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Research Article
Treatment with Tinosporaside Attenuates the Uterine Fibroid by Stimulating the Apoptosis

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Abstract

Background and Objective: Uterine fibroid is a gynecological disorder that causes infertility. Present study evaluated the protective effect of tinosporaside against the uterine fibroid (UF). **Materials and Methods:** Uterine fibroid was induced in all the animals by injecting diethylstilbestrol (2 mg mL^{-1}, i.m.) for the period of 4 weeks. All the animals were separated in to five groups such as control, UF and Tinosporaside 1, 10 and 50 mg kg^{-1} receives tinosporaside (1, 10 and 50 mg kg^{-1}, i.p.) 30 min before the injection of diethylstilbestrol for the duration of 4 weeks. At the end of protocol concentration of hormones (Estradiol and progesterone) in the serum was determined and later all the animals were sacrificed. Morphological features of uterus was estimated and activity of Nitric oxide synthase (NOS) and caspase 3 and the expressions of Bax, Bcl2, Akt and pAkt was estimated in the uterine tissues at the end of study. **Results:** Data of study revealed that treatment with tinosporaside significantly decreased the percentage of uterine coefficient and diameter of cervix compared to UF group. There was significant decrease in the serum concentration of estradiol and progesterone and activity of NOS in the uterine tissue of tinosporaside treated group compared to UF group. Moreover treatment with tinosporaside attenuated the altered expressions of several proteins (Bax, Bcl2, Akt and pAkt) in the uterine tissue of uterine fibroid rats. **Conclusion:** Present investigation concluded that tinosporaside protects the uterine fibroid by inducing the apoptosis of uterine cells.

Key words: Tinosporaside, uterine fibroid, apoptosis, nitric oxide synthase, estradiol and progesterone

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Uterine fibroid is the commonest gynecological disorder occurring in 80% of fertile females[1]. Uterine fibroid is the benign tumor of uterus. Anemia, infertility and abnormal menstruation are the clinical features of uterine fibroids[2,3]. One of the major approach used for the treatment of uterine fibroid is hysterectomy i.e., removal of uterus by surgical method and approximately 34.5 billion $ was used for the management of it[4]. Thus field of medicine is in an urgent need of a medicine that is effective in the treatment of UF.

In the recent era alternative medicine has shown potential effect in the treatment of chronic diseases. Moreover, drug molecules that are isolated from the herbal plant show the potency against the cancer. Tinospora cordifolia (Menispermaceae) was traditionally used as a medicine in China[5]. Diuretic, immunomodulatory, antioxidant, anti-diabetic, anti-inflammatory and anticancer activity of *T. cordifoliais* well documented[6-9]. Tinosporaside is a major alkaloid isolated from the root of *Tinospora cordifolia*. Literature also reveals the antihyperglycemic and anticancer (skin, liver and testis cancer) activity of tinosporaside[10-11]. However effect of tinosporaside against uterine tumor i.e., uterine fibroid was not evaluated. Thus present investigation examined the protective effect of tinosporaside on uterine fibroid.

MATERIALS AND METHODS

Animal: Female wistar rats (180-200 g) were purchased from Beijing Medical University, China. All the rats were kept under controlled condition as per the guidelines with pallet feed and water *ad libitum*. The study was performed for the period of 6 month from the month of 7th February, 2017-21st August, 2017. Investigation protocols of the present study approved by Institutional Animal Care and Use Committee (IACUC) of PLA Rocket Force General Hospital, China (IACUC/PLAGH/2017/05).

Induction of uterine fibroid: Uterine fibroid was induced in all the animals by injecting diethylstilbestrol (2 mg mL^{-1}, i.m.) for the period of four weeks and excluding control group which received equal volume of distilled water. All the animals were separated in to five groups such as control, UF and Tinosporaside 1, 10 and 50 mg kg^{-1} received tinosporaside (1, 10 and 50 mg kg^{-1}, i.p.) 30 min before the injection of diethylstilbestrol for the duration of 4 weeks.

