INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 December, 2013; received in revised form, 11 January, 2014; accepted, 09 March, 2014; published 01 May, 2014

EVALUATION OF ANTIADIPOGENESIS ACTIVITIES OF SELECTED SPECIES OF *CARALLUMA* R. BR., *BOUCEROSIA* WIGHT & ARN. AND PREGNANE STEROID ON CELL LINES.

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Keywords:

Caralluma, *Boucerosia*,pregnane steroid, antiadipogenesis and cell lines.

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ABSTRACT: Back ground: The study was designed to determine antiadipogenesis properties of crude methanolic extracts from four species of *Caralluma* R.Br. such as *Caralluma adscendens* (Roxb.) R. Brown var. *attenuata* (Wight) Grav. & Mayur. (CAA), *Caralluma adscendens* (Roxb.) R. Brown var. *fimbriata* (Wall.) Gravely & Mayur. (CAF), *Caralluma stalagmifera* C.E.C. Fisch. (CS) and *Caralluma stalagmifera* C.E.C. Fisch. var. *longipetala* Karupp. & Pull. (CSL) and as well as two species of *Boucerosia* Wight & Arn. such as *Boucerosia lasiantha* Wight. (BL) and *Boucerosia umbellata* (Haw.) Wight & Arn. (BU) at intervarietal and interspecific levels along with bioactivity studies. (BU) along with an isolated compound pregnane steroid using different cell lines. **Methods**: *In vitro* inhibitory activity of adipogenesis and facilitating activity on adipolysis were evaluated in 3T3 L1 adipocytes. **Results**: The percentage anti adipogenesis (50 µg/ml) was also observed with values of 15.29%, 4.20%, 12.27%, 21.85%, 0 and 9.75% respectively. The percentage inhibition of pregnane steroid in adipogenesis of 3T3 L1 preadipocytes by 1 and 5 µg/ml were 21.22% and 35.97% respectively. **Discussion**: Four of them are effective in inhibiting adipogenesis. When compared to methanolic extracts of *Caralluma* and *Boucerosia* species, pregnane steroid was shown to be potential in *invitro* antiadipogenesis, and showed significant variations in different cell lines at different concentrations.

INTRODUCTION: Obesity is a global health problem and current research on screening antiobesity agents from medicinal plants focuses on molecular regulation of triglyceride synthesis and to reduce fat storage, fat synthesis, size and differentiation of adipocytes as well as proliferation of preadipocytes; to increase lipolysis and fat oxidation. 3T3 L1 cell line is an *in vitro* convenient model system for research related to obesity [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(5).1919-23

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(5).1919-23

Flavonoids play an important role in the inhibition of lipogenesis related enzymes in 3T3 L1 cell differentiation for obesity prevention. Quercitin and polyphenols inhibited 3T3 L1 differentiation [2, 3], and procyanidin from grapes increased lypolysis [4].

Caralluma and *Boucerosia* belong to the family *Asclepiadaceae*, distributed in Africa, Spain, Saudi Arabia, Middle East, Pakistan and India. The species of *Caralluma* found in India are eaten raw as well as in cooked form and became a part of traditional system of the country. The medicinal activities of the genus *Caralluma* include carminative, febrifugal, anthelmintic, antirheumatic, anti-inflammatory, anti-nociceptive and anti-oxidant effects.

Caralluma acts as appetite suppressant and stimulant of central nervous system. The medicinal properties of *Caralluma* and *Boucerosia* are due to pregnane group of glycosides contained in them. Pregnane glycosides are the condensation products of sugar and non-sugar compounds along with ring structure [5]. Some of the pregnane glycosides include caratuberside A, caratuberside B and bouceroside I to X [6].

Four new pregnane glycosides comprised of genin exhibiting a hydroxymethylene instead of a methyl group at C 19 were isolated with other eight known glycosides and structures were elucidated using 2D-NMR from *Caralluma adscendens* var. *fimbriata* [7]. The extracts of *Caralluma fimbriata* have shown appetite suppressing, anti obesogenic and antiatherogenic activities in the DIO rat model[8]. The crude extracts of *Caralluma fimbriata* standardized to Pregnane glycosides inhibited proliferation 3T3-L1 preadipocytes by inhibiting the import of cyclin D1 from cytosol into the nucleus, there by arresting the cell cycle at G1 phase and reported the anti adipogenic mechanism of action of *Caralluma fimbriata* [9].

