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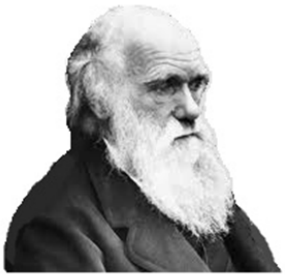

World News of Natural Sciences

An International Scientific Journal

WNOFNS 58 (2025) 270-282 EISSN 2543-5426

Acute Toxicity and Antidiabetic Effect of Polyherbal Formulation in Alloxan–Induced Diabetic Mice Model

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ABSTRACT

In Vietnam, many herbal plants with various medicinal activities can support different diseases such as anti-anemic and antimicrobial etc including diabetic treatments. Diabetes mellitus is a group of metabolic disorders that is characterized by elevated levels of glucose in the blood. When combining the herbs following a formula, the healing effect will be more effective due to synergetic actions. Our present study assesses acute toxicity and antidiabetic activity of a new polyherbal preparation, CHM-Tieu duong, in the form of capsules. It was developed based on the traditional use of six indigenous plants from the South-Western region of Vietnam, effective in fighting diabetes, namely *Andrographis paniculata* (leaves), *Centella asiatica* (leaves), *Dioscorea opposite* (tuberous rhizomes), *Gymnema sylvestre* (leaves), *Gynostemma pentaphyllum* (herbs), and *Morus alba* (leaves). Methods: The acute toxicity was evaluated following the Organization for Economic Cooperation and Development (OECD) guidelines No. 420 using five lots of female BALB/c albino mice. The antidiabetic activity was studied using an alloxan-induced diabetic mice model on thirty BALB/c albino mice of either sex. The mice were divided into five groups, namely normal mice control group with normal saline, diabetic mice fasted control group with normal saline, diabetic mice positive control group treated with the standard drug, metformin at a dose of 100 mg/kgP, and two diabetic mice groups

(Received 17 November 2024; Accepted 20 December 2024; Date of Publication 22 December 2024)

treated with different doses of herbal combination at 500 and 1000 mg/kgP. Resutls: The acute toxicity analysis showed that CHM-Tieu duong was orally non-toxic at a single dose of 5000 mg/kgP as it did not cause any pre-clinical changes in experimental mice. The results of the antidiabetic study showed that CHM-Tieu duong exhibited the lowering glucose levels in the alloxan-induced diabetic mice model at the high dose of 1000 mg/kgP dose. Conclusion: The findings demonstrated that CHM-Tieu duong could serve as safe and effective antidiabetic polyherbal combination.

***Keywords*:** Polyherbal combination, Indigenous plants, Southwestern region of Vietnam, Acute toxicity, Antidiabetic, Alloxan-Induced Mice

