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(RESEARCH ARTICLE)

In vivo effect of withacoagulin and coagulin C isolated from *Withania coagulans* Dunal fruits on blood pressure of albino rats

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Abstract

The present study was designed to evaluate the in vivo effect of withacoagulin and coagulin C isolated from Withania coagulans Dunal fruits on blood pressure of albino rats. Hypertension in rats was experimentally induced by giving DOCA (10mg/kg; i.p.) along with diet high in sodium chloride salt. Withacoagulin and coagulin C were isolated from Withania coagulans Dunal fruits. Comparative effect of withacoagulin and coagulin C was observed against standard verapamil, a known antihypertensive drug. Female Albino rats (100-120 g) were divided into seven experimental groups (n=6). Normal control, Verapamil standard control (50 mg/kg, p.o.), withacoagulin treatment groups (25, 50 mg/kg; p.o.), coagulin C treatment groups (25, 50 mg/kg; p.o.) and Toxic control (DOCA with 2% normal saline ad libitum). The hypertensive rats (>150mm Hg) were choosen for the treatment. Noninvasive tail blood pressure amplifier (NIBP200A) was used to measure blood pressure of conscious rats at a fixed interval of time every day for four weeks. A significant decrease ($P< 0.05$) in the blood pressure of withacoagulin treated and standard group were observed. Withacoagulin at dose 25 mg/kg produced significant ($P< 0.05$) antihypertensive effect. Withacoagulin at 50 mg/kg dose produced highly significant antihypertensive effect ($P< 0.001$) as compared to standard calcium channel blocker, verapamil. Coagulin C has been found to have no significant antihypertensive effect on albino rats.

Keywords: Withacoagulin; Coagulin C; *Withania coagulans*; Dunal fruits; antihypertension; verapamil

1. Introduction

Hypertension (HTN) or high blood pressure (BP) is the most common cardiovascular disease and is a major public health issue in developed as well as developing countries. It is a chronic medical condition in which the blood pressure in the arteries is elevated. Hypertension is classified as either *primary* (essential) or *secondary*. Essential hypertension in human is a complex, multifactorial, quantitative trait under polygenic control. About 90 to 95% of cases are considered primary HTN, which refers to high BP of unknown etiology [1]. The remaining 5 to 10% of cases, called *secondary* HTN, are caused by adverse effects on kidneys, arteries, heart, or endocrine system [2]. Long term HTN is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure [3]. Because of its high incidence and morbidity, various classes of drugs and regimens have been advocated for the control of hypertension. Despite the large armamentaria of drugs being available for the treatment of hypertension, the last two decades have witnessed the introduction of a number of new antihypertensive drugs. Evaluation of withacoagulin as promising antihypertensive compound of plant origin may lead to its addition into the list once it gets approval for clinical trial.

Withania coagulans Dunal belongs to family *Solanaceae* is a well-known medicinal plant in indigenous system of medicine [4]. It is mainly distributed in the eastern part of Mediterranean region extending to South Asia including many

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parts of northern and western India [5]. The drug has been reported to possess anti-inflammatory [6], antiurease effect by virtue of its prorperty to inhibit COX- 2 enzymes [7], cardiotonic activities [8], hepatoprotective [9], antifungal [10], hypoglycemic [11], hypolipidemic [12] and anticancer activity [13]. Fruits of the plant have a milk-coagulating characteristic [14]. The fruits have been used for milk coagulation which is attributed to the enzymatic charisma of the plant [15]. The aqueous extract of *W. coagulans* fruits in experimental rats has a diuretic potential [16]. The diuretic effects may be associated with the presence of the active principles of polar nature where withanolides are the main chemical protagonist of this activity.

A withanolide (steroidal lactone) having a unique chemical structure similar to the aglycone part of cardiac glycoside has been shown to exert cardiovascular effect. This withanolide produced a slight fall in blood pressure when observed in dogs. This effect could be blocked by atropine (cholinergic connection) and not by propranolol. In rabbits Langendorff preparation and ECG studies, the withanolide produced myocardial depressant effects but in perfused frog heart it caused mild positive inotropic and chronotropic effects [8].

The present study was choosen to evaluate the antihypertensive effect of isolated withanolides on further smaller mammal (albino rats). As there is slight difference in the physiology of different mammals the present study would further confirm the assumption to choose withanolides as an antihypertensive agent on the basis of which clinical studies can be inititated. The exact mechanism how the withanolide acts to control hypertension is not known and needs furthermore studies.

