

***Polygonum minus* Aqueous Extract Supplement Reduces Stress-Induced Anorexia and Anhedonia in Mice**

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ABSTRACT

Aim: To evaluate the effects of *P. minus* extract on Chronic ultra-mild stress induced anorexia and anhedonia was the main objective of this study.

Background: Anorexia (less food intake) and anhedonia (loss of interest) are very common among depressed patients which create many problems about their essential nutrition values, they can lose their body weight and immunity which can further lead towards death due to severe anorexia. Anorexia has strong relationship with anhedonia because less food intake behavior is developed due to loss of interest in food. *Polygonum minus* plant belongs to family Polygonaceae, commonly known as 'Kesum' in Malaysia. Some experimental and clinical studies showed that an aqueous extract of its leaf part improve memory and enhances positive mood in patients as well as in research animals. It contains many essential flavonoids in its leaf part.

Methodology: In this study chronic ultra-mild stress for 6 weeks had been used to induce the depression in mice (n=14), there were 6 groups in this study including three groups with different doses of *P. minus* extract. After CUMS the body weight, Food consumption and Food preference tests were performed.

Results: Results showed that stressed mice group lost their body weight significantly and *P. minus* extract improves sweet food preference and food consumption with maximum doses in comparison of Chronic ultra-mild stressed group.

Conclusion: These findings indicate that chronic administration of *P. minus* extract can reduce the anorexia and improves the anhedonia among stressed mice.

Keywords. *Polygonum minus*, Anorexia, Anhedonia, Aqueous, Extract

INTRODUCTION

Anorexia is a loss of appetite characterized by decreasing in body weight. This condition mostly occurs as a comorbidity in many disorders like depression and cancer (Hunter et al., 2021; Robinette et al., 2020). Whereas, Anhedonia is an inability to experience pleasure from rewarding or enjoyable activities and is a core symptom of depression (M.-Y. Liu et al., 2018). Anhedonia is associated with anorexia due to low interest in food or any reward in living organisms, they lose their weight because of less food intake (Haynos et al., 2021). That's why anorexia can lead severe nutritional deficiency, some time it leads emergency situation among patients (Guinhut et al., 2021). Major depressive disorder (MDD) is a common comorbidity in eating disorders (EDs), among these eating disorders anorexia is occurred in 50–75% of the patients according to American Psychiatric Association (Shilton et al., 2020). Several findings indicate a relationship between eating disorders and depression. Depressive symptoms have been significantly found in anorexia along with other major mood disorders (Herpertz-Dahlmann et al., 2020; Lenzo et al., 2020). Chronic stress is the cause of different pathological disease conditions, including psychiatric and eating disorders, such as depression, anxiety, anorexia and bulimia nervosa. In fact, changes in appetite and weight are common diagnostic markers for major depressive disorder and near about 48% of adult patients of depression show depression-related anorexia (Simmons et al., 2016). Prolonged or continuous exposure of stressful events has been linked with clinically diagnosed depression in patients and depressive-like behaviors in rodent models as well (Rincón-Cortés et al., 2020). Chronic stress induced a

disorder that causes depression-like states, including anhedonia, physical changes like reduced weight gain (Eliwa et al., 2021; Mishra et al., 2021). Previous studies show that, anhedonia is commonly found in patients with anorexia. A small sweet sensation has been observed in patients with anorexia and test subjects under influence of stressful events. In rodent model, anhedonia can be easily measured by minimum consumption of sweet foods. Repeated and chronic stress decrease the response of sweet taste in rodents, it was suggested that anhedonia may be associated with stress-induced anorexia (Kim et al., 2015). A significantly lower body weight has also strong link with severe anorexia. Reduced weight is a predictor of anorexia during examination (Garber et al., 2019). Loss of weight is also found in chronic stress induced anhedonia in many studies (Eliwa et al., 2021). Improvement in anhedonia is directly associated with anorexia (Foldi et al., 2017). Mostly, the protocol use for the measurement of anhedonia in mice is a sucrose preference test (SPT) based on sucrose and water volume intake. A reduction in the sucrose consumption as compare to control mice is an indication of anhedonia. Till now, variant and inconsistent results have been reported in the use of the sucrose preference test (M.-Y. Liu et al., 2018). Some of previous studies also referred to use of cheese as a preference food in stress induced anhedonia among mice, as they suggested that solid food can give more accuracy in anhedonia detection (Kudriavtseva et al., 2006). In present study sweet food (cookies) and cheese, both were used to detect the preference instead of liquid sucrose due to variation and inconsistency of results in sucrose preference test.