The aim of the study is to evaluate antiadipogenesis among selected four species of *Caralluma* (Asclepiadaceae) such as CAA, CAF, CL and CSF as well as two species of *Boucerosia* like BL and BU at intervarietal and interspecific levels along with an isolated compound pregnane steroid (an isolated compound from BU and BL). The present investigation has been carried out to differentiate CAF from other five species, as *Caralluma adscendens* var. *fimbriata* is commercially used to produce Genaslim capsules for antiobesity.

Antiadipogenic activity Mouse 3T3 L1 preadipocyte cell lines were treated with different concentrations of *Caralluma fimbriata* extract [9], *Taraxacum officinale* [10] and four natural extracts of *Rubi fructus*, *Cornus fructus*, *Salicis radicis*, *Geranium nepalense* [11] as well as reported that they have potential to inhibit hyperplastic obesity in dose and duration dependent manner, with results comparable to those produced by hydroxyurea**.** The study is aimed to explore and evaluate antiadipogenesis in 3T3 L1 adipocytes cell lines of methanolic extracts of *Caralluma* and *Boucerosia* species at cellular level. Once novelty and structure of the lead compound has been established, these compounds can be treated as taxonomic marker to distinguish the species. The lead compounds along with crude extracts will undergo extensive bioactivity studies. Stable standardized crude extracts along with isolated compounds were prepared, investigated and compared their immunostimulating activities of specific plants whose traditional uses are known but not experimentally proved.

MATERIALS AND METHODS: The four different species of *Caralluma* and two species of *Boucerosia* studied were collected at random, from Gooty, Tadiparthy and Penukonda regions of Ananthapur district, Andhra Pradesh. and were taxonomically identified by comparing with Gamble flora and other taxonomical literature, voucher specimens i.e. VM 46, VM 47, VM 48, VM 49, VM 50 and VM 51 were deposited in Montessori Mahila Kalasala, Vijayawada.

Adipogenesis assay with oil red O stainingCell culture reagents:

FBS-DMEM, insulin, oil red O, formaldehyde and isopropyl alcohol, DEX-dexamethasone, MIX- 3-isobutyl 1-methylxanthine. **Equipment:** Microplate reader (Bio – Rad, USA).

3T3-L1 Cell Culture: **Preadipocyte maintenance and passage**

Cell based adipogenesis inhibition assay was performed [12, 13]. The 3T3 L1 Mouse preadipocytes were procured from National centre for cell science, Pune, India. The cells were maintained in Dulbecco's modified eagle medium containing 10% fetal bovine serum and incubated at 37 ^{o}C in 10% CO_2 and grown to 80% confluence.

Differentiation of adipocytes Preadipocytes were grown to confluency in the differentiation medium containing 10% FBS-DMEM, supplemented with 500 nM insulin, 1 µM dexamethasone and 0.5 mM 3-isobutyl 1-methylxanthine for two days. After Dex- MIX treatment, equal number of 3T3 L1 mouse preadipocytes (6 X 10^4 cells/well) were seeded in each well of 24 well tissue culture plates,

the cells were further maintained in the post differentiation medium containing 10% FBS-DMEM, supplemented with 100 nM insulin in the presence or absence of respective CME test samples and pregnane steroid for further eight days and grown to 90% confluence.

The control cultures received only 0.1% v/v DMSO as the vehicle.

Preparation of plant extracts: Stock of *Caralluma* and *Boucerosia* plant extracts (CAA, CAF, CS, CSL, BL and BU) at 100 mg/ml in DMSO were prepared and stored at -0^{o}C until used. Ten-fold serial dilutions (20 µl of plant extracts in 180 µl DMEM) were then prepared from the stock solution to obtain extract working concentrations 25 µg/ml and 50 µg/ml by using DMEM. Pregnane steroid at working concentrations 1 µg/ml and 5 µg/ml was also prepared for this assay. The plant extract solutions were used immediately for cytotoxic assay.