1. INTRODUCTION

Diabetes has been identified as one of the six leading causes of disease death in the world and also various systemic complications (Lin *et al.*, 2020) [1]. Diabetes mellitus is a group of metabolic diseases caused by defects in insulin secretion or insulin action resulting in hyperglycemia. It is treated by hormone therapy (insulin) or by administering glucose-lowering agents such as alpha-glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones, which might develop adverse complications event (Garcia *et al*., 2018) [2]. Medicinal plants are effective in suppressing glucose production in the human body without any complications (Yedjou *et al.*, 2023) [3]. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Polyherbal formulations are well documented in the literature. Combining herbal powders based on their therapeutic properties is necessary to create traditional medicines for polyherbal formulations. Recently, many studies and research have been conducted to develop natural therapeutics that help to support diabetic patients (Blahova *et al.*, 2021; Bautista *et al.*, 2024) [4, 5]. Following a thorough literature review, six distinct herbal plants from local medicinal plants in the Southwestern region of Vietnam, namely *A. paniculata*, *C. asiatica*, *D. opposite*, *G. sylvestre*, *G. pentaphyllum*, and *M. alba*, were chosen with the help of a traditional medicine specialist, and a plant-derived antidiabetic formula, so-called CHM-Tieu duong, was created for the capsule formulation with pharmaceutically acceptable excipients. The polyherbal combination was fabricated into capsules. The leaf extract of *A. paniculata* at a dose of 500 mg/kg exhibited significant blood sugar-lowering properties in alloxan-induced diabetic Wistar rats (Yusuf *et al.*, 2022) [12]. A study of *C. asiatica* extract in type 2 diabetic rats showed no significant change in insulin secretion and no effect on liver glycogen deposition. The extract was found to inhibit the action of both intestinal disaccharidase and α-amylase (Ayodeji *et al.*, 2020) [10]. The crude 95% ethanol extract of the *D. opposite* tuberous rhizomes showed considerable α-glucosidase inhibitory activity (Zhang *et al.*, 2011) [9]. *G. sylvestre* is a known medicinal plant commonly used for the management of diabetes and other ailments in traditional medicine. Its extract significantly reduced blood glucose levels with a subsequent increase in plasma insulin levels and reduced total oxidant status, malondialdehyde, LDL, triglyceride, total cholesterol, and total protein levels (Muzaffar *et al.*, 2023) [6]. The *G. pentaphyllum* extract with a dose of 250 mg/kg reduced post-prandial hypertriglyceridemia, cholesterol, and LDL-cholesterol in the obese Zucker fatty diabetic rat model, improved glucose tolerance (Megalli *et al.*, 2006) [7]. Its *G. pentaphyllum* saponin extract was more effective hypoglycemic in streptozotocin-diabetic rats, and also ameliorate dyslipidemia than those of diabetic control rats by increasing SOD, GSH-px activities and

insulin levels (Gao *et al.*, 2014) [8]. The *M. alba* leaf extract at 600 mg/kg bw reduced blood glucose levels and restored the diminished β cell numbers in diabetes-induced Wistar rats (Mohammadi *et al.*, 2008) [11].

When consumed over a long period, medicinal plants might be associated with adverse effects on humans. The safety evaluation of medicinal plants intended for use is an important aspect of drug development from natural products. In vivo acute, sub-acute, chronic, and sub-chronic toxicity studies are the most widely accepted experimental strategies for assessing the safety or toxicity of medicinal plants as data generated from these studies holds a translational relevance in humans (Madhav *et al.*, 2024) [13]. Acute toxicity tests are short-term tests conducted to determine the immediate effects of any substance or formulation at a single exposure. It is useful to determine the median lethal dose (LD_{50}). Sub-acute toxicity tests, on the other hand, are designed to evaluate the effects of repeated exposure to a substance. The substance is often given to the animals daily for 4 weeks (Aydin *et al.*, 2016) [14].

This study primarily focuses on verifying any acute toxicity being produced from the prepared combination, CHM-Tieu duong. Finally, the formulation is studied for the hypoglycemic activity in alloxan-induced diabetic mice.

2. MATERIALS AND METHODS

Chemicals and reagents

Alloxan monohydrate and metformin were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). Glucocard Test Strip II kits were bought from Tokushima (Japan). Pentobarbital sodium and saline water of analytical grade were obtained from Central Pharmaceutical CPC1 JSC (Vietnam). All other reagents and chemicals were of analytical grade from Merck (USA). Serum triglyceride, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) assay kits were bought from Abcam (Cambridge, MA, USA).