Furthermore, search of appropriate treatment is very necessary for eating disorders. Many antidepressants are being used in the treatment of anorexia and other eating disorders (Marvanova et al., 2018). Due to adverse effects of antidepressant medication, new choices from medicinal plants are very important. The herbal medicine could be an effective alternative for the management of many neurological disturbances such as anxiety, depression, dementia and eating disorders. (Klemens, 2006). Previous studies have shown that flavonoids rich neuroprotective plants extract can improve the anorexia and anhedonia (Nandeesh et al., 2018). Previously reported that cognitive enhancer and mood improver herbal extracts have also potential to reduce the anorexia and anhedonia (Micheli et al., 2020; Yeh et al., 2015). Those herbal substances which have strong cognition enhancing and neuroprotective potential are strongly recommended to evaluate further for anorexia treatment research (Marvanova et al., 2018). The Polygonum minus is a cognition enhancer nutritional supplement with essential flavonoids like quercetin and quercetin 3- glucuronide, according to previously reported work these constituents can cross the blood brain barrier which is an important barrier for showing desired effects inside the brain (Bashir et al., 2020). *Polygonum minus*, synonymous to *Persicaria minus* belongs to Polygonaceae family and is well known as Kusum or laksa leaf in Malaysia. It is used as an important preventive healthcare agent in southeast Asia. These herbs are associated with strong antioxidant activities along with neuroprotective benefits (Christopher et al., 2015). The leaves of this plant have been reported to be high in antioxidants. Aqueous extract of this plant showed significant beneficial effects on memory and cognitive functions with 100mg/kg dose in mice as reported in previous study (George et al., 2014b), BioKesum is a brand name of *P. minus* leaf extract supplement produced by Malaysian company (Biotropics Malaysia). It consists of standardized constituents like quercetin and quercetin 3. Glucuronide as abundant flavonoids. A latest research of Six months BioKesum® supplementation potentially improved visual memory and negative mood among older patients which indicates that it can be proved as an option in future treatment of various neurobiological disorders (Lau et al., 2020).

In this study effects of *P. minus* aqueous extract supplement BioKesum® has been observed on chronic ultra-mild stress induced anorexia and anhedonia through food consumption and food preference tests.

MATERIALS AND METHODS

Drugs and foods: BioKesum® (an aqueous extract supplement of *Polygonum minus* leaves) was provided by Biotropics Malaysia Berhad. Amitriptyline (Apotex Inc. Canada), Cheese Slice (Almarai Malaysia), Muzic bites sweet cookies (Munchy’s Berhad Malaysia) and Altromin Diet (Altromin International Germany) were purchased from local market.

Animals and Experimental design: Male balb/c mice of 2 to 6 months age and 22 to 26 grams by weight were obtained from the Animal Research and Service Centre, Universiti Sains Malaysia, Kelantan Campus. All experimental protocols were performed after getting the approval from ethical committee of Universiti Sains

Malaysia named as USM Institutional Animal Care and Use Committee (USM IACUC). (Approval NO: USM/IACUC/2020/(123)(1075) & USM/IACUC/2021/ (127) (1129).

The animals were kept under standard atmospheric conditions (24 ± 1 °C) in the Animal Transit Room of School of Pharmaceutical Sciences, Universiti Sains Malaysia. All animals were housed on a 12-hour light/dark cycle. They were randomly distributed into the 6 different groups. Chronic ultra- mild stress technique was used to induce the stress on them. Normal control group was without any Chronic ultra- mild stress (CUMS) remaining groups were affected by Chronic ultra- mild stress (CUMS). Chronic ultra-mild stress was applied for six weeks. Treatment was continued till end of study.

Group 1: Control group (Without Stress) treated with distilled water only (n=14). Group 2: (Negative Control) Chronic ultra- mild stress (CUMS) treated with 6 weeks of CUMS and distilled water for 8 weeks (n=14). Group 3: (Positive Control) CUMS+ Amitriptyline 20mg, (n=14). Group 4. (*P. minus* 50mg + CUMS) *P. minus* 50mg/kg treated group with Chronic ultra-mild stress (n=14). Group 5: (*P. minus* 100mg +CUMS) *P. minus* treated group with 100mg/kg along chronic ultra-mild stress (n=14). Group 6: (*P. minus* 200mg+ CUMS) *P. minus* treated group with 200mg/kg with chronic ultra-mild stress (n=14). All treatments were given by oral route of administration with an oral gavage for mice. A simple and comprehensive design of study is expressed in Figure 1.



Figure 1: Schematic design of experiment.