2. Materials and methods

The study was approved by the Institutional Animal Ethics Committee (RIVTE/ IAEC/16/02) of Ram-Eesh Institute of Vocational and Technical Education Greater Noida (Animal Hose registration number: 385/PO/Re/S/01/CPCSEA). Healthy, adult female albino rats weighing approximately 100 g were used for the study. They were kept in standard laboratory conditions under 12 hours light and 12 hours dark cycle. Hypertension in rats was induced by administering DOCA (10 mg/kg; i.p.). The animals were kept on a diet high in sodium chloride (Golden Feeds, Mehrauli, New Delhi) and drinking water replaced by 2% sodium chloride solution *ad libitum*.

All AR grade chemicals including DOCA used in the study were obtained from CDH Chemicals. Withacoagulin (20β, 27-Dihydroxy-1-oxo-(22R)-witha-2,5, 24-tetraenolide) was isolated from *Withania coagulans* fruits by successive extraction and Flux chromatography. The fruits were purchased from local spice and herbs market in Delhi. The fruits were identified and authenticated by National Herbarium of cultivated plants (NHCP), Division of Plant Exploration and Germplasm Collection, National Bureau of Plant Genetic Resources, New Delhi and the specimen voucher (NHCP/NBPGR/2014-09) has been retained. The fruits were shade dried, weighed and crushed for extraction and isolation of withacoagulin and coagulin C.

2.1. Isolation scheme of withacoagulin from *Withania coagulans* berries [17]

Shade dried crushed fruits were macerated in a mixture of chloroform and ethanol (1:1) for three days by occasional shaking followed by filtration and drying in rotary evaporator. A semisolid mass so obtained after drying was fractionated by solvent-solvent extraction. Crude extract so obtained was suspended in hot water and extracted three times with n-hexane using a separating funnel. Aqueous layer was separated and extracted with ethyl acetate. The organic layer was separated and dried in rotary evaporator at 35°C and ethyl acetate fraction (WCE) was obtained. WCE was subjected to normal phase column chromatography (silica gel 60, 230-400 mesh) using the mobile phase n-Hex: EA/5:1-0:1. Multiple fractions (100ml each) were obtained and combined followed by column chromatography (sephadex LH20) using ethanol as mobile phase. Later few fractions were combined and submitted to normal phase column chromatography (silica gel 60, 5-40 µm) using the mobile phase n-Hex: EA/5:1-0:1. Finally few more fractions were collected and combined. They were subjected to RP-MPLC (Bondesil-C18, 40µm) by using the gradient mobile phase EtOH:H_2O/30:70. The collected fractions were dried to get withacoagulin. The obtained isolate was confirmed spectroscopically as withacoagulin.

2.2. Isolation scheme of coagulin C from *Withania coagulans* berries

The shade dried fruits of *Withania coagulans* were crushed and macerated in ethanol followed by filtration and evaporation to obtain ethanolic extract. The extract was dissolved in MeOH and defatted with petroleum ether followed by treatment with 10% acetic acid solution. The acidic aqueous extract (pH 2-3) was partitioned with chloroform and acidic fraction was subjected to silica gel column chromatography using *n*-hexane/$CHCl_3$ as mobile phase. The fractions so collected were combined and repeated with CH_2Cl_2/MeOH mixtures as mobile phase. The fresh fractions collected

were than subjected to silica gel coated TLC. The separated compound was washed and dried to obtain coagulin C and confirmed using chemical tests characteristic for alkaloid and spectroscopic method.

2.3. Treatment protocol

The animals were divided into seven main groups having six animals in each group. Animals were given DOCA (10 mg/kg; i.p.) for induction of hypertension along with 2% NaCl solution with drinking water. After one week the hypertensives rats were selected for the study. Verapamil (50 mg/kg) was given as standard control. Two doses of withacoagulin (25 mg/kg and 50 mg/kg) and coagulin C (25 mg/kg and 50 mg/kg) were used for the evaluation of antihypertensive effect in albino rats.

2.4. Blood Pressure measurement

Monitoring and recording of blood pressure in experimental animals was done as per CPCSEA guidelines. NIBP200A (BIOPACK Systems Inc.) small animal tail noninvasive blood pressure amplifier was used to measure blood pressure of experimentally induced hypertension in female albino rats on daily basis for four weeks. The instrument had an in built pump Tail IRCUFFSENSOR which automatically inflated the cuff and occluded the rat tail blood vessel. The cuff was deflated as soon as the pump reached the inflation point. Hereafter, a linear drop in blood pressure was recorded for each animal.