Preparation of ingredient extracts and polyherbal formulation

The herbal materials for extract preparation including *A. paniculata* leaves, *C. asiatica* leaves, *D. opposite* rhizomes, *G. sylvestre* leaves, *G. pentaphyllum* herbs, and *M. alba* leaves were collected from An Giang province (Vietnam). All the studied samples were authenticated by Dr. Hai T.V. (VAST). Among those materials, *A. paniculata*, *G. sylvestre*, *G. pentaphyllum* and *Morus alba* extracts were prepared according to standard methods of Vietnamese pharmacopeia V (Ministry of Health, 2019; pages 1280, 1139, 1178, and 1136) [15]. Dried leaves of *A. paniculata* (1 kg) were refluxed for 6 h with 10 vol. of distilled water at 90 °C, thrice. The extracts were combined and concentrated using a vacuum rotary evaporator at 50 °C to afford 31.70 g of the water extract. Dried *G. sylvestre* leaves (1kg) were cut into small pieces, extracted thrice with 1L of distilled water for 6 h at 90 °C in water bath. After removing the solvent by lyophilization, 41.03 g of the dried extract was received. Dried *G. pentaphyllum* herbs (1 kg) were infused with 1L of water during 72 h and then extracted at 90 °C for 6 h, thrice. Water solutions were collected, filtered, and vacuum-dried to obtain 25.75g of the dried water extract. Dried *M. alba* leaves (1 kg) were soaked with 10 vol. of distilled water and then extracted at 90 °C for 3 h, thrice. All water extracts were combined, filtrated, and concentrated under vacuum at 50 °C to obtain 34.46 g of the dried water extract. Other ingredient extracts, *C. asiatica* and *D. opposite* were prepared following laboratory-developed methods (Le Ngoc

Hung, 2020, unpublished data) [16]. Dried *C. asiatica* aerial parts (1 kg) were coarsely powdered and extracted with 50% ethanol (5L) by cold maceration for 24 h, thrice. The collected extract solution was evaporated to remove solvent at 50 °C and reduced pressure to collect 23.59 g of the *C. asiatica* extract. Dried rhizomes of *D. opposite* (1kg) were powdered and extracted with 50% ethanol (5L) at room temperature for 24 h, thrice. The ethanol extract was combined and evaporated to dryness under reduced pressure to yield 62.41 g of the extract.

Each hard capsule sized 0 contains 500 mg CHM-Tieu duong powder including 150 mg of *Herba Andrographii* extract, 100 mg of *Herba C. asiaticae* extract, 50 mg of *D. opposita* extract, 50 mg of *Caulis et folium Gymnematis sylvestris* extract, 50 mg of *Herba G. pentaphylli* extract, 100 mg of *Folium M. albae* extract, and excipients in sufficient quantities.

Experimental animals

BALB/c albino mice weighing 20 to 24 grams were obtained from the National Institute of Hygiene and Epidemiology (Hanoi, Vietnam) and kept in the animal house of the Pharmacy University of Hanoi. To identify the rats, a designated number was marked on the tail of each rat using a permanent marker. Experimental mice were kept in the standard laboratory conditions at 25 ± 2 °C, relative humidity of 44-56%, and 12:12 hours light and dark cycles throughout the experiment. Before the experimental procedures, all experimental animals underwent a 5-day acclimatization period to become accustomed to their surroundings. All of the animals were freely fed with a standard diet and water during the adaptation period. The design and performance of animal experiments were approved by the Ethical Committee, Pharmacy University of Hanoi, Vietnam (reference number 5-23/PCT-HĐĐĐ).

Experimental procedures

Acute toxicity study

The acute toxicity experiments followed the OECD guidelines (OECD No 420, 2001) [17]. Healthy, nulliparous, non-pregnant, female BALB/c albino mice, which had not been subjected to previous experimental procedures, were used. Sixteen mice were fasted for overnight before the commencement of experiments. Groups were observed for body weight on the first day of dosing and then after one week. Mice were observed carefully during the first 30 min. and 4 hours, and then daily for a total of 14 days for signs of toxicity like tremors, convulsions, salivation, diarrhea, lethargy, writhing, edema, opisthotonos, exophthalmos, corneal opacity, eye swelling or reddening, sleep, and coma. Changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern were also noted. The limit test was employed in the acute oral toxicity study since available information suggested that all the herbal components of the CHM-Tieu duong are likely to be non-toxic (Worasuttayangkurn *et al.*, 2019; Gayuk *et al.*, 2022; Cha *et al.*, 2021; Raji *et al.*, 2021; Chiranthanut *et al.*, 2013; and Li *et al.*, 2018) [18-23]. Administration of the stepwise dose of polyherbal preparation CHM-Tieu duong starting from 2000 mg/kg up to 5000 mg/kg was picked due to the results of the exploratory pre-test (lot 1).

