



## Music Therapy in Traditional Chinese Medicine Attenuates the Depression-Associated Breast Cancer Development in MMTV-PyMT Mice and Clinics

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### ABSTRACT

**Background:** Depression is a mental illness that disturbs an individual's health, which exacerbates the development of BC and reduces the quality of an individual's life. **Objective:** To investigate the impacts of Traditional Chinese Medicine Music (TCMM) intervention on the depressed BC mouse model and explore the potential underlying mechanisms. **Methodology:** The intervention of TCMM and its impact on the body weight, tumor size, tumor weight, and behavioral tests were conducted in Chronic Mild Stress (CMS) induced MMTV-PyMT mice. Furthermore, CMS-induced oxidative stress-related Thioredoxin-1 (TRX-1) was also determined using western blot. A clinical trial was conducted to validate the effect of stress-related TRX-1 during the intervention of TCMM. **Results:** The outcome of the study showed that mice under the environment of TCMM significantly reduced the breast tumor size, tumor weight, and behavioral changes. TRX-1 was significantly increased in the CMS-induced BC mice which were attenuated by the intervention of TCMM. More interestingly, plasma TRX-1 was also significantly reduced using the intervention of TCMM in BC patients. **Conclusion:** Based on the experimental and clinical findings, we strongly suggest that TCMM therapy could decrease CMS-induced tumor development in the breast and improves the quality of life in depressed BC individuals. Furthermore, the relationship between TRX-1 and TCMM has to be investigated, which is now underway.

**Keywords:** TCM music, CMS, Breast cancer, Experimental evidence, Clinical trial, Thioredoxin

### INTRODUCTION

Breast Cancer (BC) is one of the most common cancers and the foremost cause of mortality among women. According to the World Health Organization in 2018, BC has a higher prevalence of 11.6% among all categories of cancer, estimating about 6.5% of deaths globally [1]. The recurrence and metastasis are the primary causes of mortality in BC. In developing nations, the mortality rate related to BC is still greater because of an ineffective diagnosis and pharmacological treatment, which gives the highest death toll annually [2]. The screening and treatment of BC are often associated with unfavorable side effects, alterations in the physical states, reduced social contacts, and weakening of an individual's quality of life [3]. BC patients have been experiencing tremendous hurt from negative emotions under chronic psychological stress, anxiety, subsequent angry and depression [3,4]. Several clinical studies have also been indicated that patients with BC suffer a greater incidence of mental illnesses when compared to the common population [5-7]. Most of the prospective population-based studies have confirmed that depression is greatly linked with the demise in younger individuals with early stages of BC [3,6,8,9]. The outcome of meta-analyses data from depressed individuals of BC has shown the higher mortality rate in both depression (32.2%) and anxiety (41.9%)

[4,10]. Remarkably, most animal model studies have also related to chronic psychological stress and depression that promote tumor growth, angiogenesis, invasion, and metastasis in the liver, pancreas, lung, ovary, and breast [11-15].

Music is generally believed to aid bliss and calmness, thus giving the natural quality of healing. It gives an emotionally charging charisma of its own and facilitates relaxation by masking environmental noises and changing a person's attention to be a more pleasant emotional state [16-18]. The treatment of BC *via* Traditional Chinese Medicine (TCM) therapies has several benefits. Listening to Chinese music under TCM theory instead of metal music could help to reduce negative emotions, increase relaxation and prevent further emotional stress [19]. Music therapy is one of the non-pharmacological treatments, has been traditionally used in the treatment of emotional stress related to BC individuals [20]. Based on music therapy in TCM theory, five phases and five music scales are existing; they are Gong (do), Shang (re), Jue (mi), Zhi (so), Yu (la) [21]. TCMM and their interventions have significant effects in treating various psychological disorders, depression, stroke, Alzheimer's, dementia, reduce perinatal mental health and labor pain, various cancers and improve fibromyalgia syndrome [16,17,20,22-27]. These interventions are generally cost-effective and play crucial roles in alleviating various mental and behavioral disorders [22]. Earlier, the outcomes of a single-pilot study have recommended that music listening decrease the requirement for anesthetics, analgesics and reduce recovery time as well as decrease the duration of hospitalization [16]. Zhang, et al. reported that five-phase music therapy improves the immunological function and performs a function in adjuvant therapy in the Chinese syndrome of liver-qi stagnation and spleen qi deficiency [19].