Estimation of morphology of uterus: All the animals were sacrificed at the end of protocol by cervical dislocation and uterus was isolated from all the rats and uterine coefficient was estimated by comparing uterine weight with total body weight. Moreover diameter of cervix was also determined.

Estimation of biochemical parameters: Blood was withdrawn from the retro orbital plexus of and serum was separated out by centrifuging it at 2000× for the period of 10 min. Concentration of progesterone and estradiol by using ELISA kits. Moreover activity of NOS was also estimated in the tissue homogenate of uterus by using NOS kit. Methods used for the determination of serum concentration of progesterone and estradiol and activity of NOS in tissue homogenate as per the instruction of manufacturer of kits.

Western blot assay: Concentration of protein in the tissue homogenate of uterus was estimated by using Assay Reagent. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis 10% was used for the separation of protein and later separated protein was transferred by using Semi-Dry Transfer Cell to nitrocellulose membrane. Tris-buffer saline was used to block the membrane and thereafter primary antibodies such as caspase-3, Bax, Bcl2, Akt, pAkt and β-actin was used to incubate with the membrane after washing it. Membrane was washed and incubate with horseradish peroxidase-conjugated secondary antibodies. The density of respective bands were estimated by the Chemi-doc XRS imaging system.

Estimation of caspase-3 activity: About 50 μM of DEVD-pNA supplemented DTT Mix withan equal amount of tissue homogenate and later incubate it for the duration of 2 h at 37°C. Activity of caspase-3 was estimated at 405 nm of the cleaved substrate pNA followed by ApoAlert Caspase Colorimetric Assay kits.

Statistical analysis: All data were expressed as Mean±SEM (n = 6). The statistical analysis was performed using one way ANOVA. *Post hoc* comparison of means was carried out by Dunnett's *post hoc* test (Gradpad prism 6.1., CA, USA) multiple comparisons. The level of statistical significance was set at $p<0.05$.

RESULTS

Effect of tinosporaside on the morphological parameter: Effect of tinosporaside on the uterine coefficient and diameter of cervix in diethylstilbestrol induced uterine fibroid rat model

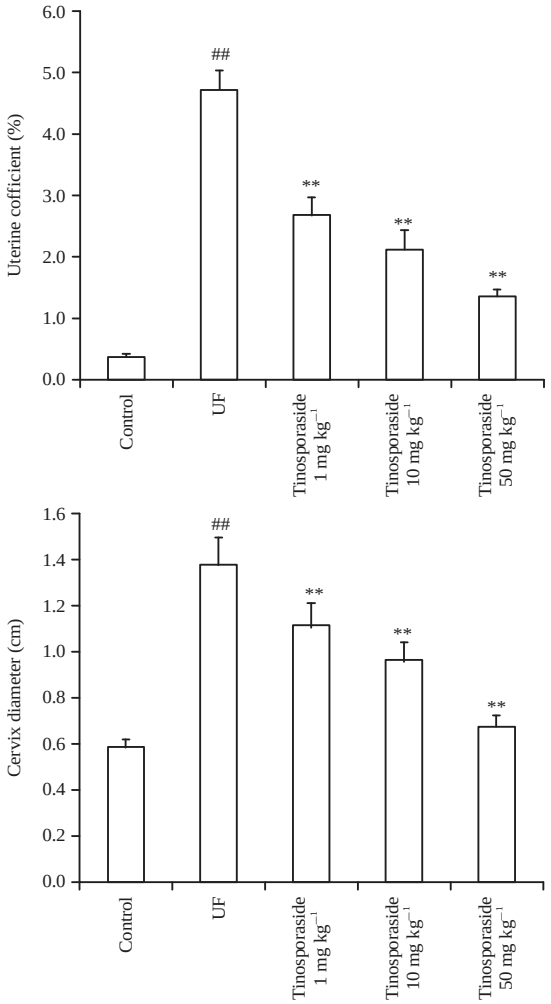

Fig. 1: Effect of tinosporaside on the uterine coefficient and diameter of cervix in diethylstilbestrol induced uterine fibroid rat model