Lot 1 (one mouse): was given CHM-Tieu duong at a dose of 2000 mg/kgP. If no mortality occurs, testing proceeds to a further stage

Lot 2 (five mice): control, 0,2-0,3 ml of water was given,

Lot 3 (five mice): CHM-Tieu duong at a dose of 3000 mg/kgP were given,

Lot 4 (five mice): CHM-Tieu duong at a dose of 4000 mg/kgP were given,

Lot 5 (five mice): CHM-Tieu duong at a dose of 5000 mg/kgP were given.

Induction of Experimental Diabetes

Animals were made hyperglycemic via intraperitoneal administration of alloxan monohydrate. Prior to experiments, mice were fed with standard rodent food for one week for acclimation to the lab conditions. The acclimated mice were fasted for 12 h with free access to water, then injected with alloxan dissolved in a freshly prepared saline (0.9%) at a dose of 150 mg/kgP. Diabetic rats were confirmed by the fasting blood glucose concentration above 15 mmol/L after alloxan administration.

Hypoglycemic effect study

In vivo doses of CHM-Tieu duong powder were determined according to intended clinical doses: a lower dose of 500 mg/kgP and a higher dose of 1000 mg/kgP, equivalent to 3 and 6 capsules per day. Diabetic mice were randomly divided into four groups (n = 6):

(1) Normal control (NC): mice treated only with normal saline per day, 5 mL/kgP;

(2) Diabetes control (D): diabetic mice treated with normal saline per day, 5 mL/kgP;

(3) Diabetes + metformin (D + M): diabetic mice treated with metformin at a dose of 100 mg/kgP per day, 5 mL/kgP;

(4) Diabetes + 500 mg/kgP CHM-Tieu duong (D + C5): diabetic mice treated with CHM-Tieu duong at 500 mg/kgP per day, 5 mL/kgP;

(5) Diabetes + 1000 mg/kgP CHM-Tieu duong (D + C10): diabetic mice treated with CHM-Tieu duong at 1000 mg/kgP per day, 5 mL/kgP.

Saline, metformin, and CHM-Tieu duong were orally administrated by gavage once a day for 7 consecutive days. Body weight and fasting blood glucose level were determined on days of the 3rd and 7th after the CHM-Tieu duong administration. On the 7th day, animals of all groups were deprived of food overnight and anesthetized by giving an intraperitoneal injection of 40 mg/kg pentobarbital sodium. The blood serum of each mouse was collected in tubes for blood glucose analysis.

Blood Tests

Fasting blood samples were collected from the tip of tail veins of rats after oral administration of CHM-Tieu duong for 7 consecutive days. The blood was used for determination of glucose concentration with a glucometer (Glucocard Test Strip II, Tokushima, Japan) immediately after collection.

Data analysis

All data presented in this study are expressed as the mean, standard deviation of the mean value. Statistical analysis consisted of the Student's t-test for comparing two mean values and a one-way analysis of variance (ANOVA) when more than two mean values were compared.

3. RESULTS

Acute toxicity study

While administrating CHM-Tieu duong powder at all the doses, there were no sign of dizziness, tremors, convulsions, writhing, sleep, and coma or lethargy. Loss of appetite was observed in one mouse of lot 4 dosing within 30 minutes that lasted only for the first day. After 14 days of experiment, experimental animals displayed no changes in skin and fur, eyes and mucous membranes, central nervous systems, behavioral pattern changes or toxic signs. No death was observed in any groups of mice. Attention was given to body weight evaluation before and during the experiment period as presented in Table 1. The weights were not statistically different.