2.5. Statistical analysis

Standard mean and deviations were calculated for all variables of each group. One-way Analysis of Variance (ANOVA) was applied for statistical analysis with post-hoc analysis (Bonferroni Multiple Range Test) and P value <0.05 has been considered as statistical significance level.

3. Results and discussion

The present study is designed to evaluate the efficacy of withacoagulin isolated from *Withania coagulans* fruits in rat model of DOCA- induced hypertension. Withacoagulin group (25 mg/kg and 50 mg/kg) have shown significant decrease in both systolic and diastolic blood pressure (P<0.05). Withacoagulin group (50 mg/kg) have shown highly significant (P<0.001) decrease in both systolic and diastolic blood pressure of experimentally induced hypertensive animals. Coagulin C group (25 and 50 mg/kg) has no significant effect on the elevated blood pressure of hypertensive rats.

Table 1 Blood Pressure recordings of animals during treatment for four weeks

Animal Group (n=6)	1st Week		2nd week		3rd week		4th Week	
	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
Normal Control	118 ± 2	82 ± 1	119 ± 2	82 ± 1	118 ± 1	91 ± 1	117 ± 2	92 ± 1
Verapamil (50mg/kg)	158 ± 1	92 ± 2	154 ± 1	94 ± 1	149 ± 2	95 ± 1	137 ± 1	92 ± 2
Withacoagulin (25 mg)	167 ± 2	134 ± 1	160 ± 1	129 ± 2	148 ± 1	125 ± 1	137 ± 1	122 ± 1
Withacoagulin (50 mg)	149 ± 1	106 ± 2	137 ± 1	103± 2	128 ± 1	86 ± 1	124 ± 1	85 ± 2
Coagulin C (25 mg)	209 ± 1	148 ± 1	196 ± 1	152 ± 1	188 ± 1	150 ± 2	187 ± 1	144 ± 1
Coagulin C (50 mg)	195 ± 2	148 ± 1	190 ± 1	153 ± 2	183 ± 1	146 ± 1	173 ± 1	141 ± 2
Toxic Control	250 ± 2	158 ± 2	258 ± 1	152 ± 1	261 ± 2	159 ± 2	268 ± 2	166 ± 2

Withania coagulans has been studied for its cardiotonic activity. The present study suggests that Withacoagulin isolated from *W. Coagulans* fruit has antihypertensive effect in experimental model of rat. Verapamil is a standard antihypertensive agent and decreases blood pressure by blocking Ca^{2+} channels in the smooth muscles of arteries. On the basis of the fact that *Withania coagulans* is having Ca^{2+} channel blocking property [18] the antihypertensive effect of withacoagulin might be through this mechanism. But the exact mechanism of action is still doubtful which needs further studies to confirm its antihypertensive effect.