Data was expressed as Mean±SEM (n = 6), ##p<0.01 compared to control group, **p<0.01 compared to UF group, UF: Uterine fibroid

was shown in Fig. 1. It was observed that there was significant increase in the uterine coefficient (%) and diameter of cervix in UF group compared to control group of rats. However treatment with tinosporaside significantly decreases the uterine coefficient (%) and diameter of cervix compared to UF group in a dose dependent manner.

Effect of tinosporaside on the biochemical parameter: Effect of tinosporaside on the serum concentration of progesterone and estradiol and activity of NOS in the tissue homogenate of diethylstilbestrol induced uterine fibroid rat was shown in Fig. 2. There was significant increase in the concentration of progesterone and estradiol in serum and activity of NOS in the tissue homogenate in tinosporaside treated group as compared to UF group of rats.

Effect of tinosporaside on the expressions of caspase-3, Bax, Bcl2, Akt and pAkt protein: Effect of tinosporaside on the expressions of caspase-3, Bax, Bcl2, Akt and pAkt protein in uterine tissue of diethylstilbestrol induced uterine fibroid rat was shown in Fig. 3. There was significant increase in the pAkt/Akt ratio and decrease in the Bax/Bcl-2 protein ration in uterine tissues of UF group compared to control group. However treatment with tinosporaside significantly attenuated the altered level of caspase-3, Bax, Bcl2, Akt and pAkt protein in uterine tissue of diethylstilbestrol induced uterine fibroid rat. Bars above the Fig. 3 was blots and according to the density of caspase-3, Bax, Bcl2, Akt and pAkt protein was shown in the bar graph.

Effect of tinosporaside on the activity of caspase-3 enzyme: Effect of tinosporaside on the activity of caspase-3 in uterine tissue of diethylstilbestrol induced uterine fibroid rat was shown in Fig. 4. It was observed that the activity of caspase-3 significantly enhanced in tinosporaside treated group compared to UF group in a dose dependent manner.

DISCUSSION

Present study evaluated the protective effect of tinosporaside on uterine fibroid in diethylstilbestrol induced uterine fibroid rat. Uterine fibroid was induced by injecting diethylstilbestrol for four weeks and at the end of protocol serum concentration of progesterone and estradiol and activity of NOS in tissue homogenate was determined. Expression of Bax, Bcl2, Akt and pAkt protein and activity of caspase-3 enzyme was estimated in the uterine tissue homogenate.

Literature revealed that hormone such as progesterone and estrogen and its receptors played important role in the pathogenesis of uterine fibroid[12]. In the development of uterine tumor concentration of hormone enhanced and the drug that used for the management of uterine tumor reduces the level of these hormones[13]. Data of the given study also represented that treatment with tinosporaside attenuated the altered level of hormone in uterine fibroid rats. In uterine tumor cell production of nitric oxide enhanced and NOS has a role in the production of nitric oxide[14]. Result of this study suggested that activity of NOS significantly reduced in tinosporaside treated group compared to UF group.