Table 1. Weight evaluation of body weight in tested mice

Lot/dose (mg/kg)	Body weight (g)			
	Before administration	**Day 1**	**Day 7**	**Day 14**
Lot 2/ control (NC)	20.67 ± 0.29	20.87 ± 0.38	21.78 ± 0.31	22.36 ± 0.28
Lot 3/ 3000 mg/kgP	20.71 ± 0.31	20.89 ± 0.25	21.63 ± 0.33	21.76 ± 0.32
Lot 4/ 4000 mg/kgP	20.48 ± 0.23	20.82 ± 0.34	21.56 ± 0.24	22.95 ± 0.27
Lot 5/ 5000 mg/kgP	20.67 ± 0.38	20.91 ± 0.31	21.58 ± 0.31	21.87 ± 0.33
P	>0.05	>0.05	>0.05	>0.05

** P<0.05 means that the difference is statistically significant.

All the acute toxicity tested results showed that the polyherbal formulation, CHM-Tieu duong was nontoxic and did not cause any abnormal clinical changes in tested animals at 5000mg/kgP dose.

Hypoglycemic activity study

Changes in the body weight profile of induced rats in different experimental groups were presented in the Table 2. Throughout the study, the diabetic mice (D) showed significant increases (~20.50%) in body weight when compared to the control animals (NC). However, the polyherbal formulation, CHM-Tieu duong (D + C5 and D + C10) and metformin (D + M) inhibited the diabetes-induced body weight reduction.

The hypoglycemic results were shown in Table 3. Diabetic control mice (D) showed severe hyperglycemia compared to NC by 1.7 fold increase. The mean blood glucose level in the D group before administration was 18.90 ± 2.26 mmol/L and on day 7th was 20.90 ± 1.75 mmol/L. It was observed that the standard drug metformin lowered the blood glucose level significantly by a decrease of 33.42% ($P < 0.05$), whereas the polyherbal capsule, CHM-Tieu duong at 500 mg/kg and 1000 mg/kg significantly ($P < 0.05$) (D + C5 and D + C10) decreased the fasting blood serum glucose level in the diabetic mice on 7th day, as compared to the D group by 22.19% and 29.79%, respectively. In the same diabetic mice groups treated with

different doses of polyherbal combination, D + C5 and D + C10, the fasting blood serum glucose levels in the diabetic mice on 7th day, as compared before administration, were decreased by 11.91% and 22.08%, respectively.

Table 2. Body weight changes of mice in different experimental groups

Lot/dose (mg/kg)	Body weight (g)		
	Before administration	**Day 3**	**Day 7**
Normal control (NC)	20.82 ± 0.31	21.35 ± 0.31 (↑2.55%)	21.85 ± 0.29 (↑4.95%)
Diabetes control (D)	25.03 ± 1.16 (↑20.22%)	25.42 ± 0.76 (↑1.59%)	24.98 ± 1.92 (↓0.20)
Diabetes + metformin (D + M)	25.10 ± 0.67 (↑20.56%)	23.57 ± 0.30 (↓6.10)	23.90 ± 0.46 (↓4.78)
Diabetes + 500 mg/kgP (D + C5)	25.93 ± 1.67 (↑24.54%)	25.22 ± 2.68 (↓2.74)	24.50 ± 1.98 (↓5.51)
Diabetes + 1000 mg/kgP (D + C10)	25.17 ± 0.93 (↑20.89%)	23.67 ± 0.67 (↓5.96)	22.50 ± 1.32 (↓10.61)
P	>0.05	>0.05	>0.05

* P>0.05 means that the difference is statistically insignificant.

Table 3. Glucose parameters of rats in different experimental groups

Lot/dose (mg/kg)	Glucose levels (mmol/L)	
	Before administration	**Day 7**
Normal control (NC)	6.81 ± 1.02	6.79 ± 1.03
Diabetes control (D)	18.90 ± 2.26 (↑170.04%)	20.90 ± 1.75 (↑10.58%)
Diabetes + metformin (D + M)	18.37 ± 2.19 (↑169.75%)	12.23* ± 1.32 (↓33.42%/ ↓41.52%)
Diabetes + 500 mg/kgP (D + C5)	18.47 ± 1.50 (↑171.22%)	16.27 ± 1.35 (↓11.91%/ ↓22.19%)
Diabetes + 1000 mg/kgP (D + C10)	18.84 ± 2.02 (↑176.65%)	14.68* ± 3.73 (↓22.08%/ ↓29.79%)

* P<0.05 means that the difference is statistically significant.