Chronic Ultra Mild Stress: Chronic Ultra mild stress technique was used for 6 weeks for induction of anorexia and anhedonia. **Figure 2** expresses the weekly plan of chronic ultra-mild stress. Each day during stress period consists of two different stresses first stress was applied on morning and second was at evening as followed by the latest study(Hu et al., 2019).



Figure 2: Weekly plan for chronic ultra-mild stress

Body Weight Test: Body weight results on zero day and after 6 weeks of chronic ultra-mild stress were observed for this study. Body weight has vital relationship with anorexia. In fact, it can predict the anorexia development in subjects, because loss of weight is the main symptom of anorexia.

Food Preference Test: Rodents are born with an interest in sweet foods or solutions. Reduced preference for sweet solution in test represents anhedonia, while this reduction can be reversed by treatment with antidepressants (Serchov et al., 2016). Previous studies shown that sweet food preference detection is more appropriate than sucrose liquid preference test due to inconvenience of accuracy and sensitivity of sucrose preference test results (Mateus-Pinheiro et al., 2014; Yin et al., 2021). Some studies report that cheese is also useful to detect the anhedonia (Kudriavtseva et al., 2006), In current study cheese was also used along sweet food (cookies) and normal diet to detect the preference behavior.

All mice were habituated to sweet cookies and cheese for two days prior to trial day, whereas Altromin diet was already part of their daily food. They were totally deprived of food for 12 hours before trial day. A special apparatus consist of a transparent glass jar was used in this experiment. It was an open deep round shaped jar (diameter 20 cm along 45 cm depth). All foods including sweet cookies, cheese pieces and Altromin diet pellets were accurately weighed and noted on label at every jar for each sample before the trial. Three different types of foods including sweet cookies, cheese and Altromin diet palette were placed in each jar, then animal was introduced in labelled jar accordingly. They were placed in the apparatus for 6 hours (9:00 am to 3:00 pm) and freely allowed to choose their preferred food. After completion of experimental duration animals were removed and remaining foods were weighed accurately and noted on label. Differences from initial readings and percentages of each food consumption were calculated.

Food Consumption Test: Anorexia is a less food consumption leading to lowered body weight. Food consumption test is a fundamental examination among eating disorders (Scharner et al., 2021). Food intake analysis is the main method to detect stress induced anorexia in mice (Yamada et al., 2020).

In current studies food consumption test was performed for 4 consecutive days after chronic ultra-mild stress in 8th week with normal routine diet (Altromin diet) of mice. Four trials were carried out just for the maximum accuracy and persistence behavior observations. Daily maximum food intake was estimated before starting the experiment. Individual animal was placed in a separate cage and controlled food pellets were weighed accurately as initial reading before providing to animals. Food pellets were only double than their daily estimated need. After 24 hours remaining food pellet were weighed and noted on label. Differences from initial readings and final readings were calculated, that showed the daily food consumption of each sample. This experiment was continued for each group with same protocol for 4 days. Comparison between each group in respect of their daily food consumption was made.

Statistical Analysis: Statistical analysis was done by using SPSS version 26 statistical software (IBM, New York, NY, USA). One-way ANOVA further followed by Post-hoc

with Tukey HSD was performed for comparisons in between different groups. Differences in mean values with $p < 0.05$ were considered as significant in all tests.

RESULTS

Effects of *P. minus* aqueous extract on body weight after Chronic Ultra-mild stress:

Figure 3 expresses the effects of *P. minus* treatment on body weight after six weeks of chronic ultra-mild stress (CUMS). Body weight of each sample were measured before and after CUMS. Results showed that CUMS treated group reduced their weight significantly $p < 0.05$ as compare to their baselines (0-day values) which indicates presence of anorexia, whereas remaining all groups showed increase in body weight after chronic ultra-mild stress. Control group showed $p < 0.01$ level of significance in increase of body weight with respect of their baseline, while Positive control group (Amitriptyline 20 mg +CUMS) and *P. minus* 50 mg treated group showed $p < 0.05$ level of significance with increasing pattern in body weight. Maximum doses groups including *P. minus* 100mg and 200mg treated groups reversed the effects of CUMS and showed an increase with $p < 0.01$ significance level in their body weight as compare to zero-day values which is same as Control group significance level and more significant than positive control (Amitriptyline 20 mg +CUMS) and *P. minus* 50mg treated group. All groups showed increase in body weight except the negative control group (CUMS treated group) which indicates that chronic ultra-mild stress reduce the body weight after 6 weeks of continuous stress procedure. Maximum doses groups including *P. minus* 200mg and *P. minus* 100mg treatment along with 6 weeks of chronic ultra-mild stress reversed the effects of stress and showed highly significant increase in body weight of mice.

