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Effect of turmeric on adiponectin, sexual function and sexual hormones in stressed mice

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ABSTRACT

Sexual function is essential for species survival. Melanocortin, progesterone, and estrogen can improve sexual function and they are modulated by adiponectin hormone which can be increased by Turmeric. In various studies shows Turmeric ability that is easily accessible to increase serum adiponectin levels. Therefore, the researchers decided to conduct a study to determine the effect of turmeric on serum adiponectin levels, sexual behavior, and profile of steroid hormones in stressed mice. Thirty female mice, six in each group (1. control group, 2. mice that received stress, 3. stress mice received 100 mg/kg turmeric (extract daily) for 4 weeks, 4. stress mice received turmeric (extract daily) for 4 weeks and also received adiponectin antagonist, and 5. stress groups received adiponectin antagonist), were used in the current study. The mice first underwent blood sampling. Then all mice were subjected to stress testing before the intervention except one group, which considered as a control group. The intervention in this study was done as a 100 mg/kg turmeric extract that was gavaged daily for each mouse. After the intervention, all mice were tested for sexual behavior, and then blood samples were taken to check serum levels of adiponectin, estradiol, progesterone and prolactin. So, the results showed before the intervention there were no significant difference among 5 group in levels of adiponectin ($p = 0.145$), estradiol ($p = 0.148$), progesterone ($p = 0.166$) and prolactin ($p = 0.206$) but after intervention there were significant difference between 5 group in levels of adiponectin, estradiol and progesterone ($p < 0.001$). Also there was significant difference among 5 groups in sexual behavior ($p < 0.001$). Therefore, consumption of turmeric, which increases serum adiponectin in the stressed mice, can improve sexual function and estradiol hormones profiling.

1. Introduction

Sexual behavior is essential for species survival and all creatures use sexual behaviors to have more pleasure and communication during sexual activity [1]. Previous studies have shown that stress has a negative effect on the sexual activity [2] and also it causes sexual dysfunction [3]. In vertebrates, hyper activation of the hypothalamic-pituitary-adrenal (HPA) axis is the main physiological response to stress [4]. The HPA axis plays an inhibitory role on the female reproductive system [5] and it regulates by corticotrophin-releasing hormone (CRH) [6]. CRH inhibits the secretion of hypothalamic gonadotropin-releasing hormone (GnRH), and also suppresses pituitary luteinizing hormone (LH), ovarian estrogen, progesterone secretion and it resists target tissues to estradiol [5]. However, sexual function is affected by the level of estrogen, progesterone, prolactin and testosterone as a reproductive hormone and it plays vital roles in the female libido. Female reveals special posture and different behavior during mating such as receptivity. It is determined by the level of E2 following the secretion of progesterone and it is limited to the estrous phase.

Adiponectin is another hormone that plays a role in reproductive system, therefore it involves in regulation of the progesterone and estrogen level and possibly improves sexual function [7]. Adiponectin and its receptors were found in the reproductive organs (such as the ovaries, oviduct and endometrium) of many animals, including rats,

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mice, humans, and pigs [8–13]. This extensive distribution shows a potential association of this hormone in the reproductive system functions. In addition, adiponectine modulates gonadotropin release and normal pregnancy [14]. Previous studies reported that adiponectin regulates oocyte maturation, granulosa cell proliferation and steroid secretion [15,16]. Animal studies have shown that in adiponectin knocking out mice decreased oocytes, increased atretic follicles and prolonged diestrus cycles, therefore adiponectin have a key role in folliculogenesis [17]. Currently, researcher reported that stress decreased adiponectin levels in an animal model of depression, post-traumatic stress disorder and other anxiety related disorders [18].

In the last half a century, large studies indicated the protective effects of turmeric in about all the metabolic disorder. It is identified to possess anti-inflammatory, anti-hypertensive, anti-hyperlipidemic, anti-diabetic, and many other beneficial effects [19]. Li and colleagues reported that turmeric decreased the level of serum corticosterone in rats and effectively improved chronic unpredictable mild stress [20]. The importance of turmeric in traditional medicine in the treatment of sexual reluctance has been discussed in the study of Qureshi et al. Besides, turmeric is a cost-effective and easily accessible plant and also common in daily consumption [21]. In various studies, it increases serum adiponectin levels [22], but the effect of turmeric on serum adiponectin and sexual hormones levels and also sexual behavior following stress remains unclear. Therefore, this study was done with the aim of evaluation of turmeric effects on adiponectin, sexual function and sexual Hormones in stressed mice (Table 1).

