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Safety Evaluation of *Dendrobium officinale* Combined with Black Tea Extract in Rodents

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Abstract

Background: Potential acute and chronic oral toxic effects of *Dendrobium officinale* (DO) and black tea extract (BTE) mixture, a health-care food for osteoporosis, were evaluated to assess its suitability for therapeutic exploitation.

Materials & Methods: Acute toxicity was examined via intragastric administration of DO-BTE twice within 24 h at 5 g/kg for a 14-day observation. In the subchronic toxicity test, normal rats were orally administered the combination at doses of 0.5, 1 and 2 g/kg twice daily for 90 days. Female ovariectomized (OVX) rats were intragastrically administered DO-BTE at 0.84 g/kg for 90 days.

Results: Acute toxicity test disclosed no death or marked signs of toxicity, except lower activity in the combination treatment group. The maximal tolerance dose (MTD) of DO-BTE was 10 g/kg. In the chronic toxicity experiment, the rates of body weight growth in the combination treatment groups were slower than that in the control group. Plasma fibrinogen (FIB) and platelet (PLT) levels showed a decreasing trend.

Conclusion: Acute and long-term toxicity tests revealed no toxic side-effects at the given doses in rodents, supporting further clinical investigation.

Keywords: *Dendrobium officinale*; black tea extract; acute toxicity; chronic toxicity; ovariectomized rats; hematological analysis

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1. Introduction

The demand for natural nutrients and medicinal agents has become increasingly popular with elevated awareness of nutrition, health and safety. Integration of natural substances into modern medicine is widely applied to facilitate improvement of health worldwide. As a consequence, issues of efficacy, quality and safety of naturally produced substances are an emerging focus of research [1]. In particular, proof of safety takes precedence over efficacy in the history of drug regulation [2]. Toxicological evaluation and dose regulation of these natural products is essential for maximizing benefits while minimizing side-effects.

Dendrobium officinale Wall. Ex Lindl (DO), a prized medicinal species of the Orchidaceae family, and black tea (*Camellia sinensis*, BTE) are extensively utilized in traditional practices and modern healthcare products. DO is widely recognized for its immunomodulatory and anti-inflammatory properties [3–6], BTE is rich in bioactive polyphenols that offer significant cardiovascular, metabolic, and bone-protecting benefits [7–11], due to their diverse pharmacological benefits. Despite their ubiquitous daily consumption and widely accepted health-promoting effects, the comprehensive safety profiles of these ingredients require rigorous evaluation, particularly when used in novel formulations.

Previous experiments by our group showed that a combination of DO and BTE could effectively ameliorate osteoporosis, both *in vivo* and *in vitro* [12], and exerts effects on estrogen activity via an estrogen receptor α -dependent signaling pathway [13]. Despite the widely accepted health benefits of the combination treatment, the potential problem of safety should not be overlooked. Acute toxic and cytotoxic effects exerted by *Dendrobium moniliforme* [14] and acute and sub-chronic toxicity of Pu-erh black tea [15] have been reported. However, the toxicological effects of our novel DO-BTE mixture remain to be established.

In this study, we evaluated the potential acute and subchronic oral toxicity of the DO-BTE combination. Acute toxicity in mice was examined following oral administration of 10 g/kg of the combination twice within 24 hours, followed by 14 days of observation. Subchronic oral toxicity tests were conducted in normal rats using doses of 0.5, 1 and 2 g/kg DO-BTE twice a day and ovariectomized rats by treatment with 0.84 g/kg DO-BTE continuously for 90 days. This safety assessment following the guidelines of the Organization of Economic Co-operation and Development (OECD) [16, 17] and combining with the Maximum Feasible Dose (MFD) [18] should provide a reliable foundation for further development and effective utilization of the DO-BTE mixture in the clinic.