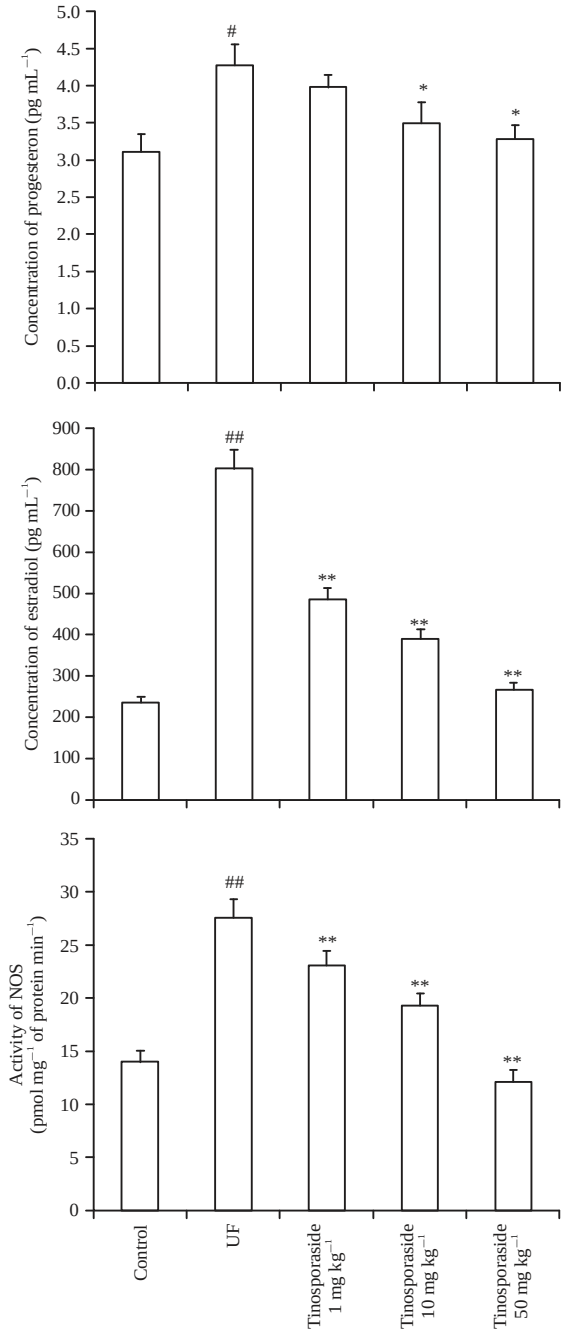

Fig. 2: Effect of tinosporaside on the biochemical parameters in diethylstilbestrol induced uterine fibroid rat model
Data was expressed as Mean±SEM (n = 6), ##p<0.01 compared to control group, *p<0.05, **p<0.01 compared to UF group, UF: Uterine fibroid