2. Materials and methods

Female C57BL/6J mice were purchased from Pasteur Institute of Iran and were housed under controlled room temperature 23 ± 2 °C, and relative humidity of 50 ± 6%, in a 12:12 h light–dark-cycle (light on at 7 am) with ad libitum access to food and water. Female mice at 8 weeks of age were used for the experiments. The protocol of animal care was approved by the ethics committee of the Research Institute for Tarbiat Modares University with ethics code of IR.MODARES.REC.1397.206.

2.1. Experimental design

Mice were randomly divided into 5 groups of 6 mice each: A control group were kept without any intervention (C); control mice that received stress (S); stress mice received 100 mg/kg turmeric (extract daily) for 4 weeks (ST); stress mice received 100 mg/kg turmeric (extract daily) for 4 weeks and also received 6 nmol/day adiponectin antagonist (STA); stress groups received 6 nmol/day adiponectin antagonist (SA).

2.2. Turmeric extraction

Rhizome of turmeric (*Curcuma longa* of the ginger family, Zingiberaceae) was bought from Perfumes of Tehran at 2019 on February then was milled. 100 mg powder with 200 ml of ethanol %80 was extracted in Soxhlet extractor. In this method, 11.5 g of dry extract was obtained from 100 g of turmeric rhizome.

2.3. Forced swim stress test

The mice were put into a transparent cylinders (25 cm in height and 10 cm in diameter) filled with 19 Cm water. The water temperature was 25 °C and the test was acceptable when mice floated and held its head above the water (this position must last for at least 4 min). This test was performed once before the intervention [23,24], because stress decreases adiponectin level.

2.4. Measurement of adiponectin and sexual hormones

After 12–14 h fasting, blood samples were collected and centrifuged at 4000 rpm for 10 min [25]. Serum adiponectin, estradiol, progesterone and prolactin concentration were measured using a mice ELISA kit (Zell Bio GmbH, Ulm, Germany); the intra-assay coefficient of variation (CV) was 6.3%. The sensitivity of the assay was 0.1 μg/ml. A standardized checklist was utilized to gather anthropometric and laboratory data.

2.5. Sexual function

Sexual behavior was studied when the female mouse was in the estrous stage because the highest rate of sexual activity of female mice is in this phase (the female mouse is in her estrous phase every 4 or 5 days) and it was assessed by vaginal cytology [26]. For this purpose, vaginal samples were collected through cotton swabs soaked in saline. Cotton swabs were completely rotated in the posterior and anterior walls of the mouse's vagina and were rolled on glass lam after that it was dried in the air. In the next step, staining was performed for 45 s with 400 ml of stain. The lams were washed and were immediately observed under a bright background microscope with magnifications of 200×. In the proestrus stage, nucleated epithelial cells and several mature epithelial cells and leukocytes may be seen. The characteristic of the estrous stage is the emerge of mature epithelial cells [27].

After confirmation of estrous stage, a female mouse was placed in a glass box 45 × 60 × 60 cm for 5 min to get used to the environment and then the male mouse was added. Each test took 10 min. If the intromission was not observed within the first 10 min of the test, the male was called non copulatory and was replaced by another male mouse. The sexual behavior of the female mouse was calculated by dividing the number of times the female mouse was receptive (receptivity) by the number of times the mount multiplied by 100 [28]. In the receptivity state, the female mouse bends her spine, get bending posture and put her legs in a standing position. We also measured the rate of sexual function with the rate of pregnancy in mice in each group.

$$Sexual\ behavior = \frac{Receptivity}{The\ mount} \times 100$$

Table 1
Comparison of adiponectin, estradiol, progesterone and prolactin hormone levels in different groups before the intervention.