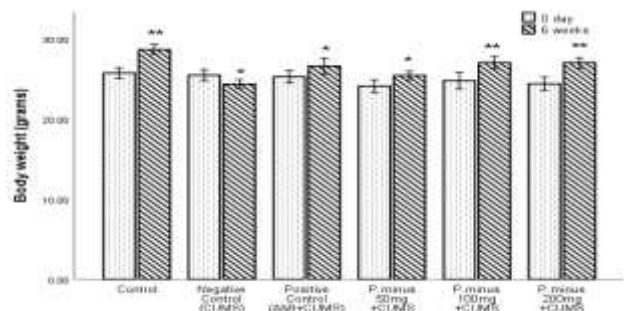


Figure 3: Effects of *P. minus* aqueous extract on body weight in after 6 weeks of chronic ultra-mild stress in mice. * ($p < 0.05$) ** ($p < 0.01$), vs. 0-day measurements.

Effects of *P. minus* aqueous extract on sweet food preference in CUMS induced Anhedonia:

Food preference behaviors are shown in Figure 4. Results showed that chronic ultra-mild stress induced anhedonia in negative control group as difference between control and negative control group is highly significant by $p < 0.01$ which show that negative control group showed significantly reduced preference for sweet food as compare to control group, that is indication of anhedonia development in CUMS treated group. This low preference for sweet food is

significantly ($p < 0.01$) reversed in Positive control (Amitriptyline 20 mg + CUMS), *P. minus* 100mg and *P. minus* 200 mg with CUMS treated groups. In addition, there was no any significance improvement observed in *P. minus* 50mg + CUMS treated group for sweet food. Maximum doses improved the CUMS induced anhedonia significantly but not more than Amitriptyline 20 mg + CUMS treated group.

In case of cheese, no any significance difference observed in all groups due to presence of sweet foods. All groups preferred sweet food and Altromin diet more than cheese. Furthermore, Altromin diet preference in negative control group was higher than all groups.

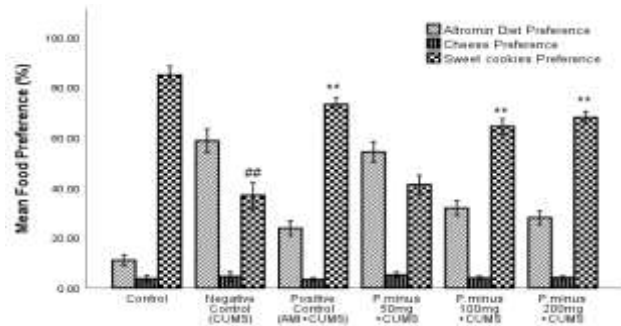


Figure 4: Effects of *P. minus* aqueous extract on sweet food preference in CUMS induced anhedonia. # ($p < 0.05$) ## ($p < 0.01$), vs. Control; * ($p < 0.05$) ** ($p < 0.01$), vs. Negative Control (CUMS) group.

Effects of *P. minus* aqueous extract on food consumption in CUMS induced anorexia:

Figure 5 indicates that chronic ultra -mild stress reduced the food consumption in negative control group throughout 4 days of experiment. Reduced food intake shows anorexia among mice belong to negative control group in comparison of control group, while positive control (Amitriptyline 20mg + CUMS), *P. minus* 200mg +CUMS and *P. minus* 100mg +CUMS showed significant ($p < 0.01$) increase in food consumption as compare to CUMS group. Maximum doses groups reversed CUMS induced anorexia significantly but minimum dose group (*P. minus* 50 mg +CUMS) did not show any significant improvement in anorexia in comparison of negative control (CUMS) group throughout 4 days of observations.

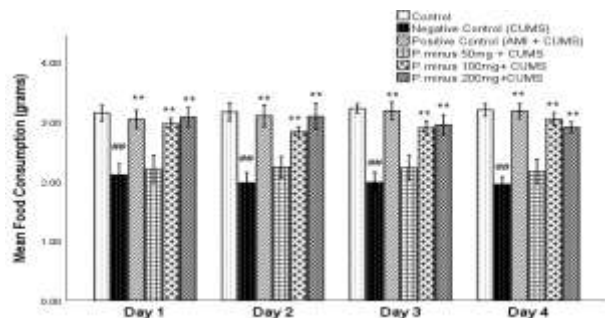


Figure 5: Effects of *P. minus* aqueous extract on food consumption in CUMS induced anorexia in mice. # ($p < 0.05$) ## ($p < 0.01$), vs. Control; * ($p < 0.05$) ** ($p < 0.01$), vs. Negative control (CUMS) group on each day separately.