2. Materials and Methods

2.1. Preparation of the DO-BTE combination

Dendrobium officinale stem was obtained from the Yunnan Province in 2011 and identified by Kaicong Fu, a professor at the Pu'er National Institute of Traditional Medicine. It should be explicitly noted that *Dendrobium candidum* is a widely recognized botanical synonym for *D. officinale* in traditional and previous pharmacological literature. Consequently, prior findings citing *D. candidum* are directly transferable to the species investigated herein. The voucher specimen was preserved under the reference number 2011-DPE-1 and maintained in the Key Laboratory of Pu-erh Tea Science of Yunnan Agricultural University. The plant material was crushed and screened by a mesh size of 80. Black tea (*Camellia sinensis* L.) was provided by Yunnan Tea Home Biotechnology Co. Ltd. Every 100 g sample of black tea was extracted twice with 1.2 L water for each reflux (1.5 h). After filtration and vacuum drying, BTE was finally obtained by 20 g of aqueous crude extract. DO powder and BTE were mixed at a proportion of 5:2 and crude polysaccharides or caffeine used as quality control standards. The crude polysaccharide and the caffeine contents were 31.12% and 1.97%, respectively [12].

2.2. Experimental animals

Specific pathogen-free Sprague-Dawley (SD) rats (170–250 g) and ICR mice (18–20 g) were purchased from Kunming Medical University (license number SCXK K2015-0002). Rats were housed in a room at 24 ± 1 °C and 50–60% relative humidity under a 12 h light–dark cycle. Animals were acclimatized to the laboratory environment for 7 days and allowed free access to water and a standard diet prior to the experiment. Experiments were performed in the animal facility according to the institutional guidelines and approved by the Institutional Animal Care and Use Committee of the Yunnan Agricultural University (approval number: YNAU2019LLWYH003-1; approval date: March 21, 2019). All animals were fasted but provided water for 10–12 h before the start of the experiment.

2.3. Acute toxicity study

Acute toxicity of the DO-BTE combination was evaluated in mice according to the OECD guidelines for testing of chemicals (No. 423) and combined with the Maximum Feasible Dose (MFD). For the experiment,

fasted mice were randomly divided into two groups (20 mice per group, 50% male and female). The control group received an equal volume of distilled water while the combination treatment group was orally administered the maximal concentration (0.125 g/mL) and maximal volume (40 mL/kg) of DO-BTE twice within 24 h, leading to a total dose of 10 g/kg. Symptoms of toxicity and mortality were recorded systematically at 1, 2, 4 and 6 h after test substance administration. The number of living mice was noted after 24 h and once-daily observations performed for a further 14 days.

2.4. Subchronic toxicity in normal animals

The subchronic 90-day oral toxicity test was conducted according to the OECD guidelines 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents) [17]. To comprehensively assess the safety profile and maximize systemic exposure, 20 male and 20 female rats were randomly divided into four groups: Control, DO+BTE (0.5 g/kg×2, 1 g/kg×2, 2 g/kg×2), each containing 10 rats. The combination treatment was orally administered at doses of 0.5 g/kg, 1 g/kg and 2 g/kg, given twice daily (BID), leading to accumulated doses of 1 g/kg, 2 g/kg and 4 g/kg per day, respectively, while the control group was given the same volume of double-distilled water every day. The split-dosing regimen (BID) was specifically employed to overcome the ethical and physical limitations of maximum single oral gavage volume in rodents, allowing us to achieve an extraordinarily high Maximum Feasible Dose (MFD) of 4.0 g/kg/day [18]. The animals were continuously administered for 90 consecutive days, during which toxicological signs, mortality, physiological and behavioral changes were recorded. Body weight alterations were recorded every week and the dosage adjusted in relation to body weight. Food consumption and water intake were additionally measured each week. Rats were anesthetized using ketamine-xylazine solution (60 mg/kg/10 mg/kg via intraperitoneal injection) and blood samples collected from the common carotid artery with EDTA-2K, sodium citrate (1:9), vacuum blood collection tubes at the end of the experiment. Following blood collection, internal organs (heart, liver, spleen, kidney, thymus, lung, testes, epididymis, uterus and ovary) were isolated, weighed, examined for gross lesions and stored in 10% neutral buffered formalin.

2.5. Hematological analyses

Blood samples were treated with EDTA-2K to perform hematological assessments using the Mindray BC-3000 automated hematology analyzer for measurement of red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), platelet distribution width (PDW), red blood cell distribution width coefficient variation (RDW-CV), red blood cell distribution width standard deviation (RDW-SD), and platelet (PLT) levels. Blood was treated with sodium citrate for measuring prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen (FIB) content.