There are several studies on the pathogenesis of depression that promotes the development of BC, however, no study has interpreted the pathogenesis from a single systematic investigation, as the development of BC involves multiple factors [23]. The treatment is extremely complicated, and the effect of advanced prescriptions is inadequate. Hence, the present study aimed to investigate the impacts of TCMM intervention on the Chronic Mild Stress (CMS) induced BC mouse model and BC patients and explore the potential underlying molecular mechanisms.

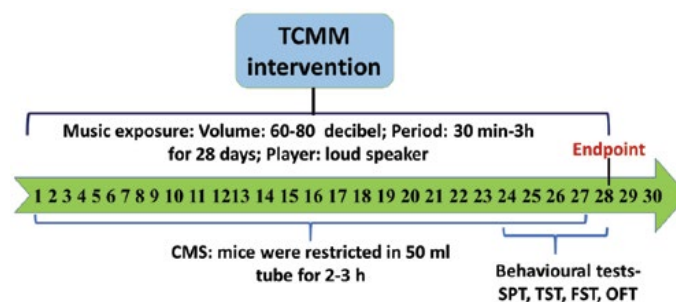
## MATERIAL AND METHODS

### Traditional Chinese Medicine Music (TCMM) Intervention

TCMM was composed by Wu Shen using Wood sound (Jiao). This folk music was prepared under the guidance of TCM, based on the five elements with emotions.

### Experimental Animals

Female MMTV (Mouse Mammary Tumor Virus)-PyVT (Polyomavirus middle T antigen) (+/-) mice (4 weeks old) were housed and maintained in the Laboratory Animal Unit, the University of Hong Kong, a specific pathogen-free and climate-controlled room ( $25 \pm 2^\circ\text{C}$ , 60% relative humidity) with a 12 h light/dark cycle and provided with diet and water ad libitum. All experiments were approved by the Institutional guidelines of Laboratory Animal Care and Committee on the Use of Live Animals in Teaching and Research (CULATR No.: 3228-14). Animals were randomly divided into four groups based on treatment with or without the intervention of TCMM, *viz.*, sham control group (Sham, n=15); Chronic Mild Stress (CMS, n=15) group; CMS with the intervention of TCMM (CMS+TCMM, n=15) group; the intervention of TCMM (TCMM, n=15) group. At the endpoint (day 28) (Figure 1), body weight and tumor weight were determined, and collected mammary tissues for the analysis of Thioredoxin (TRX-1) expression.



**Figure 1** Schematic representation of TCMM intervention in CMS induced mice; music exposure of TCMM was 30 min to 3 h with a volume of 60 dB-80 dB for 28 days; behavioral tests were conducted during 24<sup>th</sup> to 28<sup>th</sup> days; after the endpoint (day 28), animals were sacrificed

### Chronic Mild Stress Paradigm

The CMS paradigm was made using restraint in a plastic tube (2 h). Briefly, all mice were freely accessed food and water in the same environment. The experimental animals were carried out CMS on day 12, which was placed in the plastic centrifuge tube (50 ml) with 7 breathing holes of 0.4 cm-0.5 cm in diameter. Mice were plugged in the centrifuge tube and filled with a piece of tissue (20 cm × 20 cm) to limit their movement. Mice were initially restricted to 2 hours per day for a week and extended 3 h per day in the subsequent weeks. The same stress was applied on regular basis until the endpoint. The mice in the sham group and TCMM were not disturbed except for feeding and cage cleaning.

### Behavioral Testing

**Sucrose preference test:** Sucrose preference test is employed as symptoms of anhedonia, which is a main sign of depression. This test was conducted as described in the earlier publication with some modifications [28]. All the mice were offered two pre-weighed bottles containing sucrose solution (1%) or Milli-Q water on day 24, until the endpoint. The locations of the bottles were changed every 12 h to avoid the impacts of location preference on drinking behavior. The consumption of Milli-Q water and sucrose solution was measured 12 h after weighing the bottles.

**Open field test:** The experimental mice were subjected to an open field box, which was a white plastic box (40 × 40 × 40 cm) constructed with plywood. The box was divided into 2 parts, a central field (center, 20 cm × 20 cm) and an outer field (periphery) [29]. Mice were placed individually in the corner of the open field apparatus, total distance moved by the mice in 30 min which was analyzed using a video-tracking program (EthoVision, version 7.0, Noldus, Wageningen, The Netherlands). After each test, the box was cleaned with 70% ethanol using gauze to remove odor cues.