4. DISCUSSION

Effects of polyherbal combination on fasting blood glucose of normal and diabetic mice

The use of traditional medicine is expanding globally, and public health and safety issues are also receiving increased attention. Tables 2 showed changes in body weights of individual

groups of mice at 3rd and 7th days after a model of diabetic rats being established. The weights were changed in similar manner in all the 4 experimental groups (P>0.05). It is well known that loss of body weight is one of the most intuitive indicators of diabetes. In acute toxicity study, no dead mouse was found after taking the combination of CHM-Tieu duong powders in all experimental and control groups.

According to OECD, GHS (Globally Harmonized System of Classification and Labelling of Chemicals), and WHO classifications based on oral toxicity, the studied CHM-Tieu duong was non-toxic at maximum doses, 5000 mg/kg body weight. Previously, each constituent of the new formulation, CHM-Tieu duong, had been studied for acute toxicity. The standardized *A. paniculata* leaves extract had no significant acute toxicological effects at an upper fixed dose of 5000 mg/kgP (Worasuttayangkurn et al. 2019) while its sub-acute repeated-dose toxicity at 1 g/kg for 8 weeks did not reveal any obvious toxic effects (Tian *et al.***,** 2022) [24]. The standardization *C. asiatica* water extract showed no evidence of acute toxicity with a single dose of 10 g/kg in male or female mice and no severe pathological abnormalities in any of their organs. Its subchronic toxicity was safe at a dose of 1,000 mg/kg/day for 90 days (Gayuk *et al.*, 2022) [19]. The *D. villosa* water extract administered by rats at any dose lower than 5000 mg/kgP showed no adverse effects on clinical signs, body weight, food and water consumption, ophthalmic examination, urinalysis, hematology, or organ weights (Cha *et al.*, 2021) [20] while the *G. sylvestre* extract was safe at sub-acute doses of 100 mg/kgP for diabetic management (Raji *et al.*, 2021) [21].

The standardized water extract of *G. pentaphyllum* did not cause acute oral toxicity with the single oral dose of 5000 mg/kg in female Sprague-Dawley rats. Its subchronic toxicity with the daily administration of the oral dose of 1000 mg/kgP for 90 days did not produce lethal or harmful effects (Chiranthanut *et al.*, 2013) [22]. The *M. alba* leaf extract study showed the acute toxicity LD50 was higher than 15.0 g/kgP as no mortality or behavioral changes were observed. Its subacute toxicity was at the highest dose of 7.50 g/kgP as the hematological, biochemical, or histopathological parameters of the tested rats were not significantly changed (Li *et al.*, 2018) [23].

Effects of polyherbal combination on fasting blood glucose of normal and diabetic mice

Diabetes is characterized by hyperglycemia with an increase of glucose in blood. Alloxan is a classical diabetogenic chemical which exerts selective cytotoxic influences on pancreatic β-cells, resulting in destruction of β-cells and type 1 diabetes. It is well known that loss of body weight is one of the most intuitive indicators of diabetes (Li *et al.***,** 2015) [25]. Tables 3 showed changes in fasting blood glucose levels of individual groups of mice within 7 days after a model of diabetic rats being established. Levels of fasting blood glucose of diabetic rats which were injected with alloxan at a dose of 100 mg/kg after 7 days were all higher than 20 mmol/L. These high levels of fasting blood glucose could confirm that the model of diabetic rats had been successfully established. Moreover, by comparing glucose levels of D + M mice group with those of D mice group in Table 3, it is obvious that there was significant decrease of fasting blood glucose after oral administration of metformin at a dose of 100 mg/kg for as long as 7 days. The metformin exhibited no effect on fasting blood glucose in diabetic rats, implying that the pancreatic β-cells might be damaged too severely by alloxan to synthesize insulin. In diabetic rats, oral administrations of the polyherbal combination at 2 doses of 500 and 1000 mg/kgP for 7 days could reduce fasting blood glucose but the decreases were all lower compared with those of the DC rats.