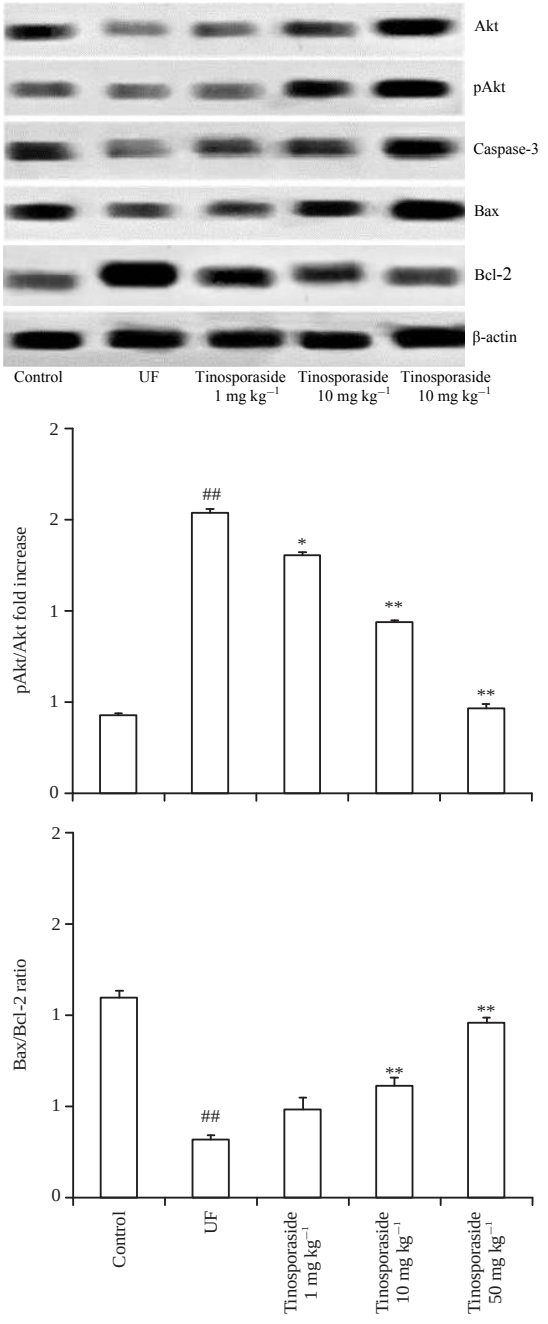

Fig. 3: Effect of tinosporaside on the expressions of caspase-3, Bax, Bcl2, Akt and pAkt protein in uterine tissue of diethylstilbestrol induced uterine fibroid rat model
Data was expressed as Mean±SEM (n = 6), ##p<0.01 compared to control group, *p<0.05, **p<0.01 compared to UF group, UF: Uterine fibroid

This study revealed that anti cancer drug enhanced the apoptosis of cells for the management of cancer[15]. Expression of several proteins such as Bax, Bcl-2 and caspase-3 alters in the cancer which blocks the process of apoptosis[16]. Treatment with tinosporaside attenuated the altered expressions of Bax, Bcl-2 and caspase-3 like proteins and thereby induced apoptosis of uterine cells. Several cellular functions including apoptosis controlled by Akt pathway and in cancerous cell regulation of Akt pathway altered[17]. Data of the given study revealed that tinosporaside attenuated the altered Akt pathway in tumor cells and thereby showed protective effect

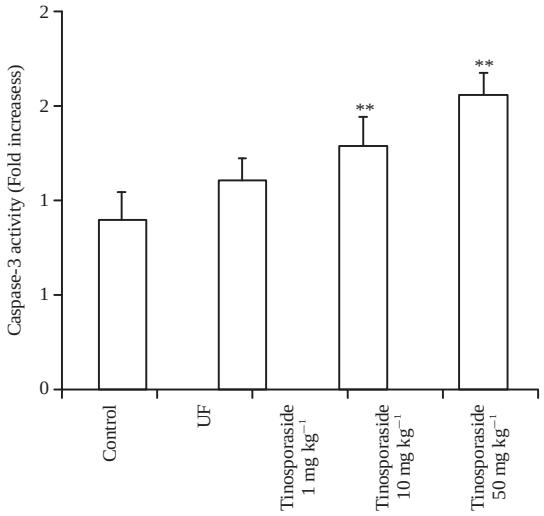

Fig. 4: Effect of tinosporaside on the activity of caspase-3 in uterine tissue of diethylstilbestrol induced uterine fibroid rat model

Data was expressed as Mean±SEM (n = 6), **p<0.01 compared to UF group

against uterine fibroid. Data of the study postulated the mechanism of protective effect of tinosporaside on uterine fibroid, as it induced apoptosis in uterine cells. However in future effect of tinosporaside on the modulation of gene need to be studied for understanding the mechanism of it on genetic level. Moreover tinosporaside effect required to estimate on uterine fibroid clinically.

CONCLUSION

Present investigation concluded that tinosporaside protected the uterine fibroid by inducing the apoptosis of uterine cells. Tinosporaside attenuated the altered concentration of hormones and activity of NOS in uterine cells and thereby inducing the process of apoptosis.

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SIGNIFICANCE STATEMENT

Present investigation confirms the beneficial effect of tinosporaside against the uterine fibroid. Study also postulates that tinosporaside ameliorates the uterine fibroid by inducing the apoptosis in uterine cells. Data of the study reveals that tinosporaside could be used clinically for the management of uterine fibroid.

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