2.6. Biochemical analyses

Blood samples in vacuum blood collection tubes were centrifuged for 10 minutes at $4000 \times g$, and serum was collected in 1.5 mL centrifuge tubes for measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLB), albumin/globulin ratio (A/G), total bilirubin (T-BIL), alkaline phosphatase (ALP), urea, creatinine (CRE), glucose (GLU), triglyceride (TG), total cholesterol (TC), sodium (Na), potassium (K) and chloride (Cl) levels using the Beckman AU680 automatic biochemistry analyzer (Kraemer Boulevard Brea, USA).

2.7. Histopathological examination

Liver and kidney samples from the control and high-dose combination treatment (2 g/kg×2) groups were rinsed with running tap water for 4 h, dehydrated via a graded ethanol series, de-waxed in xylene, embedded in paraffin, cut into 5 μ m sections using a microtome and stained with hematoxylin and eosin (H&E). Pathological sections of tissues were examined under a light microscope and photomicrographs recorded. When lesions were observed in the high-dose group, the corresponding organs from the low-dose group were also examined. The histopathological evaluation was conducted by an experienced researcher who was fully blinded to the treatment group assignments.

2.8. Chronic toxicity in ovariectomized rats

Rats were allowed to acclimatize for one week and anesthetized via intraperitoneal injection of a ketamine-xylazine solution (60 mg/kg/10 mg/kg), followed by bilateral ovary resection (OVX). A further 10 rats (sham group) were also anesthetized but only a small sample of fat resected rather than entire bilateral ovaries. All rats were monitored for 15 days before initiation of the therapeutic regimen. We randomly divided ovariectomized rats ($n = 20$) into two groups (Model and 0.84 g/kg DO-BTE treatment [12]). The sham and model animals were given a volume of 15 mL/kg distilled water for a consecutive 90 days. All animals were weighed and observed daily for toxicological signs. After the end of the experiment, rats were euthanized via CO₂ asphyxiation followed by cervical dislocation in strict accordance with the AVMA Guidelines for the Euthanasia of Animals, and blood samples obtained for biochemical analyses.

2.9. Statistical analysis

Data are presented as means $\pm$ standard error of mean (SEM). Statistical evaluation was performed using SPSS 17.0 software (Chicago, IL, USA) and significance analyzed via one-way analysis of variance (ANOVA) and Student's two-tailed t -test. Results were considered significant at P values < 0.05 .

3. Results

3.1. Acute toxicity

Animals from both the control and combination treatment groups showed no mortality or obvious changes during the treatment and observation periods, except lower activity after administration of the second dose of DO-BTE. We detected no marked changes in body weight (Table 1) and other physiological activities or signs of toxicity in mice after administration of DO-BTE at a dose of 5 g/kg twice within 24 h. Based on the collective findings, the maximal tolerated dose (MTD) was calculated as 10 g/kg for both sexes.

Table 1. Body weights of mice following the acute toxicity test.

Day	Control	DO+BTE (5 g/kg×2)
0	20.0 $\pm$ 0.2	19.8 $\pm$ 0.2
3	24.3 $\pm$ 0.3	25.0 $\pm$ 0.3
7	29.1 $\pm$ 0.6	30.4 $\pm$ 0.3
14	34.5 $\pm$ 0.7	35.7 $\pm$ 0.4

3.2. Body weights, food and water consumption

In the subchronic toxicity study, normal rats orally received the combination at doses of 0.5, 1 and 2 g/kg twice daily for 90 days. Rats showed no clinical toxicity signs or mortality over the entire experimental period. Weights of all animals increased with time, except that the growth rates of the three groups treated with DO-BTE were slower compared with the control group, although these differences were not statistically significant ($P > 0.05$, Fig. 1A). Moreover, food and water consumptions were not significantly different between the control and DO-BTE treatment groups ($P > 0.05$, Fig. 1B and 1C).

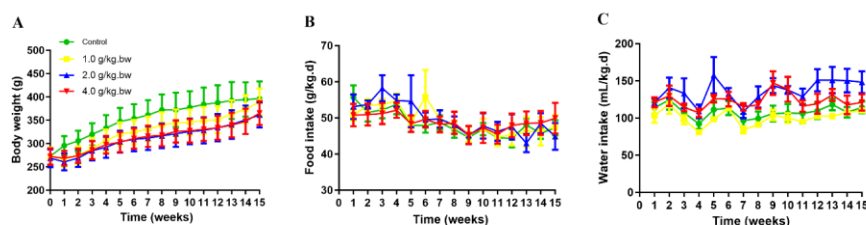


Figure 1. Body weights, food and water consumption of normal rats orally administered DO-BTE for 90 days. Results are expressed as means $\pm$ SEM ($n = 10$, male/female = 5/5). No statistical significance compared to control ($P > 0.05$).