The results demonstrated that CHM-Tieu duong could reduce the fasting blood glucose of diabetic rats, with no side-effects on both body weights**.** Each herbal component of the polyherbal formulation, HM-Tieu duong has been shown to have antidiabetic properties in published research.

In a hypoglycaemic study, the *A. paniculata* crude extract reduced the blood glucose level of diabetic induced rats and found no significant difference with the hypoglycaemic activity of insulin at dose of 500 mg/kgP (Victoria *et al.*, 2020) [26] and another study in the diabetic dogs model showed that its supplementation did not alter the blood glucose levels and did not have any adverse effects (Suemanotham *et al.*, 2023) [27]. The *C. asiatica* ethanol extract at a dose of 1000 mg/kgP showed no significant change in insulin secretion in-vivo and in isolated rat islets, and no effect on liver glycogen deposition (Kabir *et al.*, 2014) [28]. In a streptozotocin-induced diabetic rats model study, the crude *D. batatas* powder maintained glucose levels and exerted antidiabetic effects by modulating oxidative stress, antioxidant activities, and lipid profiles; improving kidney and liver function; promoting the release of GLP-1; and improving the function of β-cells (Go *et al.*, 2015) [29].

Avanish *et al.*, (2021) [30] showed that *G. sylvestre* extract with a dose of 4.7 mL/kg had the ability to lower blood glucose levels in streptozotocin-induced diabetic rats. The *G. pentaphyllum* extract maintained glucose levels and significantly enhanced 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose uptake and Glucose Transporter 4 translocation via activating the AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) signaling pathway (Wang *et al.*, 2018) [31]. The water extract of *M. alba* leaf at the dose of 600 mg/kg significantly reduced blood glucose levels and improved the histological features of pancreatic islets in diabetic rats (Suphaket *et al.*, 2011) [32]. The isolated compounds of the herbal components of the polyherbal formulation, HM-Tieu duong, and their combinations with other compunds also have been shown to have antidiabetic properties in published research. From the *M. alba* root bark, three isolated compounds, moracin M, steppogenin-4'-O-beta-D-glucosiade, and mullberroside A produced hypoglycemic effects. In a dose of 100 mg/kg, moracin M could make the fasting blood glucose level decreased (Zhang *et al.*, 2009) [33].

The combination of *A. paniculata* and *C. sappan* extracts had a moderate antihyperglycemic effect; however, a single extract may have better potential than the combined extract (Wediasari *et al.*, 2020) [34]. In a vivo activity of a combination of *Centella asiatica* leaves and *Zingiber officinale* rhizome was evaluated in streptozotocin-induced diabetic albino rats model maintained the blood glucose levels at a dose of 200 mg/ kgP (Ahmed *et al.*, 2023) [35]. All the results of the present study showed that the treatment of diabetic mice with the polyherbal formulation, HM-Tieu duong for 7 days showed hypoglycaemic effect as compared with the diabetic non treated mice.

5. CONCLUSION

In conclusion, the present study demonstrated that the polyherbal CHM-Tieu duong preparation at 5000 mg/kg body weight/day did not cause any death in experimental animals and belongs to the orally non-toxic group. The polyherbal combination also exhibited noticeable hypoglycemic activity in the induced mice model at a higher dose of 1000 mg/kg. However, a longer treatment course study is needed for more sufficient effects.

AUTHOR CONTRIBUTION

N.T. Huong: Conceptualization, design of experiments, and draft manuscript. **D.N. Thuy**: Acute toxicity experiments. **L.N. Hung**: Experimental diabetes and hypoglycemic effect. **L.M. Ha** and **P.V. Trung**: Polyherbal preparation and data analysis. **M.V. Nam:** Final manuscript

ACKNOWLEDGMENTS

The project was supported by the National Science and Technology Program the south-western region development, grant number KHCN-TNB /14-19/C21.

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