**Tail suspension test:** The tail suspension test was employed as depicted in the earlier publication [30]. The tails were sheathed with a long straw (5 cm), in such a situation mouse was not mobilized or hold on to nearby surfaces. This test was typically six minutes in duration, ensuing escape adapted behaviors were calculated using EthoVision® XT. The test was recorded using video-tracking (EthoVision, version 7.0, Noldus, Wageningen, The Netherlands).

**Forced swimming test:** The forced swimming test is a commonly applied animal behavioral paradigm to determine depression-like behavior. This paradigm was employed as described in the earlier publication with slight modifications [28]. The mice were placed in a glass beaker (2 Litre) filled with 25°C water. This behavioral paradigm was documented by a video camera. The test generally lasted about 6 min and the immobility time during the last 4 min was then examined and calculated using EthoVision® XT (EthoVision, version 7.0, Noldus, Wageningen, The Netherlands).

### Western Blot Analysis

Western blot assay was conducted as previously described [31]. The proteins from the cell were lysed in RIPA buffer (pH=7.4) comprised of protease inhibitors cocktail (10 µg/ml, Cat# 5872S, Cell Signaling Technology, MA, USA). The contents were centrifuged at 10,000 g at 4°C for 15 min and the concentration of protein in the supernatants was determined using Bradford reagent (BioRad) with bovine serum albumin (BSA, Sigma Aldrich, St. Louis, MO, U.S.A.) as the standard. The protein samples were separated by electrophoresis on SDS-PAGE 10% or 12.5% gels. After blocked in 3% BSA, the membrane was incubated with primary antibodies, β-actin (Cat# 4967, Cell Signaling Technology), and Thioredoxin (Cat# 2298, Cell Signaling Technology), as needed. For secondary antibodies, antibodies to mouse (Cat# 7076, Cell Signaling Technology) and rabbit (Cat# 7074, Cell Signaling Technology) were used. To visualize protein bands, a chemiluminescence (ECL) system (Cat# WBLUF0500, Millipore, MA, USA) was used.

### Perspective Clinical Trials

To verify the impact of TCMM on BC in the clinic, we recruited 32 BC patients in which they were randomly divided into 2 groups namely, the control (n=16) and treatment group (n=16) who had undergone the surgery within a month.

### Ethical Consideration

Ethical approval was granted from the research ethical committee of The Guangdong Hospital of Traditional Chinese Medicine, Guangzhou, China (Reference No. B2016-043-01). The study must be performed under international ethical standards and approved by the equivalent human ethics committee. Confidentiality and anonymity were guaran-

teed throughout the study. An informed consent form was signed by all breast cancer patients who met the inclusion criteria. Participants also gave verbal agreement to receive the intervention of TCMM before each session.

**Inclusion criteria:**

- A clinical diagnosis of BC stratified by the stage of cancer (I, II, and III) and were receiving recent surgery
- Aged 28-65 years old

**Exclusion criteria:**

- A history of repeated suicidal behavior
- Acute and severe substance misuse
- The severity of depression prevented them from participating in the measurements or engaging in a verbal conversation
- Insufficient knowledge of filling the questionnaire.
- Current participation involved in any other behavioral or pharmacological study or instructor-led exercise program
- A music background or job-related to music

For the TCMM intervention, folk music was used for treatment groups (n=16) based on the five elements with emotions which were used one hour/daily for 30 days under the guidance of TCM. After 30days, the blood samples were collected from both control and treatment groups to determine TRX-1.

**Determination of Thioredoxin Levels in the Blood**

Before and after TCMM intervention, blood samples were collected from the forearm vein of the individual in the morning, after overnight fasting. 5 ml of EDTA-containing tubes were used to collect the blood. The samples were centrifuged at 3000 rpm for 5 minutes, and plasma was obtained and stored at -80°C for the assay. Plasma TRX-1 levels were established using the method of sandwich ELISA (Bio-Rad, Model # 680, with Microplate Reader). Briefly, the 100 µl of standard, sample, and blank were added in designated wells. Wells were blocked with bovine serum albumin and then, incubated at room temperature for 90 min. Wells were incubated with diluted biotinylated detection antibody at room temperature for 1h, followed by incubation with HRP conjugate at room temperature for 30 min. In the next step, each well was incubated with the substrate at room temperature for 15 min to measure the chromogenic reaction that forms an Ag-Ab complex. Finally, an appropriate cessation solution was added to stop the reaction. The absorbance was measured using 450 nm filters by an ELISA reader.

