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PHYTOCHEMICAL AND *IN VIVO* BIOLOGICAL STUDIES OF *TANAECIUM BILABIATUM* (SPRAGUE) L.G. LOHMANN

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Abstract

Phytochemical analysis of *Tanaecium bilabiatum* leaf led to the isolation and identification of salacinin C, daturadiol and a mixture of triterpenes, α-amyrin and *β*-amyrin. Besides chemical analysis, the methanol extract of *T. bilabiatum* leaf, METBL was evaluated for analgesic, anti-diarrheal and hypoglycemic potential in mice model. For analgesic activity test by tail immersion technique, the extract significantly extended the percent elongation time of thermal nociception. METBL (400 mg/kg bw) also established the highest inhibition of formalin-induced abdominal writhing (69.39%) in mice, which was comparable with the standard aspirin (75.51%). Likewise, dose-dependent antidiarrheal activity was observed in this test. After oral administration, METBL demonstrated maximum inhibition (40.93%) of castor oil-induced diarrhea in mice. In oral glucose tolerance test, the plant extract produced significant ($p < 0.05$) hypoglycemic activity. Therefore, it is concluded that *T. bilabiatum* is a promising source of bioactive compounds with analgesic, antidiarrheal and hypoglycemic potential.

Introduction

Medicinal plants are important sources of diverge chemical compounds (Newman and Cragg 2020) that have been found to show beneficial pharmacological effects in living system. Therapeutic potential of a medicinal plant is attributed to the multifunctional natural compounds like terpenoids, flavonoids, alkaloids, glycosides and tannins etc. (Mercy and David 2018). Currently, phytochemicals represent over 50% of all therapeutic agents in the world (Pan *et al*. 2013, Barkat *et al*. 2021). Therefore, medicinal plants can provide limitless prospects for research and development of new pharmaceuticals for human. Isolation and characterization of such phytochemicals are scientific processes in discovering the bioactive profiles of a medicinal plant. Biological investigation is an important part of phytochemical research which is performed to define the pharmacological activities of a medicinal plant.

Bignoniaceae is a family of flowering plants which is familiar as the Trumpet Creeper family. This family consists of about 104 genera and 810 species. Plant species under Bignoniaceae family are commonly found in the tropical and sub-tropical countries. Bignoniaceae family members are traditionally significant for their stated bioactive compounds and various pharmacological actions. Phytochemical studies with plants of this family afforded various pharmacologically important compounds including ursolic acid, oleanolic acid, lapachol, verbascoside, quercetin, apigenin, pomolic acid and isoacteoside. In Bangladesh, the plant species belonging to this family are used by folk people for the treatment of diverse ailments (Rahmatullah *et al.* 2010). *Tanaecium bilabiatum* (Sprague) L.G. Lohmann is a flowering plant in the genus *Tanaecium* (Family: Bignoniaceae). The plant species grows in tropical climates in northern South America, western South America and in northern Brazil. The leaves are compound

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with lanceolate to oval shaped leaflets while flowers are trumpet-shaped and white in color with a yellow throat (Website-1). *T. bilabiatum* in Brazil causes acute cardiac failure in livestock and is known to contain monofluoroacetate (Lee *et al.* 2012).

From extensive literature review, very little information is known about the chemical composition and biological effects of *T. bilabiatum.* Therefore, in continuation of the study with medicinal plants (Islam *et al.* 2019, Moniruzzaman *et al.* 2021), it was aimed to conduct phytochemical studies of *n*-hexane fraction of methanol extract of *T. bilabiatum* leaf by frequent chromatographic techniques. In addition, for the first time, the pharmacological activities such as analgesic, anti-diarrheal and hypoglycemic potential of *T. bilabiatum* leaf were determined in mice model.

Materials and Methods

Leaves of *T. bilabiatum* were collected from Bangladesh National Herbarium, where the plant's identification was verified by a taxonomist. Later the plant samples were washed properly with tap water and then subjected to shade drying with sufficient ventilation. The dry leaves were crushed into a fine powder by means of a suitable grinder. About 600 g of powder sample was soaked with methanol (1.5 L) in a flat bottom flask for a period of 15 days. The mixture was filtered with Whatman filter paper (No. 1) and was then concentrated by a rotary evaporator to obtain a semisolid, gummy residue of methanol extract of *T. bilabiatum* leaf (METBL). The extract (5 g) was subjected for solvent-solvent partitioning (VanWagenen *et al.* 1993) to get *n*-hexane, chloroform and aqueous solvent fractions.