Oil red O staining: The intracellular lipid accumulation was measured by staining cells with oil red O. Briefly, the 10% formaldehyde- fixed cells were washed with 60% isopropyl alcohol and the air dried cells were stained with oil red O for 10 min at room temperature. Unbound stain was washed with distilled water. The dye incorporated into the fat vesicles was eluted with 100% isopropyl alcohol, and optical density was read at 550 nm using a microplate reader. Percent inhibition of adipogenesis was expressed in terms of inhibition of fat accumulation or lipid load in the treated cell, calculated by using following formula. Percent inhibition in adipogenesis = (OD in vehicle control – OD in treated well) X 100/ OD in vehicle control.

RESULTS: The intracellular lipid accumulation was measured by staining cells with oil red O. Two concentrations (25 µg/ml and 50 µg/ml) of crude extracts of *Caralluma* and *Boucerosia* such as CAA, CAF, CS, CSL, BL and BU were treated against 3T3 L1 preadipocytes. Percent inhibition of adipogenesis was expressed in terms of inhibition of fat accumulation or lipid load in the treated 3T3 L1 preadipocytes. The percentage inhibition of all the selected species in adipogenesis of 3T3 L1 preadipocytes by 25 µg/ml and 50 µg/ml has shown dose dependent responses **(Table 1).**

TABLE 1: COMPARATIVE DOSE DEPENDENT PERCENTAGE INHIBITION IN ADIPOGENESIS IN 3T3 L1 PRE ADIPOCYTES BY SIX TARGET *CARALLUMA* EXTRACTS AND PREGNANE STEROID AT DIFFERENT CONCENTRATIONS.

S. No.	*Caralluma* / *Boucerosia* species	Concentration of sample (µg/ml)	% inhibition in adipogenesis
1	*Caralluma adscendens* var.*attenuata* (CAA)	25	12.06
		50	15.29
2	*Caralluma adscendens* var.*fimbriata* (CAF)	25	-4.43
		50	4.20
3	*Caralluma stalagmifera* (CS)	25	10.69
		50	12.27
4	*Caralluma stalagmifera* var. *longipetala* (CSL)	25	7.48
		50	21.85
5	*Boucerosia lasiantha* (BL)	25	-25.50
		50	-8.40
6	*Boucerosia umbellata* (BU)	25	-0.31
		50	9.75

The percentage inhibition of pregnane steroid in adipogenesis of 3T3 L1 preadipocytes by 1 and 5 µg/ml were 21.22% and 35.97% respectively **(Fig 1).** CAA, CSL, CS and BU are effective ininhibiting adipogenesis, while CAF and BL were not effective, when 50 µg/ml of crude methanolic extract of *Caralluma* and *Boucerosia* was treated with 3T3 L1 preadipocytes **(Table 2).**

TABLE 2: PERCENTAGE INHIBITION IN ADIPOGENESIS OF 3T3 L1 PREADIPOCYTES BY SELECTED SPECIES OF *CARALLUMA* AND *BOUCEROSIA*.

S. No.	*Caralluma* and *Boucerosia* species	Percentage inhibition Mean±SD
1	*Caralluma adscendens* var. *attenuata* (CAA)	15.29±0.94
2	*Caralluma adscendens* var. *fimbriata* (CAF)	4.2±0.7
3	*Caralluma stalagmifera* (CS)	12.27±0.89
4	*Caralluma stalagmifera* var. *longipetala* (CSL)	21.85±1.50
5	*Boucerosia lasiantha* (BL)	nil
6	*Boucerosia umbellata* (BU)	9.75±0.43

All test samples run in triplicates and one way ANOVA test was carried. Values are expressed as mean ± standard deviation (n = 3). The results of ANOVA analysis show significant differences ($p<0.05$) in the means of inhibition of cellular adipogenesis (%) at 50 µg/ml of *Caralluma* and *Boucerosia* extracts.