**Statistical Analysis**

Quantifications and statistical analysis were carried out on GraphPad Prism 8.43. Data were analyzed with one-way analysis of variance (ANOVA) followed by group comparisons using a post hoc Tukey's multiple comparison test. For the assay of TRX in clinical samples, a two-way Analysis of Variance (ANOVA) was followed by group comparisons using a post hoc by Sidak multiple comparison test. Data were presented as Means ± SEM. Results of the significance tests were indicated in the illustrations.

**RESULTS****The Intervention of TCMM and Its Impact on the Bodyweight of the MMTV-PyMT Mice**

The bodyweight of MMTV-PyMT mice in the CMS-induced sham and experimental groups were shown in Figure 2. The analysis of data demonstrated that the bodyweight of the CMS group was significantly (p=0.0429) decreased relative to the sham group at day 28. The intervention of the TCCM significantly (p=0.0668) improved the bodyweight in CMS-induced mice. However, there was no significant variation among interventions of TCMM with and without CMS-induced mice.

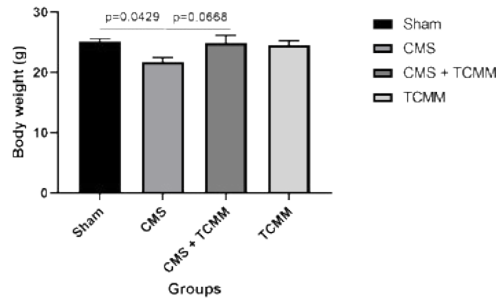


Figure 2 TCMM intervention reduced body weight in CMS induced mice; data were presented as Means ± SEM; results of the significance tests were indicated in the illustrations

**TCMM Intervention Reduces the Mammary Tumor Size and Tumor Weights in the MMTV-PyMT Mice**

To study the effects of stress exposure on breast tumor development in the transgenic MMTV-PyMT mouse line, we had primarily tested the social isolation-induced stress, which augmented mammary tumor growth based on the earlier breast tumor models [14,32]. Nevertheless, social isolation tested in the MMTV-PyMT mice did not show any significant variation in the tumor growth (data not shown). Driven by earlier reports that heightened CMS induced stress, which was consistent and had greater impacts [15,28]. Exposure of TCMM (Duration: 30 min to 3h, volume: 60 dB-80 dB) was provided for 28 days to all mice except the sham group. MMTV-PyMT mice generally produced primary tumors in all mammary fat pads. At the endpoint, all experimental animals were sacrificed; solid mammary tumors were dissected and weighed. Tumor sizes were also measured. The size of the mammary tumor and its weights were increased in the CMS-induced mice and the intervention of TCMM to the CMS mice significantly reduced the mammary tumor size ( $p < 0.0001$ ) and tumor weights ( $p < 0.080$ ) (Figure 3). The intervention of TCMM provided harmony to the transgenic mice (without exposure to CMS) that significantly decreased mammary tumor size ( $p = 0.0036$ ) when compared to the intervention of TCMM in CMS mice.

