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Anticancer effect of *Cenchrus ciliaris* L

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ABSTRACT

Cenchrus ciliaris L total alcohol and successive extracts of both aerial and root parts were tested for their anticancer activities against lung (A-549), intestinal (CACO), colon (HCT-116), cervical (Hela), hepatocellular (HepG-2), and breast (MCF-7) (PC3) cell lines and compared with the standard drug vinblastine sulphate. The obtained results exhibited direct cytotoxic effect with variable inhibiting effect on the growth of the listed cell lines comparing to vinblastine sulphate as reference standard drug, these effects showed different IC_{50} ranged from 11.1 ± 0.3 to 267 ± μg/ml.

All root extracts showed the best activities against most of the tested cell lines specially HepG-2 (Hepatocellular carcinoma) (9 ± 2.1 μg/ml) which was somewhat closely related to the effect of vinblastine sulphate (2.93 ± 0.3 μg/ml).

The highest anticancer effect of Cenchrus *ciliaris* L aerial parts and root extracts were recorded on HepG-2 (Hepatocellular carcinoma) their IC_{50} were 12 ± 0.8 & 9 ± 2.1 respectively, CACO (colorectal carcinoma) their IC_{50} were 27.2 ± 1.6 & 20.5 ± 0.6 respectively, A-549 (Lung carcinoma) their IC_{50} were 14.5 ± 0.7& 11.1 ± 0.3 respectively which were better than the standard drug especially in case the anticancer effect on CACO (colorectal carcinoma) and A-549 (Lung carcinoma). Chloroform extracts of both aerial and roots achieved the best anticancer activities on all of the cell lines especially with colorectal (CACO) and Lung carcinoma (A-549). *Cenchrus ciliaris* could be a promising source of new chemical moieties used to target cancer cells.

1. Introduction

Cancer is a multifactorial disease with a multistep pathogenesis. Based on National Cancer Institute (NIH), cancer available treatments depend on type of cancer and include; surgery, radiation therapy, chemotherapy, Immunotherapy, targeted therapy, hormone therapy, stem cell therapy and precision medicine. However, these treatments have multiple side effects that can affect mortality rates. Global Snapshot done by World health organization (WHO) in 2015, states that cancer is a leading cause of mortality and morbidity globally, affecting more than 14 million people annually. Cancer cells are known to continuously develop resistance to current treatments and for that, the need for new treatments arises (Housman et al., 2014).

Natural products have been used extensively as a source of anticancer compounds. Multiple anticancer "lead" compounds were isolated and identified from many natural sources. After chemical modifications and bio evaluations these compounds gave rise to important drugs. Many pharmaceutical agents have been discovered by the testing of natural products found in microorganisms, animals, plants, marine organisms. Irinotecan, vincristine, paclitaxel, etoposide are plant-derived compounds that have been used for the treatment of cancer. Plants produce a large variety of secondary metabolites that can exceed a hundred thousand molecules and promising anticancer effects of plants intensifies the need for investigational studies on plants. (Greenwell and Rahman, 2015).

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Cenchrus ciliaris L. (Buffel grass) is a pasture grass belonging to *Poaceae* family formerly called "Gramineae". *Poaceae* is very big family which included many genera and species with variety of chemical contents and biological activates such; anticancer (Alves et al., 2016; Premlata et al., 2016), increase milk production (Marshall, 2012), antifungal, antibacterial (Singariya et al., 2012), anthelmintic, anti-amoebic, Cyclooxygenase (COX) I and II inhibitory activity (Light et al., 2002), dysmenorrheal and uterine relaxing activity.

The present study aims to determine the anticancer effects of aerial and root parts of *Cenchrus ciliaris* and investigate the phytochemical groups which may be responsible about this effect.

2. Material and methods

2.1. Phytochemical study

2.1.1. Plant material

Cenchrus ciliaris L. aerial and root parts were collected in March 2017, from the region of Alkharj in Saudi Arabia and identified by Dr. Jacob Thomas, Taxonomist, College of Science, King Saud University. The plant materials of aerial parts and root were air dried, grounded to powder, packed and stored in a tightly closed container for phytochemical analysis and biological activities.

2.1.2. Total plant extraction of aerial and root part

The air dried powdered plant material 1 kg of each aerial and root parts were separately extracted by percolation in 2 L of ethanol (95%) for 2 days and the solvents of each part (aerial and root) was filtered over filter paper, the marks lifted of each part (aerial and root) were extracted four times by the same method (Awaad et al., 2015). The total alcohol extracts were concentrated at a temperature not exceeding 35 °C. The obtained alcohol free extracts were symbolized as; **A & R** for Arial parts and root respectively).

The total alcohol free extracts (**A** & **R)** were separately dissolved in hot water and filtered using peace of cotton, the non-filtered parts was symbolized as; **AL** indication for lipoidal matters of aerial only no lipoidal matter was obtained from root. The aqueous filtered portions were symbolized as; **AP** and **RP** (for aerial and root respectively).