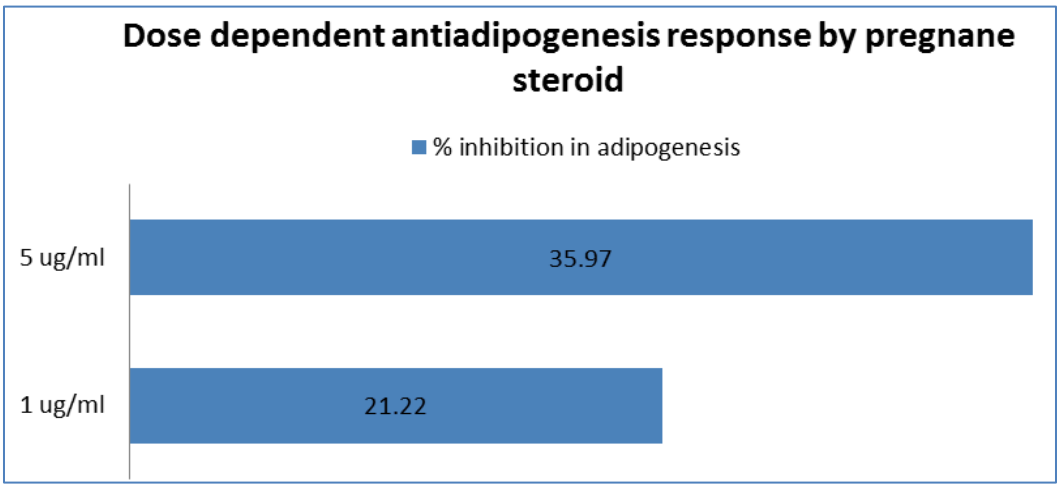

FIG 1: DOSE DEPENDENT PERCENTAGE INHIBITION OF ADIPOGENESIS IN 3T3 L1 PREADIPOCYTES BY PREGNANE STEROID. *µg/ml represents concentration of pregnane steroid used

DISCUSSION: CAA, CS, CSL and BU were effective in inhibiting adipogenesis, while CAF and BL were not effective when 50µg/ml of crude methanolic extract of *Caralluma* was treated with 3T3 L1 preadipocytes. The metabolic process of adipogenesis and has been well documented with the use of 3T3 L1 cell line [14, 15].

Under the control of adipose specific regulatory genes, transcriptional cascade gets activated namely peroxysome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer binding protein (C/EBP) during adipocyte differentiation. DEX activates C/EBP and IBMX inhibits soluble cyclic nucleotide phosphodiesterases that lead to increase in cAMP levels intracellularly [16], resulting in activation of C/EBP genes and PPARγ, which in turn activate adipocyte specific genes such as fatty acid synthetase, fattyacid binding proteins, leptin and adiponectin.

Insulin and insulin like growth factors activates P13-kinase and Akt activity, leading to adipocyte differentiation. By using the 3T3 L1 model of adipogenesis and adipogenic agents like 0.5 mM IBMX, 1 µM DEX and 10 µg/ml of insulin, four species of *Caralluma* and two species of *Boucerosia* as well as pregnane steroid marker compound were screened.

The methanolic extract of CAA, CAF, CS, CSL and BU inhibited lipid accumulation of 3T3 L1 cells in a concentration dependent manner.

Caralluma and *Boucerosia* extracts showed 50% or more adipogenesis inhibition at 25 and 50 µg/ml (**Table 1**). Among six extracts tested, methanolic extract of CAA, CS, CSL and pregnane steroid showed potent inhibitory activity on intracellular lipid accumulation in 3T3 L1 adipocytes.

In the study, it was also observed that the antiadipogenesis effect of pregnane steroid, where the potency was comparatively higher than that of crude extracts. This is the first study to investigate comparative analysis of antiobese characteristics of four species of *Caralluma* and two species of *Boucerosia*, so that the result gave useful information to take another look at differentiation of species. Further investigations are needed to identify the active constituents responsible for anti adipogenesis properties of crude methanolic extracts (CME) from four species of *Caralluma* and two species of *Boucerosia* CAA, CAF, CSL, CS, BL and BU.

ACKNOWLEDGEMENT: The authors are thankful for support from the management, Laila IMPEX for providing for providing necessary facilities for completion of this work.