3.3. Organ coefficients of normal rats

The organ-to-weight ratios of experimental rats are presented in Table 2. No significant differences in coefficients of all the organs examined were evident between the experimental (DO-BTE) and control groups ($P > 0.05$).

Table 2. Organ coefficients in normal rats treated orally for 90 days.

Index	Control	0.5 g/kg $\times$ 2	1 g/kg $\times$ 2	2 g/kg $\times$ 2
Heart ^a	0.86 $\pm$ 0.07	0.80 $\pm$ 0.05	0.80 $\pm$ 0.05	0.76 $\pm$ 0.04
Liver ^a	5.26 $\pm$ 0.75	5.90 $\pm$ 0.58	5.34 $\pm$ 0.33	5.41 $\pm$ 0.23
Spleen ^a	0.67 $\pm$ 0.05	0.58 $\pm$ 0.03	0.62 $\pm$ 0.07	0.60 $\pm$ 0.04
Kidney ^a	1.27 $\pm$ 0.13	1.22 $\pm$ 0.10	1.32 $\pm$ 0.15	1.21 $\pm$ 0.08
Thymus ^a	0.22 $\pm$ 0.01	0.23 $\pm$ 0.01	0.22 $\pm$ 0.01	0.18 $\pm$ 0.01
Lung ^a	0.99 $\pm$ 0.07	0.96 $\pm$ 0.05	1.00 $\pm$ 0.04	1.03 $\pm$ 0.07
Testes ^a	1.77 $\pm$ 0.09	1.75 $\pm$ 0.05	1.87 $\pm$ 0.04	1.89 $\pm$ 0.13
Epididymis ^a	0.79 $\pm$ 0.07	0.76 $\pm$ 0.02	0.83 $\pm$ 0.02	0.72 $\pm$ 0.03
Uterus ^a	0.45 $\pm$ 0.06	0.42 $\pm$ 0.05	0.38 $\pm$ 0.03	0.45 $\pm$ 0.05
Ovaries ^b	87.37 $\pm$ 8.92	94.83 $\pm$ 8.66	98.35 $\pm$ 7.18	81.61 $\pm$ 5.98

3.4. Hematological parameters

Hematological analysis of rats treated with DO-BTE (Table 3) revealed no notable changes except decreasing trends in PLT and FIB values relative to the control group. However, these differences were not statistically significant ($P > 0.05$).

Table 3. Hematological values in normal rats treated orally for 90 days.

Index	Control	0.5 g/kg $\times$ 2	1 g/kg $\times$ 2	2 g/kg $\times$ 2
WBC ($\times 10^9/L$)	3.68 $\pm$ 0.75	3.41 $\pm$ 0.60	4.38 $\pm$ 0.73	3.72 $\pm$ 0.46
RBC ($\times 10^{12}/L$)	5.56 $\pm$ 0.22	5.50 $\pm$ 0.27	5.40 $\pm$ 0.20	4.97 $\pm$ 0.41
HGB (g/L)	117.70 $\pm$ 4.14	118.88 $\pm$ 3.59	120.50 $\pm$ 2.81	111.40 $\pm$ 9.56
HCT (%)	0.28 $\pm$ 0.01	0.28 $\pm$ 0.01	0.28 $\pm$ 0.01	0.26 $\pm$ 0.02
MCV (fL)	51.17 $\pm$ 0.73	51.81 $\pm$ 0.68	52.57 $\pm$ 0.57	52.79 $\pm$ 0.78
MCH (pg)	21.19 $\pm$ 0.45	21.78 $\pm$ 0.85	22.40 $\pm$ 0.51	22.34 $\pm$ 0.65
MCHC (g/L)	415.60 $\pm$ 6.44	421.50 $\pm$ 15.87	427.60 $\pm$ 9.64	424.50 $\pm$ 11.67
MPV (fL)	10.93 $\pm$ 0.36	10.48 $\pm$ 0.39	10.67 $\pm$ 0.34	10.30 $\pm$ 0.47
PDW	19.17 $\pm$ 0.24	19.00 $\pm$ 0.29	19.43 $\pm$ 0.17	19.36 $\pm$ 0.20