DISCUSSION

Polygonum minus supplement showed neuroprotective effects in previous studies like improvement in memory and positive mood (Shahar et al., 2015; Yahya et al., 2017). BioKesum is a commercial supplement in Malaysia which consist of *P. minus* leaf aqueous extract. This supplement contains standardized flavonoids and total phenolic constituents. Quercetin (not less than 0.15%) and quercetin 3 glucuronide (not less than 0.45%), are among the abundant and vital flavonoids of this extract (Lau et al., 2020). These constituents are involved in many neuroprotective activities as they can cross the blood brain barrier (George et al., 2014a). Anorexia and anhedonia are neurological and severe psychiatry disorders (X. Liu et al., 2021; Wentz et al., 2020). In current study, the effects of *P. minus* extract on these activities have been evaluated first time.

Anorexia and anhedonia were developed under influence of 6 weeks of chronic ultra-mild stress. A previously done work show that CUMS can develop the anorexia and anhedonia in animal model (Géa et al., 2019), it can also reduce the body weight as per previous research studies (Deng et al., 2015; Ding et al., 2017) reduced body weight is the main symptom of anorexia induction (Trinh et al., 2021). As per current results, shown in Figure 3 chronic ultra-mild stress reduced the body weight of negative control group significantly ($p < 0.05$) which was similar to previously done research study on CUMS induced decrease in body weight (W. Liu et al., 2019), *P. minus* 100mg and 200mg treated groups along CUMS showed highly significant ($p < 0.01$) restoration in body weight of mice which was more than amitriptyline 20 mg treated group (Positive control). According to previous study it was observed that chronic administration of quercetin treatment with (15mg/kg) dose significantly restored the weight loss in mice caused by chronic ultra-mild stress (Ma et al., 2021), while Quercetin 3 glucuronide (0.06 $\mu\text{mol}/\text{mouse}$ i.p. injection) treatment showed increase in body weight in experimental mouse (Liao et al., 2015). Furthermore, anhedonia (low interest in sweet food) is also a negative effect of chronic ultra-mild stress that indicates lack of joy able moments (Pignatelli et al., 2020). As shown in Figure 4, *P. minus* maximum doses (100mg and 200mg) reduced the CUMS induced anhedonia significantly through sweet food preference test. In presence of sweet food cheese preference was very low that show that sweet food is the more reliable diagnostic marker for anhedonia detection as compare to cheese. Current sweet food preference results are supported by previous studies on major constituents of *P. minus* (Quercetin and Quercetin 3 glucuronide), those studies reported that quercetin as a single constituent and Quercetin 3 glucuronide as a part of Hypericum Perforatum has an ability to reduce the anhedonia in depressed animals (Gambarana et al., 2001; Mehta et al., 2017). Anhedonia is directly associated with anorexia as it can decrease the interest in eating that leads an eating disorder (Foldi et al., 2017; Jahng, 2014). Figure 5 showed that CUMS significantly reduced the food intake throughout 4 days of experimental period that is strongly supported by previous research on stress induced loss of appetite (Li et al., 2021). According to results of current study *P. minus*

aqueous extract treatment with maximum doses (100mg and 200mg) reversed the CUMS induced appetite among mice, whereas low dose (*P. minus* 50mg) treatment showed non-significant reduction in anorexia. It is suggested that reduction behavior in anorexia is also due to presence of quercetin and quercetin 3- glucuronides in leaf extract of *P.minus* (BioKesum). This recommendation is strongly supported by reported studies about these constituents, in which quercetin (2mg/kg) improves lipopolysaccharide-induced anorexia and increase the food intake in rats significantly (Sah et al., 2011) while quercetin 3- glucuronides significantly increase the food intake in imbalance hippocampal depressive mice model (Xu et al., 2020). Current study suggested that *P.minus* aqueous extract reduce the anorexia and anhedonia might be due to the anti-stress activities of its abundant constituents (quercetin and quercetin 3- glucuronides).

CONCLUSION

P. minus aqueous extract has an ability to reduce the chronic ultra-mild stress induced anorexia and anhedonia significantly with its maximum doses (100mg and 200mg). Further evaluation is recommended to detect the mechanisms involved in anti-anorexia and anti-anhedonia activities of *Polygonum minus* aqueous extract

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Author Contributions: Muhammad Irfan Bashir performed the experimental work. Nur Hidayah Kaz Abdul Aziz and Dzul Azri Mohamed Noor conceptualized and design this experiment. Dzul Azri Mohamed Noor and Muhammad Irfan Bashir performed data analysis. Nur Hidayah Kaz Abdul Aziz and Muhammad Irfan Bashir wrote the article.

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