CONFLICT OF INTEREST: The authors report no conflicts of interest

REFERENCES:

1. Kwak DH, Lee JH, Kim DG, *et al*: Inhibitory Effects of Hwangryunhaedok-Tang in 3T3-L1 Adipogenesis by Regulation of Raf/MEK1/ERK1/2 Pathway and PDK1/Akt Phosphorylation. Evidence Based Complementary and Alternate Medicine 2013; 2013: 1-19.
2. Zebisch K, Voigt V, Wabitsch M and Brandsch M: Protocol for effective differentiation of 3T3-L1 cells to adipocytes. Analytical Biochemistry 2012; 425: 88-90.
3. McGarvey BD, Liao H, Ding K and Wang X: Dereplication of known pregnane and structural characterization of novel pregnanes in *Marsdenia tenacissima* by high-performance liquid chromatography and electrospray ionization-tandem mass spectrometry. *Journal* of *Mass Spectrometry* 2012; 47:687-693.
4. Kim Y, Choi Y, Lee J and Park Y: Downregulated lipid metabolism in differentiated murine adipocytes by procyanidins from defatted grape seed meal. Bioscience Biotechnology. Biochemistry. 2013; 77:1420-1423.
5. Astell KJ, Mathai ML, McAinch AJ, *et al*: A pilot study investigating the effect of *Caralluma fimbriata* extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial. Complementary Therapies in Medicine 2013; 21:180-189.
6. Al-Massarani SM, Bertrand S, Nievergelt A, *et al*: Acylated pregnane glycosides from *Caralluma sinaica*. Phytochemistry 2012; 79: 129-140.
7. Dutt HC, Singh S, Avula B, *et al*: Pharmacological review of *Caralluma* R. Br. with special reference to appetite suppression and anti-obesity. Journal of Medicinal Food 2012; 15: 108-119.
8. Kamalakkannan S, Rajendran R, Venkatesh RV, *et al*: Antiobesogenic and antiatherosclerotic properties of *Caralluma fimbriata* extract. Journal of Nutrition Metabolism 2010; 2010: 285-301.
9. Kamalakkannan S, Rajendran R, Venkatesh RV, *et al*: Effect of *Caralluma fimbriata* extract on 3T3-L1 pre-adipocyte cell division**.** Food Nutrition Science 2011; 2: 329-336.
10. Srividya AR, Balaji PH, Raghu H and Vijayan P: Antioxidant and anti-adipogenic activity of *Taraxacum officinale* by in vitro screening methods. The Pharmacist 2008; 3: 41-44.
11. Roh C and Jung U: Screening of crude plant extracts with anti-obesity activity. International Journal of Molecular Science 2012; 13: 1710-1719.
12. Dentesano G**,** Straccia M**,** Ejarque-Ortiz A**,** et al: Inhibition of CD200R1 expression by C/EBP beta in reactive microglial cells. *Journal of Neuroinflammation* 2012; 9:165-172.
13. Zhu HJ, Ding HH, Deng JY, *et al*: Inhibition of preadipocyte differentiation and adipogenesis by zinc-α2-glycoprotein treatment in 3T3-L1 cells. Journal of Diabetes Investigation 2013; 4: 252-260.
14. Lowe CE, O'Rahilly S and Rochford JJ: Adipogenesis at a glance Journal of Cell Science 2011; 124: 2681-2686.
15. Zebisch K, Voigt V, Wabitsch M, Brandsch M, *et al*: Protocol for effective differentiation of 3T3-L1 cells to adipocytes**.** Analytical Biochemistry 2012; 425: 88–90
16. Li W, Vogel CFA, Matsumura F. Studies on the cell treatment conditions to elicit lipolytic responses from 3T3-L1 adipocytes to TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin. Journal of Cellular Biochemistry 2007; 102: 389-402.

How to cite this article:
Madhuri V, Audipudi AV, Murthy KSR and Krishna CSR: Evaluation of antiadipogenesis activities of selected species of *Caralluma* R.Br. *Boucerosia* Wight & ARN. and pregnane steroid on cell lines.. *Int J Pharm Sci Res* 2014; 5(5): 1919-23.doi: 10.13040/IJPSR.0975-8232.5 (5).1919-23.