±0.06	37.7±0.02[b]	39.9±0.03[b]	38.7±0.05	37.9±0.04[b]
Paracetamol 150 mg/kg	37.8±0.02[b]	39.9±0.03[b]	38.9±0.02	38.5±0.02[b]	39.3±0.04[b]	39.7±0.03

[a] The results given are mean ± S.E.M.; number of animal used (n=6)
[b] $P < 0.01$ Experimental groups were compared with control

Table 3: Antipyretic activity of *Bidens pilosa* extracts and fractions on Rabbits

Test Sample	MFI ± SEM (° C)*
BPnHE	0.05
BPCE	0.04
BPME	0.03
BPEAE	0.03
BPAF	0.04
BPWF	0.02
Paracetamol	0.09

* Average of three independent determinations three

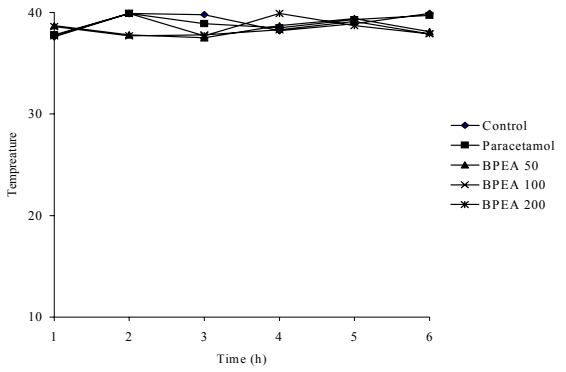
Results and discussion

A rush to very specific, in vitro, robotic mechanism was cautiously carried out however, with mechanism based assays. The field of vision of such specific microscopes is very narrow; they must be assured that the scope of their bioassays can be wide enough to include diverse and unknown mechanism as well as new chemical entities. In addition, in such specific bioassays the same extract, fractions or standard compounds have to be analyzed many times over and over again, and permits a large number of samples and dilutions within the shorter time than using the original test vials, before detecting activities.[19, 20]

These results suggest the presence of bioactive compounds, which would be helpful for further examinations, by using elaborated bioassays for detection of more specific pharmacological properties. It is important to separate active candidates and to identify the major active biomolecules that is present in plant, which can be suggested for further investigations to determine that any other components are responsible for the cytotoxic activity or the existence of synergic effect.

On the other hand, based on the possible relationship between comet assay and plant bioactivity[21, 22] this work encouraged the research on biological assays in vivo of this plant. The antipyretic effects were produced after intraperitoneal administration of extracts to fever induced rabbits. In our tests, the intraperitoneal administration of methanolic extracts from whole part of *Bidens pilosa* (500mg/kg body weight) to normal rabbits produced a significant reduction in body temperature. Compared with the control and experimental groups (Fig.1) whose body temperature decreased after a 4 hrs treatment. Studies are currently in progress in our laboratories to determine the active principles of *Bidens pilosa*.

Fig.1. Effect of the ethyl acetate extract of *Bidens pilosa* (500 mg/kg) on body temperature in normal rabbits treated during 6 hrs all t values are mean ± SEM, n=6(**), significant differences between treated and control group were evaluated by co-efficient correlation.



Conclusion

We have adopted a microtitter assay based on metabolic reduction of MTT to evaluate the cytotoxic effect on different cells and on the basis of rabbit pyrogen test lowering the body temperature of fever therapeutic index to evaluate the body temperature. This technique allows evaluating dose dependent effect, by linear regression analysis shows acceptable R^2 values and correlation coefficients.

Table 1 and 3 shows concentration of extract to produce a reduction viability of cell cultures of 50% (CC_{50} values) as determined by comet assay and it produces a lower body temperature of rabbit as determined by rabbit pyrogen test. The ethyl acetate fractions of methanolic extract from *Bidens pilosa* on Hela and KB cells present in CC_{50} values of 965.2 and 586.2 at 24 h respectively[23]. The methanolic extract of *Biden pilosa* on rabbit presented lower body temperature of 1° C at 4 h. The comparison of the CC_{50} values of the methanolic extract from this plant proves to conclude that this extract would be helpful for further studies of activity monitored fractions to identify the active principles. The extract examined showed an important cytotoxicity and antipyretic activity.

In our test, the intraperitoneal administration of Methanolic extracts from whole plant of *Bidens pilosa* (500mg / kg body weight) to normal rabbits produced a significant reduction in body temperature. Compared with control and experimental groups of rabbits (Fig. 1) there is a reduction in the body temperature after 4 treatments with Methanolic extract. Studies are currently in progress in our laboratories to determine the active principles of *Bidens pilosa*, which is responsible for the antipyretic activity.

Table 1 and 3 compound whose anticancer and antipyretic activity has been proved, with the values of *Bidens pilosa* extract and it concludes that they have an acceptable anticancer and antipyretic activity.

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