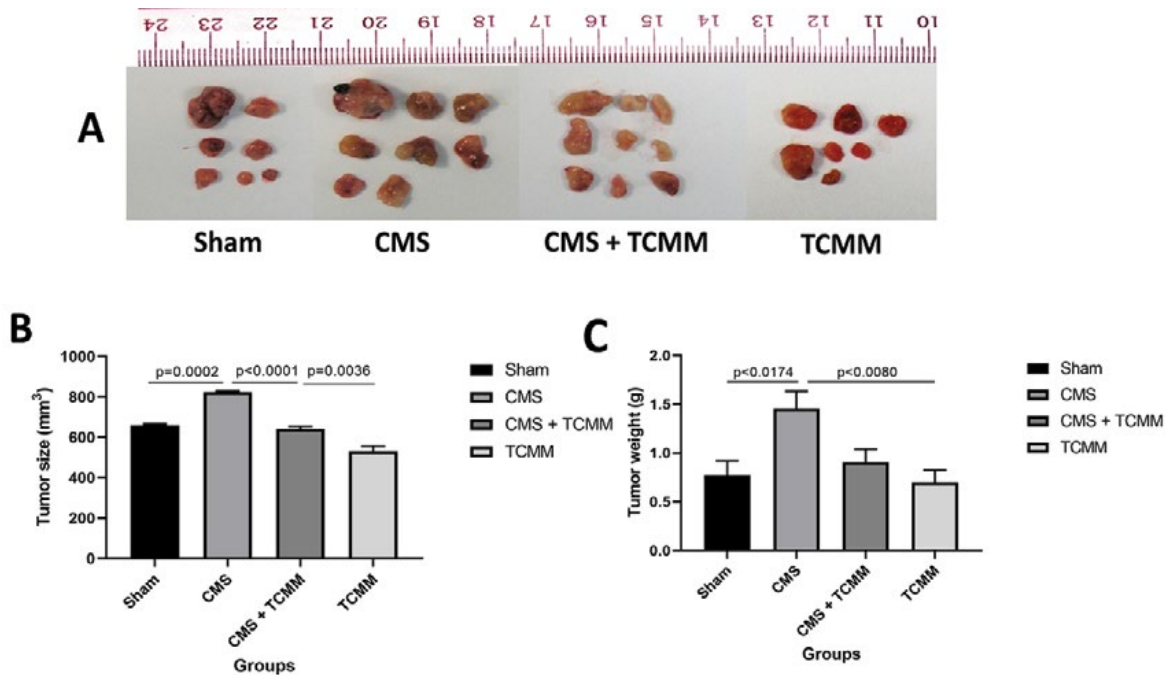
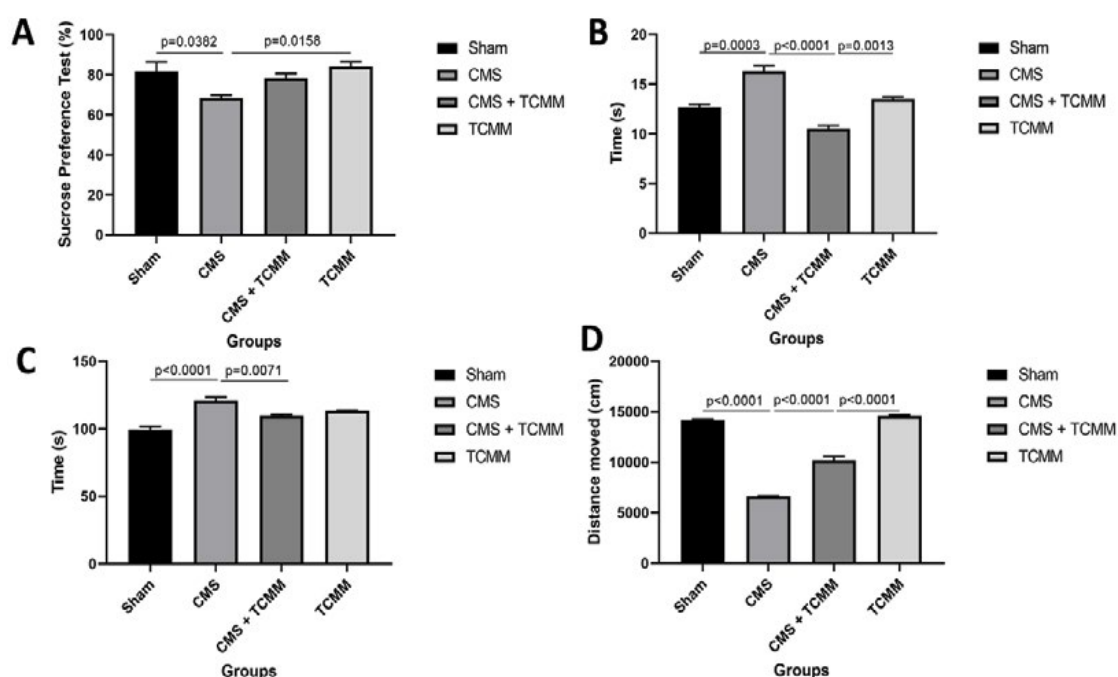