Group	Hormone			
	Adiponectin	Estradiol	Progesterone	Prolactin
C	4.50 ± 0.212	69.260 ± 4.235	12.200 ± 0.644	9.240 ± 0.581
S	3.983 ± 0.491	61.800 ± 5.031	10.657 ± 1.110	8.257 ± 0.680
ST	4.0714 ± 0.402	67.133 ± 4.578	12.116 ± 1.248	8.950 ± 6.123
STA	8.100 ± 1.644	10.583 ± 2.528	59.733 ± 11.186	4.500 ± 0.469
SA	4.140 ± 0.482	61.725 ± 6.983	10.480 ± 1.454	8.140 ± 0.838
p-Value	0.206	0.166	0.148	0.145

2.6. Statistical analysis

Data was reported as means ± SEM. Statistical analysis was performed using Graph Pad Prism software (Version 6). Shapiro-Wilk test was used to check the data normality and parametric or non-parametric statistical tests were used for the analysis of normal and non-normal data distribution, respectively [23]. Two-way mixed (between-within) analysis of variance (ANOVA) test used to compare adiponectin, estradiol, progesterone and prolactin hormone level. One-way ANOVA was used for comparing of sexual behavior. Two-sided p values < 0.05 were considered statistically significant. Chi square test used to compare rate of pregnancy between 5 groups (Table 2).

3. Results

3.1. Adiponectin hormone levels

The results showed that before the intervention, there was no significant difference in adiponectin hormone level between the five groups ($p = 0.145$). After intervention, compared to controls (4.662 ± 0.130), adiponectin hormone levels was significantly ($p < 0.05$ and $p < 0.01$) lower in STA (3.666 ± 0.647) and SA (3.060 ± 0.151) mice respectively. Adiponectin hormone level was not changed in stress (4.225 ± 0.446) and ST (5.166 ± 0.665) compared to control groups (Fig. 1). In ST group oral administration of turmeric significantly ($p < 0.01$) increased the adiponectine level compared before turmeric administration and using turmeric antagonist significantly ($p < 0.05$) decreased adiponectin level in STA groups, but in this study adiponectin levels was not changed in stress and SA groups.

3.2. Estradiol hormone levels

Estradiol levels were similar between all groups before starting the intervention ($p = 0.148$). After intervention in STA (55.26 ± 7.538) and SA (51.948 ± 2.378) groups the level of estradiol significantly ($p < 0.01$) was lower than control (69.460 ± 4.235) but it was not changed in stress (63.037 ± 5.609) and ST (71.633 ± 2.967) groups. After oral administration of turmeric the level of estradiol significantly ($p < 0.01$) increased in ST group (71.633 ± 2.967) (Fig. 2).

3.3. Progesterone hormone levels

The level of progesterone was similar in all groups before intervention ($p = 0.166$). As shown in Fig. 3, compared to the controls, the level of progesterone hormone decreased in SA groups. Turmeric significantly ($p < 0.05$) increased the progesterone level compared to stress group. Progesterone level was not changed in stress (10.525 ± 1.516), ST (13.650 ± 0.845) and STA (10.333 ± 1.399) groups compared to control (12.380 ± 0.672) groups (Fig. 3).

3.4. Prolactin hormone level

The level of prolactin was not significant difference in all groups before intervention ($p = 0.206$). After intervention the level of prolactin was not significant difference but decreased and analysis of Cohen's d showed an effect size was large (0.836, 1.285, 1.581 and 2.417) in ST, S and STA groups respectively (Fig. 4).

3.5. Sexual function

Sexual activity significantly decreased in S ($p < 0.001$), SA ($p < 0.0001$) and STA ($p < 0.0001$) groups. Mean ± SEM of S, SA, and STA groups was (10.525 ± 1.516), (8.380 ± 0.952) and (10.333 ± 1.399) respectively. Compared to stress group, the sexual behavior was significantly decreased in S, STA and SA groups. There was a significant difference between the groups in terms of the number of pregnant mice ($p = 0.042$). The highest rate of pregnancy was in ST group (100%). Compared to stress group, the rate of pregnancy was significantly decreased in S, STA and SA groups.

4. Discussion

According to our knowledge, this study is the only one that has focused on the effect of turmeric extract on adiponectin levels, sex hormones and sexual behavior.

In our study consumption of turmeric extract increased sexual behavior in the intervention group, noting that sexual behavior in groups under antagonist injections was milder so this could due to the increasing in adiponectin levels and its effect on sexual behavior. Different types of stress reduce sexual behavior. Also, stress increases catechol amines in the brain that regulate sexual behavior [29–31].