RDW-SD (fL)	25.79 ± 0.39	26.23 ± 0.49	27.14 ± 0.50	26.63 ± 0.41
PLT (×10 ⁹ /L)	907.40 ± 98.92	902.38 ± 97.25	856.00 ± 50.59	806.30 ± 83.44
PT (s)	8.49 ± 0.17	8.41 ± 0.11	8.34 ± 0.12	8.12 ± 0.21
APTT (s)	17.75 ± 0.63	17.31 ± 0.31	21.10 ± 2.00	19.18 ± 1.15
FIB (g/L)	2.07 ± 0.16	1.88 ± 0.14	1.74 ± 0.14	1.58 ± 0.15

3.5. Biochemical indices

Data from biochemical analyses are presented in Tables 4 and 5. AST values of DO-BTE-treated groups showed a decreasing trend relative to the control group without statistical significance ($P > 0.05$). For ALP, while pairwise comparisons between individual treatment groups and the control did not reach statistical significance, a formal linear trend analysis revealed a highly significant dose-dependent increase across the groups ($P = 0.0059$). Urea and CRE levels of DO-BTE-treated rats showed a minor decrease compared with the control group ($P > 0.05$). No significant changes in other parameters were detected. The main biochemical parameters were not markedly different between the combination-treated and control ovariectomized rats ($P > 0.05$).

3.6. Histopathological changes

Pathological findings in liver and kidney samples of normal and ovariectomized animals are depicted in Fig. 2. No abnormalities in the color and texture of organs between the groups were detectable in macroscopic analyses.

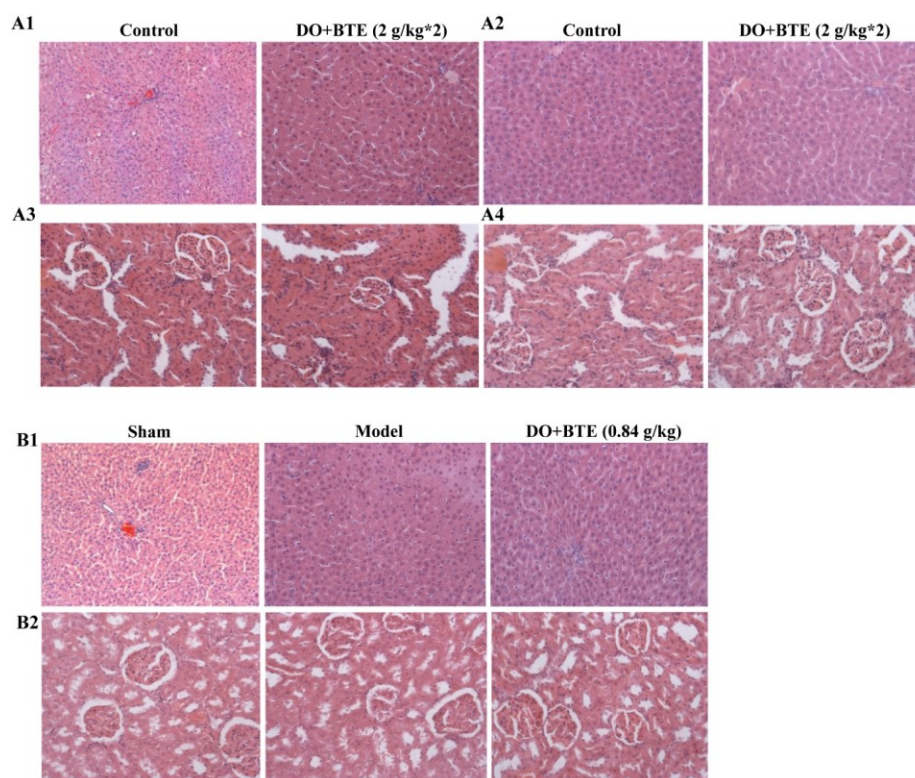


Figure 2. Histopathology of liver and kidney sections in normal and ovariectomized rats observed via H&E staining at 40× magnification. A1. Liver of normal male rats. A2. Liver of normal female rats. A3. Kidney of normal male rats. A4. Kidney of normal female rats. B1. Liver of ovariectomized rats. B2. Kidney of ovariectomized rats.