Figure 3 TCMM intervention reduced breast tumor size and weight in CMS induced mice; A): Representative pictures of different mammary tumor sizes of experimental mice; B): tumor size; C): tumor weight; data were presented as Means ± SEM; results of the significance tests were indicated in the illustrations

### TCMM Intervention Ameliorates CMS-Induced Depressive-like Behaviors in MMTV-PyMT Mice

In this study, the antidepressant effects of TCMM on the MMTV-PyMT animal model were evaluated. The Sucrose Preference Tests (SPT), Forced Swimming Tests (FST), Tail Suspension Tests (TST), and Open Filed Tests (OFT) are extensively used for the evaluation of the antidepressant activity, which is shown in Figure 4 (A-D). In SPT, there was a statistical significance ( $p=0.0382$ ) in total liquid intake between the sham and CMS groups, indicating that CMS groups were in greater anhedonic behavior. The total sucrose solution preference was significantly ( $p=0.0158$ ) increased in the intervention of the TCMM group (Figure 4A) which indicated that the mice were relaxed due to the impacts and harmony of TCMM.



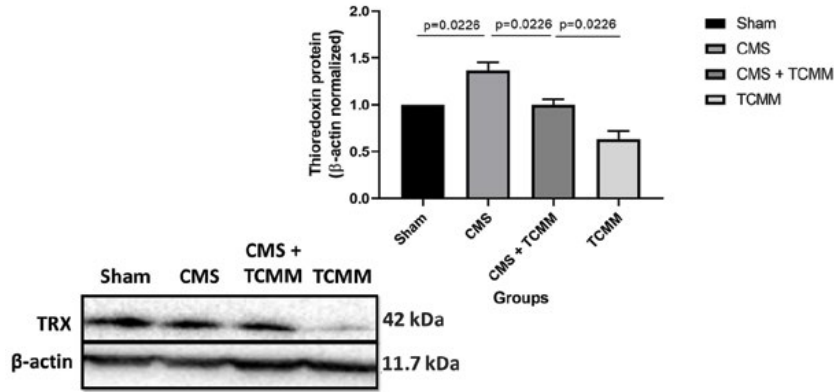
**Figure 4** TCMM intervention reduced CMS induced depressive-like behaviors in MMTV-PyMT mice; A): sucrose preference tests; B): forced swimming tests; C): tail suspension tests; D): open filed tests; data were presented as Means ± SEM; results of the significance tests were indicated in the illustrations

The mouse model of the FST is a relatively short and low-cost behavioral test that entails no training of the mice and can be operated with negligible equipment. In the present study, FST, the CMS mice were significantly immobile for more time (16.33 s) than the sham (12.7 s) ( $p=0.0003$ ), CMS+TCMM (13.5 s) ( $p<0.0001$ ), and TCMM (10.53 s) ( $p<0.0001$ ) intervention (Figure 4B) which indicated that the animals lost their hope to escape from the stressful environment. Like FST, TST mice were also positioned in an inescapable but relatively stressful environment. Lack of escape-related behavior was measured immobility. In the TST, the CMS mice were significantly immobile for more time (121 s) than the sham (99.7 s) ( $p<0.0001$ ), CMS+TCMM (109.9 s) ( $p=0.0071$ ), and TCMM (113.5 s) ( $p=0.0548$ ) intervention (Figure 4C) which indicated that the animals lost their confidence to evade from the traumatic situation. In the OFT, there were significant differences among sham, CMS, CMS+TCMM, and TCMM in total distance traveled over the 10-minute test, while CMS mice spent significantly less time in the center zone than the sham ( $p<0.0001$ ), CMS+TCMM ( $p<0.0001$ ), and TCMM ( $p<0.0001$ ) mice (Figure 4D), indicating decreased exploratory behavior.

### TCMM Intervention Reduces Oxidative Stress-Related TRX-1 in MMTV-PyMT Mice

TRX-1 involves in several cellular processes including redox signaling, response to oxidative stress. Oxidative stress is often linked to ROS, which is significantly involved in the progression of depression during the conditions of malignancies [33,34]. TRX-1 plays a key role in cancer progression and the expressions that are significantly greater during stress-induced BC [35]. Hence, we had evaluated the expression of TRX-1 levels in CMS-induced MMTV-PyMT

mice. Mammary TRX-1 was significantly ( $p=0.0226$ ) elevated during CMS and however, the intervention of TCMM in mice in CMS significantly ( $p=0.0226$ ) reduced TRX-1 expression (Figure 5).