On the other hands, according to various studies, stress testing causes lower adiponectin levels in mice [18,32]. In the present study, the consumption of turmeric extract increased serum adiponectin levels. Consumption of turmeric extract in intervention group even increased serum adiponectin levels more than control group mice (without stress). As expected, the stress reduced serum adiponectin levels. Adiponectin reduces TNFα levels and increases interleukin 10 levels. There is a strong association between the endocrine system and the nerves. Adiponectin is found in cerebrospinal fluid and affects the neuronal excitability of synaptic plasticity and the release and remodeling of neurons [18,32]. Also, in the study of Ismaili et al., turmeric is mentioned to have effects in reducing stress. Alcoholic extract of turmeric controls anxiety and depression by neurotransmitters such as dopamine, serotonin and norepinephrine. In fact, alcoholic extract of turmeric regulates serotonin by regulating the expression of mRNA5HT1A, and curcumin can prevent Alzheimer's and Parkinson's disease by regulating neurotransmitters. Teixeira et al.'s study investigates the effects of adiponectin in the treatment of Parkinson's disease [33]. Many studies have also addressed the issues of anxiety and stress in obese people with metabolic syndrome, which can be attributed to their low adiponectin levels [33,34]. The study of Salahshooh and colleagues also showed the effect of increasing adiponectin following the turmeric consumption. Also, in a study by Salahshooh et al. fat cells

Table 2
Comparison of adiponectin, estradiol, progesterone and prolactin hormone levels in different groups after the intervention.

Group	Hormone				Sexual behavior
	Adiponectin	Estradiol	Progesterone	Prolactin	
C	4.662 ± 0.130	69.460 ± 4.235	12.380 ± 0.672	9.040 ± 0.487	71.917 ± 2.720
S	4.225 ± 0.446	63.037 ± 5.609	10.525 ± 1.516	8.137 ± 0.866	73.952 ± 2.953
ST	5.166 ± 0.665	71.633 ± 2.967	13.650 ± 0.845	8.266 ± 1.214	65.094 ± 2.22
STA	3.666 ± 0.647	55.26 ± 7.538	10.333 ± 1.399	8.000 ± 0.792	58.087 ± 2.528
SA	3.060 ± 0.151	51.948 ± 2.378	8.380 ± 0.952	7.560 ± 0.716	54.751 ± 2.057
p-Value	<0.001	<0.001	<0.001	0.133	<0.001

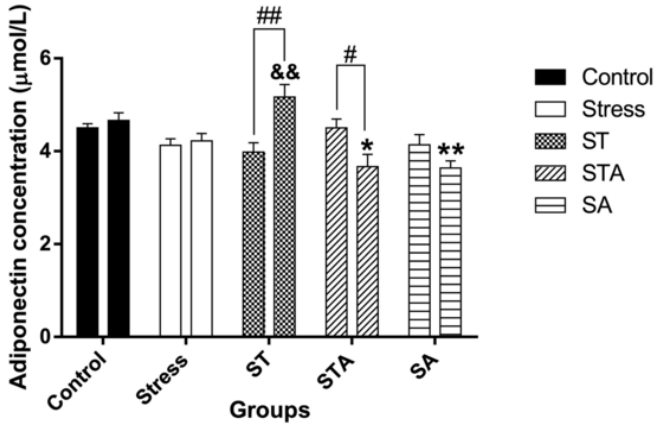

Fig. 1. The effect of turmeric on adiponectin level. Results are mean ± SEM (N = 10/group). *p < 0.05 and **p < 0.01 compared to control, &&p < 0.01 compared to stress group and #p < 0.05 and ##p < 0.01 compared to before intervention.