4. Discussion

Natural products are becoming increasingly popular in healthcare worldwide but their safety and effectiveness remain a major public health concern. Although the bioactive compounds in medicinal plants

clearly exert a variety of beneficial biological effects in humans, knowledge of their potential toxicities is limited. Both *Dendrobium officinale* and black tea are natural products with a long history of common usage worldwide. Previously, our group demonstrated the efficacy of a combination of these two constituents in osteoporosis management [12]. Although the safety of *Dendrobium moniliforme* [14] and Pu-erh black tea have been evaluated individually [15], the potential toxic effects of the mixture remain unknown. Accordingly, the present study was performed to evaluate the acute and chronic oral toxicity of DO-BTE in rodents.

In 14-day acute toxicity tests, oral exposure to the combination treatment at a dose of 10 g/kg triggered no significant toxic effects except lower activity. In the 90-day subchronic toxicity study, both male and female rats treated with DO-BTE at doses of 0.5, 1 and 2 g/kg twice daily showed no signs of clinical toxicity or mortality. Changes in body weight and food/water ingestion are generally used as indicators of harmful effects of drugs and chemicals [20, 21]. In our experiments, these three parameters showed no significant differences, except for a slower growth rate of body weight in the combination treatment group relative to the control group, which may be attributable to frequent administration (twice daily).

Organ weight is one of the main parameters used to evaluate the effects of test substances in toxicity studies [22, 23]. No significant alterations were observed in the organ indices of the treatment groups relative to the control group. Moreover, oral administration of repeat doses did not induce atrophy or swelling of organs, including heart, liver, spleen, kidneys, thymus, lung, testes, epididymis, uterus or ovaries [15].

The hematopoietic system, which is highly sensitive to toxic substances, can undergo alterations upon ingestion of poisonous plants [2], and is therefore used as an important indicator of physiological and pathological conditions [24, 25]. Hematological parameters reflect adverse effects on bone marrow function, such as anemia and hemolysis [26]. Our results showed that WBC and RBC counts of the treatment groups were comparable to those of the control group. Other hematological parameters examined ranged within normal reference values [21, 22].

FIB, a known risk factor for coronary heart disease, is considered a critical part of the coagulation pathway [28, 29]. While slight decreases in FIB and PLT were observed in this study, the exact pharmacological mechanisms underlying these changes remain unclear. Therefore, the specific impact of the extracts on the coagulation system represents a limitation of the current study, which warrants further targeted investigations.

Clinical blood biochemical examinations are commonly used to assess toxic effects on liver function (ALT, AST, TP, ALB, GLB, T-BIL and ALP) [30], kidney function (urea and CRE) [31] and pancreas function (GLU) [19]. The liver is a central organ of detoxification for most drugs [30]. Moreover, ALT and AST are well-known enzymes used as indices of liver function and predictive biomarkers of potential toxicity [32, 33]. In our study, a slight decreasing trend in AST was observed in the 2 g/kg×2 DO-BTE treatment groups. For ALP, however, a significant dose-dependent increase was noted across the treatment groups. While this monotonic upward trend suggests that higher doses may impose a mild metabolic burden on the liver, other key indicators (TP, ALB, GLB, T-BIL) remained stable [21, 34]. Most importantly, histopathological evaluation revealed no structural damage in liver tissues. We postulate that this ALP elevation likely represents an adaptive metabolic response rather than overt hepatotoxicity. CRE and urea values of the combination treatment groups were slightly lower than those of the control group but remained within the normal range [34]. Other biochemistry indices were within normal limits [21, 34, 35], signifying that DO-BTE does not cause damage to liver, kidney or pancreas function.

Histopathological examination is a fundamental step in preclinical toxicology research [26, 34]. According to the OECD guidelines, microscopic examination of organs is not required in the low-dose group when no histopathological alterations are observed in the high-dose group [17, 36]. Our results disclosed no apparent histopathological changes in liver and kidney of rats treated with high doses of DO-BTE in chronic toxicity experiments and OVX-induced rats.

5. Conclusion

At the oral doses tested, the DO-BTE combination did not produce any signs of acute or chronic toxicity in both normal and ovariectomized rats. No lethality or significant alterations in hematological and serum chemical indices or adverse histopathological effects were evident. The maximum tolerance dose in mice was 10 g/kg. However, long-term toxicity in non-rodents and coagulation function require comprehensive evaluation for clarifying the clinical safety of DO-BTE and providing evidence to support its further development as a pharmaceutical or health product.