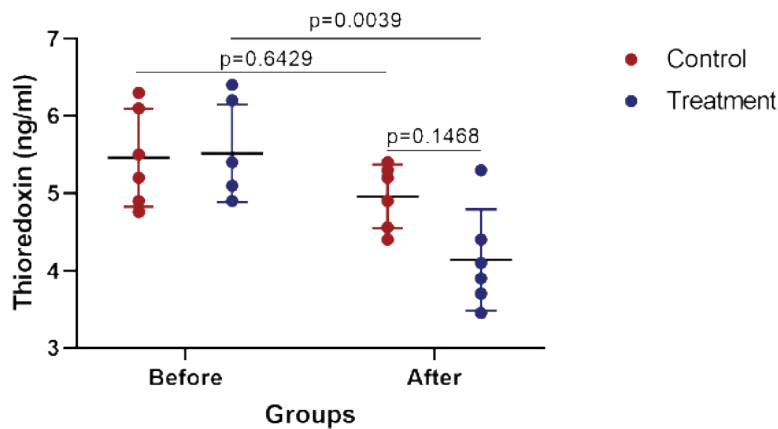


**Figure 5** Protein bands of TRX-1 were determined in sham, CMS, CMS+TCMM, and TCMM by western blotting; bar charts showing protein expression of TRX-1 after  $\beta$ -actin normalization; each bar represents means  $\pm$  SEM; data were analyzed with one-way Analysis of Variance (ANOVA) followed by group comparisons using a post hoc Tukey’s multiple comparison test with p-value stated in the illustrations

**Validation of and Quantification of Thioredoxin in the Clinical Trial**

During the CMS conditions, TRX-1 was significantly increased in the BC mice which were attenuated by the intervention of TCMM. To validate the outcome of this animal study, we had conducted the TCMM intervention on the BC subjects, who had undergone recent surgery. More interestingly, we had found similar outcomes in the BC clinical trials in which the plasma TRX-1 was significantly reduced ( $p=0.0039$ ) after the intervention of TCMM.

The clinical outcomes robustly suggested that TCMM had a significant positive impact on depression. A comparison testing of the time points and the mean TRX-1 of the control group showed no significant ( $p=0.6429$ ) changes in the pre-therapy (baseline) and post-therapy results. However, the TRX-1 levels were significantly ( $p=0.1468$ ) different among the control and treated groups during post-therapy of TCMM intervention (Figure 6).



**Figure 6** Quantification of TRX-1 was determined in sham, and treated groups by ELISA; each bar represents means  $\pm$  SEM; data were analyzed with two-way Analysis of Variance (ANOVA) followed by group comparisons using a post hoc Sidak’s multiple comparisons test with the p-value stated in the illustrations

## DISCUSSION

BC is the most common invasive cancer in women, with nearly 1,671,149 new diagnoses and 521,907 mortality globally occurred in 2012 [36]. According to GLOBOCAN, BC is estimating for around 25% of all cancers [37,38]. In preclinical models of BC, psychological stress and depression have been highly connected to tumor progression and metastasis in the mammary organ [39]. Female MMTV-PyMT mice instinctively produce estrogen-progesterone-Her2-Neu positive ductal carcinoma, which is the most widespread clinical expression of BC [15]. This mouse model produces spontaneous and luminal-like BC from the normal mammary epithelium and can recapitulate similar pathological processes and characteristics found in human BC. Mammary hyperplasia can be detected in this model as early as 4 weeks of age, and nearly 100% of mice develop BC by 8-9 weeks [15]. In the present study, the impact of CMS exposure in the mouse model was studied by initiating social isolation and depression during malignant transformation with subsequent exposure to CMS. This dual stressor elicited the activation of tumor growth throughout the malignant transformation and, intriguingly, reduced primary tumor size, tumor weight, and improved body weight was noticed in the intervention of TCMM stimulated MMTV-PyMT mice.