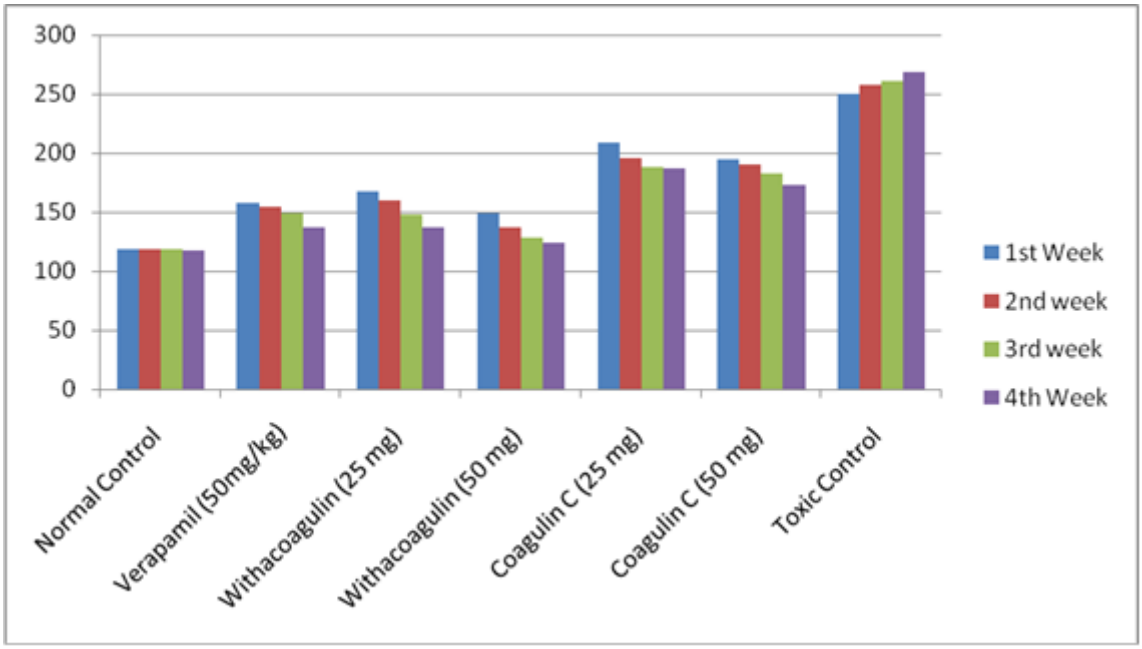

Figure 1 Comparative effect of withacoagulin and coagulin C on Systolic Blood Pressure of experimentally induced hypertensive animals

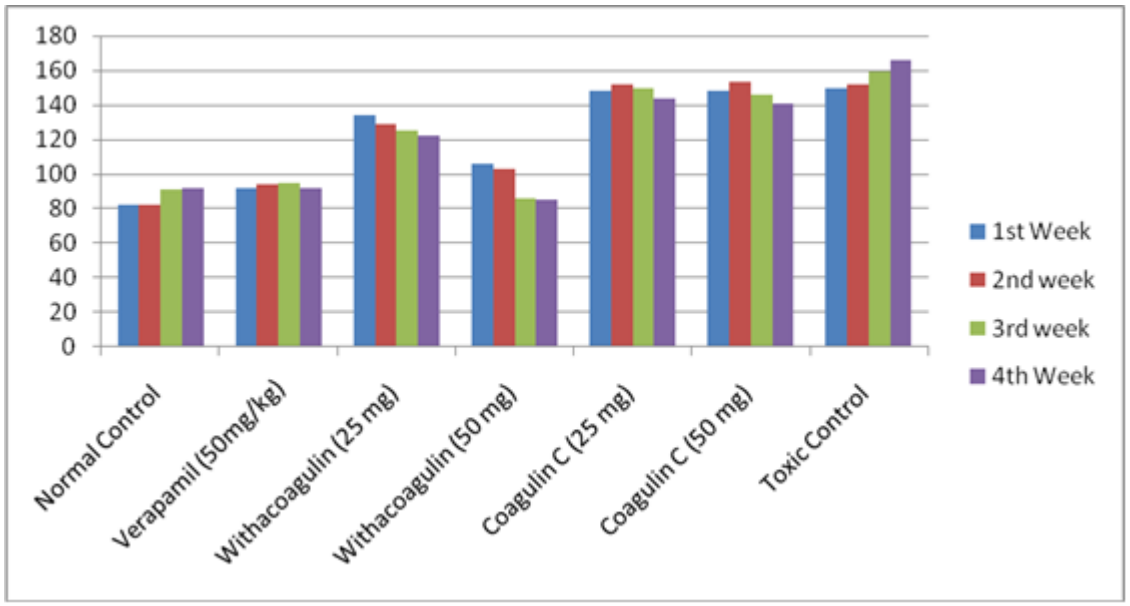

Figure 2 Comparative effect of withacoagulin and coagulin C on Diastolic Blood Pressure of experimentally induced hypertensive animals

Withacoagulin at a dose of 50 mg/kg in rats has better effect than standard verapamil which is a known antihypertensive drug in clinical use. Further studies can open a way for its clinical trial if studies confirm its safe use in experimental animals.

4. Conclusion

In vivo effect of withacoagulin and coagulin C isolated from *Withania coagulans* Dunal fruits were observed on blood pressure of albino rats. Withacoagulin at dose 25 mg/kg showed a significant decrease ($P< 0.05$) in the blood pressure of rats. Withacoagulin at 50 mg/kg dose produced highly significant antihypertensive effect ($P< 0.001$) as compared to standard calcium channel blocker, verapamil. Coagulin C has been found to have no significant antihypertensive effect on albino rats. Hence it can be concluded that withacoagulin may have potent antihypertensive effect although the exact mechanism of action of its antihypertensive effect needs further studies to confirm.

Compliance with ethical standards

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Disclosure of conflict of interest

The author(s) have no conflicts of interest to disclose with anyone else.

Statement of ethical approval

The study was approved by the Institutional Animal Ethics Committee (RIVTE/ IAEC/16/02) of Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, Uttar Pradesh (Animal Hose registration number: 385/PO/Re/S/01/CPCSEA).

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