Table 4. Serum chemistry values in normal rats treated orally for 90 days.

Index	Control	0.5 g/kg×2	1 g/kg×2	2 g/kg×2
ALT (U/L)	63.11 ± 7.50	63.75 ± 3.42	79.60 ± 5.60	63.80 ± 3.38
AST (U/L)	107.44 ± 22.15	94.63 ± 22.79	92.60 ± 28.70	48.90 ± 15.74
TP (g/L)	54.44 ± 1.44	54.00 ± 1.58	51.10 ± 1.04	51.20 ± 1.20
ALB (g/L)	34.22 ± 1.85	35.13 ± 1.43	32.90 ± 0.86	32.80 ± 0.99
GLB (g/L)	20.22 ± 0.97	18.88 ± 0.58	18.20 ± 0.53	18.40 ± 0.45
A/G	1.76 ± 0.16	1.88 ± 0.10	1.81 ± 0.08	1.79 ± 0.06
T-BIL (μmol/L)	1.23 ± 0.19	1.14 ± 0.16	1.30 ± 0.27	1.25 ± 0.22
ALP (U/L)	126.00 ± 17.84	131.86 ± 11.13	157.70 ± 13.00	195.90 ± 26.17
Urea (mmol/L)	5.13 ± 0.26	4.73 ± 0.40	5.03 ± 0.22	4.37 ± 0.26
CRE (μmol/L)	69.22 ± 2.67	64.00 ± 2.31	61.30 ± 1.29	62.90 ± 1.83
GLU (mmol/L)	5.88 ± 0.38	6.11 ± 0.53	5.38 ± 0.22	5.88 ± 0.41
TG (mmol/L)	0.46 ± 0.03	0.54 ± 0.03	0.48 ± 0.03	0.41 ± 0.03
TC (mmol/L)	1.08 ± 0.05	1.34 ± 0.11	1.28 ± 0.08	1.37 ± 0.07
Na (mmol/L)	140.74 ± 0.38	140.09 ± 0.57	140.70 ± 0.43	140.40 ± 0.38
K (mmol/L)	4.94 ± 0.14	5.08 ± 0.24	5.28 ± 0.13	5.07 ± 0.12
Cl (mmol/L)	108.26 ± 0.71	109.08 ± 0.59	108.68 ± 0.65	107.58 ± 0.57

Table 5. Serum chemistry values in ovariectomized rats treated orally for 90 days.

Index	Sham	Model	DO+BTE (0.84 g/kg)
ALT (U/L)	49.32 ± 3.11	59.35 ± 3.63	60.96 ± 2.19
AST (U/L)	201.16 ± 12.62	207.86 ± 14.63	214.59 ± 10.54
BUN (mmol/L)	8.30 ± 0.39	7.67 ± 0.32	7.27 ± 0.40
CRE (μmol/L)	46.65 ± 2.34	46.58 ± 1.99	47.14 ± 1.23
GLU (mmol/L)	4.76 ± 0.30	5.72 ± 0.47	4.89 ± 0.23
TG (mmol/L)	0.69 ± 0.03	0.59 ± 0.07	0.55 ± 0.06
TC (mmol/L)	2.07 ± 0.14	2.23 ± 0.11	2.46 ± 0.78

Author Contributions

Conceptualization: X.-J.W., Y.-L.Z.; Investigation: M.-Y.W., G.-T.L., Y.-R.X., S.-Y.Z.; Methodology: M.-Y.W., M.-F.A., Y.-R.L., R.Z., Q.-H.Z.; Writing – original draft: M.-Y.W.; Writing – review & editing: X.-J.W., Y.-L.Z.; Supervision: X.-J.W., Y.-L.Z.; Funding acquisition: X.-J.W., Y.-L.Z.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Statement

All animal experiments were performed according to institutional guidelines and approved by the Institutional Animal Care and Use Committee of the Yunnan Agricultural University (approval number: YNAU2019LLWYH003-1; approval date: March 21, 2019). Euthanasia was performed via CO₂ asphyxiation followed by cervical dislocation per AVMA Guidelines.

Data Availability

All data are included in the article.

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