Many models have been used to determine the animal depressive behaviors, namely sucrose preference test, tail suspension test, forced swimming test, and open field test, which are based on the notion that immobility reflects the determination of behavioral despair [40,41]. In these experiments, immobility has confirmed the inability or reluctance, which indicates the patients suffering from depression imply psychomotor impairments [42]. Relaxation interventions are generally recognized to decrease clinical depressive symptoms and benefits for anxiety, fatigue, hypertension, various psychic disorders, and improve immune systems [43]. Notably, the intervention of TCMM has been demonstrated to elevates immune systems, beta-endorphins, and to reduce hypertension and stress hormones *viz.*, adrenocorticotropic hormone, dehydroepiandrosterone, norepinephrine, epinephrine, and cortisol in the blood [43-45]. Lei, et al. have also confirmed that TCMM decreases depressive behaviors, tumor growth, and stress hormones, and enhances the effects on antitumor immunologic functions in the mice [46].

TCMM is one of the Chinese popular music in historic roots of modern TCM theory and practice that allows individuals to nurture the natural force or energy ("Qi") in TCM, associated with physiological and psychological functionality [47]. Earlier studies have also suggested that intervention of TCMM reduces various mental disorders including, anxiety, depression, stress, mood, fear of falling, self-esteem, and improves an individual's health-related quality of life [47-49]. Electroencephalography studies have also confirmed that the intervention of TCMM elevates the brain frontal  $\alpha$ ,  $\beta$ , and  $\theta$  wave activity and increases relaxation, and attentiveness [47]. The TCMM is not only reducing the clinical implications of depression but also involves depression-inducing BC development. Hence, TCMM is unique to overwhelm the common depression and disabling result during and after treatment of BC.

Most of the clinical studies indicate that about 80% of BC patients have depression or distress, a sense of physical, emotional, and/or cognitive tiredness or exhaustion due to the treatment of chemo and radiotherapy [50]. This stress also exacerbates mammary tumor growth and limiting the overall quality of an individual's life. However, the intervention of TCMM interventions could be a promising therapy in the management of depression and improve the quality of life. A randomized, single-blinded, three-arm intervention pilot study suggests that an 8-week intervention of TCMM improves cognitive function and reduces stress in BC survivors who have undergone the treatment about 2 months to 8 years post achievement of chemo and radiation therapy [51]. A systematic and meta-analysis have also confirmed that TCMM intervention reduces cancer-related symptoms including depression, pain and improved the quality of life [52].

Recently, oxidative stress has been extensively studied, which plays a critical role in the occurrence of cognitive impairment, depression, and mental disorders [53,54]. TRX-1 is a ubiquitous antioxidant protein involved in the oxidative stress and redox mechanisms that has numerous biological functions against oxidative stress, and to prevent depression, cognitive dysfunction, and mental illness [55]. TRX-1 has been well-recognized as a biomarker for several psychiatric disorders, including, schizophrenia, bipolar disorder, and autism [55].

The present study confirms that CMS-induced mice exhibited a high expression of TRX-1, in which the levels were down-regulated by the intervention of TCMM. This study was consistent with earlier investigations related to plasma TRX-1 levels which were elevated during the oxidative stress-related depression in animal models and patients with psychosis and schizophrenia [56]. Harris, et al. reported that elevating this plasma TRX-1, as a promotor and enhanc-



ing the initiation and progression of cancer [57]. Our study was also consistent with Harris, et al. investigation, in which the elevated plasma TRX-1 was found in the basal value of TCMM treatment [57]. However, the clinical intervention of TCMM reduced plasma TRX-1 levels after 30 days of treatment in BC patients. Based on the findings, the intervention of TCMM attenuates depression-associated BC development in MMTV-PyMT mice and clinics through the TRX-1 system. Further, extensive in vivo and clinical studies are greatly required to investigate the relationship between depression-induced TRX-1 and BC.

### CONCLUSION

Depression is a mental illness that disturbs an individual's health, which exacerbates the development of BC and reduces the quality of an individual's life. Hence, we aimed to investigate the impacts of TCMM Intervention on the depressed BC mouse model and explore the potential underlying mechanisms. The intervention of TCMM reduced the body weight, tumor size, tumor weight, and behavioral changes in CMS-induced MMTV-PyMT mice. Furthermore, Intervention of TCMM reduced oxidative stress-related TRX-1 levels in the BC mice, which were also confirmed by the outcome of TRX-1 levels in the clinical trials. Based on the experimental and clinical findings, we strongly suggest that TCMM therapy could decrease tumor development in the breast and improves the quality of life. Additionally, the relationship between TRX-1, BC, and TCMM has to be comprehensively investigated, which is now underway.

### DECLARATIONS

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#### Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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