The aqueous fractions residues (**AP** and **RP)** were separately extracted successively using chiroform and butanol saturated with water. Each extract was passed over anhydrous sodium sulphate and evaporated using rotatory evaporator and low temperature to obtained residual gummi extracts with different weight. The obtained dry chiroform extracts were symbolized as **APC & RPC** for aerial parts & root respectively while the butanol extracts were symbolized as **APB** & **RPB** for aerial parts & root respectively. During butanol extraction one resinous compound was isolated from **APB** & **RPB** this compound symbolized as **AR & RR** for aerial and root respectively.

2.2. Biological activities

2.2.1. Anticancer activity

2.2.1.1. Cell lines. The cell lines A-549 (Lung carcinoma), CACO (Colorectal carcinoma), HCT-116 (Colon carcinoma), Hela (Cervical carcinoma), HepG-2 (Hepatocellular carcinoma), and MCF-7 (Breast carcinoma) were carried in Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt

2.2.1.2. Anti-tumor assay. The antitumor assay was carried out on cell lines as published by Ooi et al. (2016) for all of the extracts which have been obtained in Section 2.1.2 **(A**, **R, AL**, **APC, RPC, APB, RPB, AR &RR**). Briefly, the cell lines were suspended in medium at concentration 5 × 104 cell/well in Corning® 96-well tissue culture plates and then incubated for 24 h. The tested extracts were then added into 96-well plates (six replicates) to achieve seven concentrations for each extract. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubation for 24 h, the numbers of viable cells were determined by the MTT assay method. The media was then removed from the 96 well plate and replaced with 100 μl of fresh culture RPMI 1640 medium without phenol red, then 10 μl of the 12 mM MTT (Sigma) stock solution (5 mg of MTT in 1 mL of PBS) was added to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% CO2 for 4 h. An 85 μl aliquot of the media was removed from the wells, and 50 μl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader to determine the number of viable cells. The percentage of viability was calculated as: (ODt/ODc) × 100%, where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells.

The relation between surviving cells and extract concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified extract. The 50% inhibitory concentration (IC50), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA. USA).

2.2.2. Test for toxicity

2.2.2.1. Animals. Swiss albino mice of both sexes (30–35 g) were purchased from Alazahar University animal house, Cairo, Egypt. The animal was kept in standard polypropylene cages and maintained under standard conditions (Awaad et al., 2016)

2.2.2.2. Preparation of the extracts for biological studies. Each Dried alcohol extracts of ***Cenchrus ciliaris*** (Arial parts and root separately) was suspended in distilled water; freshly just before administration using few drops of Tween 80 as emulsifying agent (El-Meligy et al., 2017).

2.2.2.3. Acute Toxicity (LD_{50}) test. Each Dried alcohol extracts of ***Cenchrus ciliaris*** (Arial parts and root separately) was orally given to the animal for median lethal dose (LD50) as described by El-Meligy et al. (2017).

2.2.2.4. Sub-chronic toxicity. For determination of the sub-chronic toxicity, rats were divided into 2 groups each of 6 rats. The 1st group was administrated with the vehicle orally and left as a control, while the groups from 23 were separately administrated the total alcohol extracts of arial parts and root respectively in a dose of 400 mg/kg for 15 days. After the examination period, the collected sera were used for determination of liver and kidney functions (El-Meligy et al., 2017).

2.3. Statistical analysis

All values were expressed as mean ± S.D. Comparisons between means were carried out using a one-way ANOVA test followed by the Tukey HSD test using SPSS, version 14 (SPSS, Chicago, IL). Differences at p = 50.05 were considered statistically significant.

Table 1
The IC_{50} values of *Cenchrus ciliaris* L extracts on cell lines.

Extracts		Cell line IC_{50} (μg/ml)					
		A-549 (Lung carcinoma)	CACO (Colorectal carcinoma)	HCT-116 (Colon carcinoma)	Hela (Cervical carcinoma)	HepG-2 (Hepatocellular carcinoma)	MCF-7 (Breast carcinoma)
Vinblastine Sulphate		24.6 ± 0.7	30.3 ± 1.4	3.5 ± 0.2	59.7 ± 2.1	2.93 ± 0.3	5.9 ± 0.4
Arial part	A	124 ± 6.2	168 ± 9.4	248 ± 13.8	204 ± 8.6	40.5 ± 3.9	78.2 ± 7.6
	AL	59.7 ± 2.3	40.6 ± 2.6	54 ± 2.9	107 ± 6.3	30.9 ± 2.8	58.4 ± 4.3
	APC	14.5 ± 0.7	27.2 ± 1.6	24.5 ± 0.9	27.5 ± 1.4	12 ± 0.8	50.8 ± 3.4
	APB	48.9 ± 4.2	55.8 ± 3.4	267 ± 14.6	123 ± 8.3	51.2 ± 5.7	237 ± 9.8
	AR	45.2 ± 3.7	22.5 ± 3.1	154 ± 6.4	162 ± 8.1	23.6 ± 3.2	121 ± 4.7
Root	R	47.1 ± 1.8	56 ± 2.8	99 ± 3.8	93 ± 5.4	37.5 ± 2.9	93.3 ± 12.1
	RPC	11.1 ± 0.3	20.5 ± 0.6	18.8 ± 2.9	22.6 ± 2.4	9 ± 2.1	46.5 ± 2.6
	RPB	49.2 ± 1.7	28.8 ± 1.6	155 ± 6.3	113 ± 10.2	46.9 ± 3.4	51.5 ± 4.3
	RR	43.9 ± 2.4	29.2 ± 2.4	234 ± 7.9	234 ± 4.9	28.2 ± 1.9	111 ± 3.7

A; total alcohol aerial parts, R; total alcohol root, AL; lipoidal matter aerial parts, APC; chloroform extract aerial parts, RPC; chloroform extract roots, APB; butanol extract arial parts, RPB; butanol extract root, AR; Resinous Compound aerial parts, RR; Resinous Compound root.