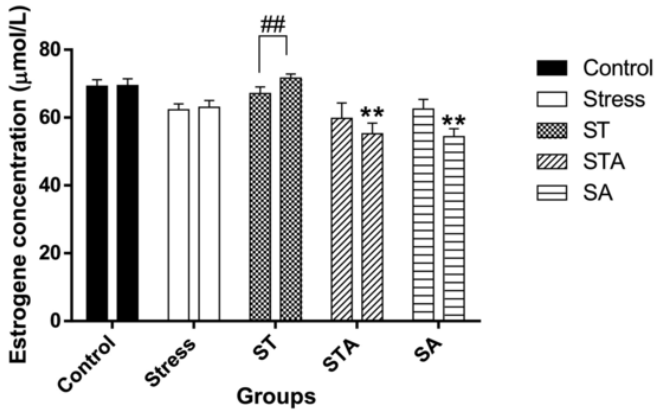

Fig. 2. The effect of turmeric on estradiol level. Results are mean ± SEM (N = 10/group). **p < 0.01 compared to control and ##p < 0.01 compared to before intervention.

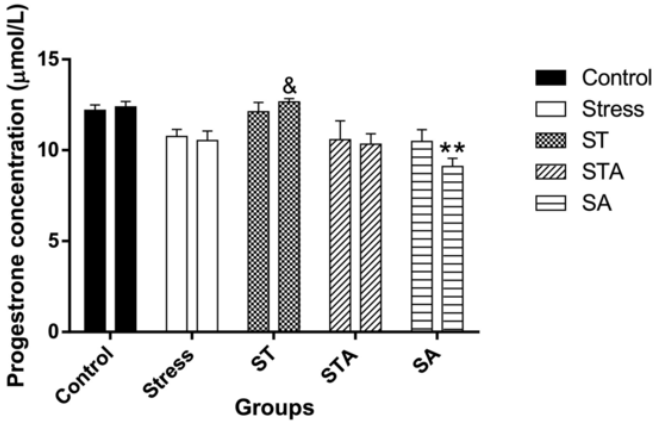

Fig. 3. The effect of turmeric on progesterone level. Results are mean ± SEM (N = 10/group). **p < 0.01 compared to control and &p < 0.05 compared to stress group.

were picked up following visceral fat surgery and were treated with curcumin. As a result, the secretion of interleukin 6 and adiponectin from these cells increased [35]. Turmeric extract protects against oxidative stress by creating a balance between antioxidant defense and the ROS (reactive oxygen species) system, and we can consider the balance process as a way of impact on the endogenous system [36] (Fig. 5).

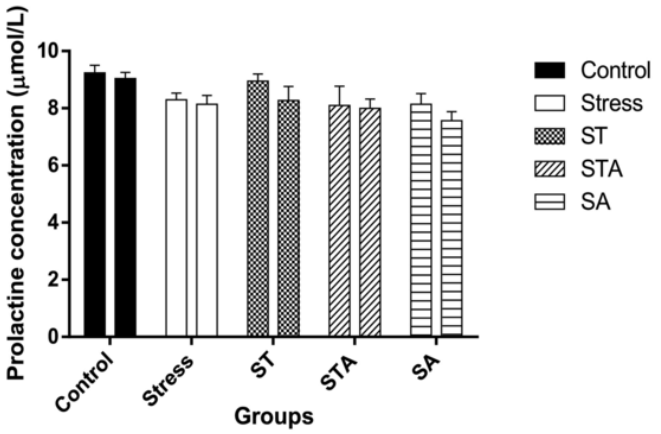

Fig. 4. The effect of turmeric on prolactin level. Results are mean ± SEM (N = 10/group).

According to various studies, there is a link between adiponectin levels and ovarian steroidogenesis in stressed mice [37]. Many articles have suggested a link between increase in the level of steroid hormones and improved sexual behavior. In fact, the antioxidant and anti-inflammatory activity of curcumin improves sexual activity [6]. In our study consumption of turmeric extract also increased estradiol and progesterone levels in the intervention groups to a level measured in the controlled group. In this regard, our study is consistent with the study of Takor et al. According to the findings of their study, the alkaloids and flavonoids in the alcoholic extract of turmeric increase the level of estradiol, which in turn increase the size of the uterus and ovaries [38].