Table 2
Effect of the total alcohol extracts *Cenchrus ciliaris* L on liver and kidney functions.

Parameter	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Urea (mg/dL)	Creatine (mg/dL)
Plant part							
Control	61.23 ± 1.4	47.70 ± 1.3	1.60 ± 0.3	8.90 ± 0.5	3.7 ± 0.5	36.66 ± 0.2	0.48 ± 0.4
Arial parts	60.41 ± 1.3	48.90 ± 1.6	1.61 ± 0.3	8.80 ± 0. 3	3.3 ± 0.2	35.33 ± 1.3	0.50 ± 0.3
Root	62.30 ± 1.2	46.12 ± 1.4	1.62 ± 0.4	8.75 ± 0.9	3.1 ± 0.6	36.13 ± 1.2	0.47 ± 0.5

3. Results and discussion

3.1. Phytochemical analysis

Phytochemical screening of *Cenchrus ciliaris* L showed the presence of the following groups: carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, anthraquinones, protein and/or amino acids, and tannins and absence of saponin, alkaloids, cardinolides, and oxidase enzyme in all the investigated parts.

Total alcohol extracts were 30.3 & 40.5 for aerial part and root respectively (**A**, **R**). The lipoidal matter was only obtained from the Arial parts (**AL**) and it gave 3.24 g.

Chloroform extracts evaporation produced 10.9 and 7.8 g (from aerial part & root respectively). But butanol evaporation gave 14.5 & 15.3 g from aerial part & root respectively. The isolated resinous matter were 8.9 and 16.6 g respectively (**AR &RR**).

3.2. Anticancer activity

The *in vitro* antitumor activities of *Cenchrus ciliaris* L extracts were evaluated on six cell lines. The obtained results exhibited direct cytotoxic effect (Table 1) with variable inhibiting effect on the growth of the listed cell lines comparing to vinblastine sulphate as reference standard drug, these effects showed different IC_{50} ranged from 11.1 ± 0.3 to 267 ± μg/ml.

In general all root extracts showed the best activities against most of the tested cell lines specially HepG-2 (Hepatocellular carcinoma) (9 ± 2.1 μg/ml) which was somewhat closely related to the effect of vinblastine sulphate (2.93 ± 0.3 μg/ml**).**

The highest anticancer effect of *Cenchrus ciliaris* L arial parts and root extracts were recorded on HepG-2 (Hepatocellular carcinoma), CACO (colorectal carcinoma), A-549 (Lung carcinoma) which were better than the standard drug especially in case the anticancer effect on CACO (colorectal carcinoma) and A-549 (Lung carcinoma).

Chloroform extracts of both aerial and roots achieved the best anticancer activities on all of the cell lines specially with colorectal (**CACO**) and Lung carcinoma (**A-549**). The effect of root chloroform extract the effect was 11.1 ± 0.3 and 20.5 ± 0.6 μg/ml on colorectal (**CACO**) and Lung carcinoma (**A-549**) respectively, while in case of aerial chloroform extract the effect was 14.5 ± 0.7& 27.2 ± 1.6 μg/ml on colorectal (**CACO**) and Lung carcinoma (**A-549**) respectively and all of these effects were better than vinblastine sulphate (24.6 ± 0.7, 30.3 ± 1.4 for colorectal and Lung carcinoma respectively). Phenolic compounds are knowns to possess antioxidant and anticancer activity (Carocho and Ferreira, 2013).

3.3. Test for toxicity

After oral admiration of *Cenchrus ciliaris* L alcohol extracts (arial parts and root) with different doses, up to 5000 mg/kg, the results did not produce any sign of toxicity or animal death during 24 h of observation. Therefore, LD_{50} of the tested extracts, up to 5000 mg/kg, is considered safe for human use (Awaad et al., 2016).

The sub-chronic toxicity also supported the safety of the plant extracts (arial parts and root). The oral dosing (400 mg/kg) which given to rats for 14 days, did not affect the levels of ALT, AST, total bilirubin, total proteins, albumin, urea and creatinine as compared to control (Table 2). It means that the investigated extracts are not hepatotoxic (Rysz et al., 2017).

4. Conclusion

Upon the testing of aerial and root part of *Cenchrus ciliaris* it can be concluded that extracts of the two parts were effective in inhibiting the growth of lung (A-549), intestinal (CACO), colon (HCT-116), cervical (Hela), hepatocellular (HepG-2), and breast (MCF-7) carcinomas with high safety margin for human use.

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