Turmeric extract in rat mice has a proapoptotic, pronerotic and antiproliferative effect and protects oogonia cells against stress. The mechanism by which turmeric affects the reproductive system is still unknown but it can be dependent on the growth of follicular blood vessels by gonadotropins, ovarian steroids, and peptide hormones such as leptin and adiponectin. In the present study, the use of turmeric extract in antagonist injection groups did not increase estradiol and progesterone levels. According to these findings, it can be claimed that turmeric extract has an increasing effect on estradiol and progesterone levels through adiponectin [39]. Barbe et al. have studied the effect of adiponectin on increasing estradiol and progesterone, and according to

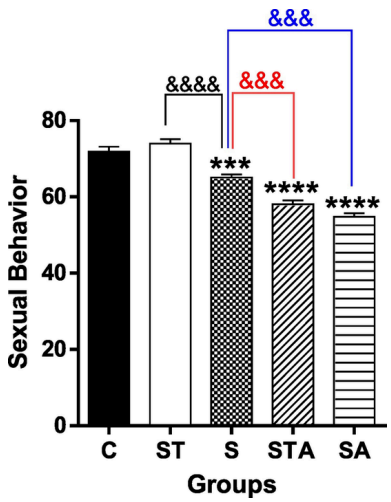

Fig. 5. The effect of turmeric on sexual behavior. Results are mean ± SEM (N = 10/group). ***p < 0.001 and ****p < 0.0001 compared to control, &&&p < 0.001 and &&&&p < 0.0001 compared to stress group.

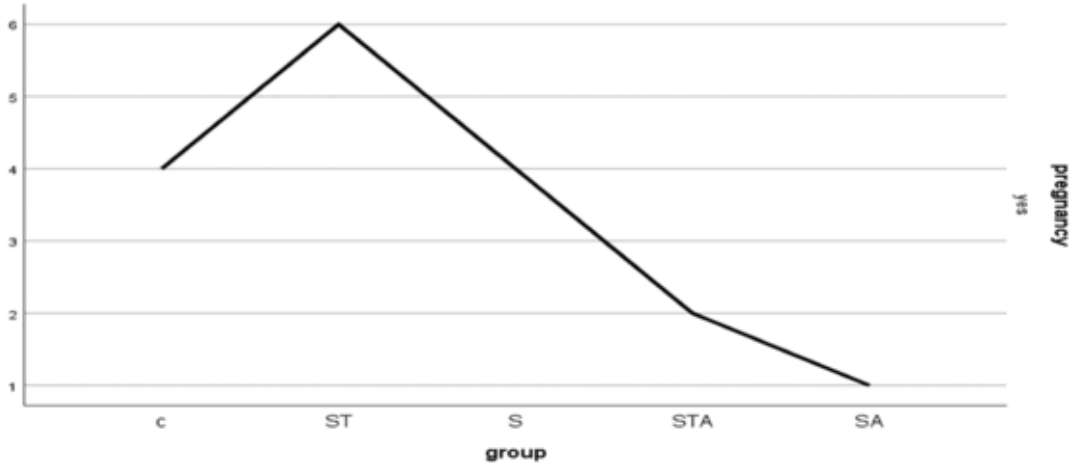

Fig. 6. The effect of turmeric on pregnancy rate in each group.

their findings, adiponectin increases the steroidogenesis of ovarian granulosa cells. There is evidence of existing of adiponectin receptors Adipor1 and ADIPOR2 in the hypothalamus, which can play a role in the maturation and reproductive. A mutation in the adiponectin gene reduces GNRH and disrupts the mice's estrous cycle. The presence of adiponectin in the cerebrospinal fluid may indicate its role in the pituitary hypothalamic axis [37]. A study by Rahal et al. showed that adiponectin increases the secretion of estradiol and progesterone from granulosa cells. In addition, the removal of the adiponectin gene in mice causes problems with steroidogenesis and impairs the fertility and maturation of follicles. In chickens, adiponectin can induce IGF1, which increases the secretion of progesterone from follicle cells F2 and F3 by follicles. Contact with isoflavonoids increases adiponectin levels [22] (Fig. 6).

5. Conclusion

According to the data obtained in this study, it can be expected the consumption of turmeric which increase serum adiponectin in the stressed population can improve sexual behavior and profile of steroid hormones by increasing the serum adiponectin levels.

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Ethics approval

The ethics committee of study Tarbiat Modares University with ethics code of IR.MODARES.REC.1397.206.

Availability of data and material

Data supporting our findings can be sent upon request.

Declaration of competing interest

The authors declare that there is no conflict of interest between authors.

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