For phytochemical studies, the *n*-hexane fraction of *T. bilabiatum* was further fractionated by size exclusion chromatographic technique using Sephadex (LH-20) with *n*-hexane-dichloromethane-methanol (2:5:1) solvent system. A total of 50 fractions were obtained. Repetitive preparative TLC of the sub-fractions 9-14 on silica gel using chloroform-ethyl acetate (90:10) afforded compound **1**, **2** (a mixture of two compounds **2a** and **2b**) while sub-fractions 27-31 yielded compound **3**.

For pharmacological studies, Swiss albino mice of both sex, were purchased from icddr,b. Mice were placed in polypropylene cages under normal environmental condition (24ºC temperature, 60% relative humidity). They were served with icddr,b formulated food and acclimatized for 7 days before experiment. Mice were arbitrarily allocated into four groups of three mice in each for single test. Negative and positive control received saline (10 ml/kg) and standard drug, while the test groups were given 200- and 400 mg/kg bw of METBL, respectively.

In tail immersion method (Aziz *et al.* 2019) for central analgesic activity, the standard group was given morphine solution (2 mg/kg bw) by subcutaneous route while the negative control group of mice were treated orally with normal saline. Two doses (200- and 400 mg/kg bw) of *T. bilabiatum* leaf extract, METBL were loaded to the mice of test groups by oral route. After administration of the test sample and standard drug, tip of mouse tail marked up to 1-2 cm was dipped in a water bath at 55°C temperature which acted as the thermal stimulus for pain sensation. The reaction time (tail flick response) was observed and documented at 0, 30, 60 and 90 min. Then, the percentage of time elongation was determined from the following equation:

$$\% \text{ Time elongation} = \frac{\text{Latency of test} - \text{Latecny of control}}{\text{Latency of control}} \times 100$$

Formalin induced writhing test (Bukhari *et al.* 2016) was used to evaluate the peripheral analgesic activity of METBL in mice. About 20 µl of 1.0% formalin was administered subcutaneously to create pain sensation in mice 30 min after oral administration of extractives and

aspirin. Abdominal writhing was recorded for five min. The peripheral analgesic activity was calculated by the following equation:

$$\%\ \text{Inhibition of writhing} = \frac{N_{\text{Control}} - N_{\text{Test}}}{N_C} \times 100\%$$

Here, N = Mean number of writhing in the respective group.

Anti-diarrheal activity was carried out by castor oil induced diarrheal model (Abdela 2019). Briefly, following 1 hr of administration of plant extracts (200- and 400-mg/kg bw) and standard loperamide (10 mg/kg bw), castor oil (0.5 ml) was given orally to induce diarrhea in each mouse. Then, each mouse was positioned in an isolated cage and the blotting paper was altered at each hour. Next, defecation was observed for 4 hrs. The percentage (%) decrease in diarrhea was determined using the following formula:

$$\%\ \text{Reduction in diarrhea} = \frac{D_{\text{Control}} - D_{\text{Test}}}{D_{\text{Control}}} \times 100\%$$

Here, D = Average number of diarrheal defecations in the respective group.

Hypoglycemic activity of plant extractives was determined by oral glucose tolerance test in mice (Islam *et al.* 2019). Briefly, mice in control group, standard group and trial groups orally received distilled water, glibenclamide and plant extracts, respectively. Blood was taken from the tail vein at 0, 30, 60, 120 and 180 min following the oral glucose (10%) load in each mouse.

The statistical analysis was conducted by Microsoft Excel. Data were expressed as mean ± SEM. $p < 0.05$ were considered as statistically significant.

Salacinin C (**1**): Colorless crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83 (1H, dd, *J* = 15.5, 6.4 Hz, H-1), 6.09 (1H, d, *J* = 11.6 Hz, H-2), 3.25 (1H, dd, *J* = 11.2, 4.8 Hz, H-21*β*), 2.54 (1H, m, H-10α), 2.37 (1H, m, H-4α), 2.03 (1H, m, H-6β), 1.79 (1H, m, H-22α), 1.23 (3H, s, H_3-28), 1.17 (3H, s, H_3-27), 1.02 (6H, s, H_3-29, H_3-30), 0.99 (3H, s, H_3-23), 0.96 (3H, s, H_3-26), 0.93 (3H, s, H_3-25), 0.91 (3H, s, H_3-24).

α-Amyrin (**2a**): Colorless crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.28 (1H, t, *J* = 4.6 Hz, H-12), 3.23 (1H, dd, *J* =10.2, 4.8 Hz, H-3), 1.16 (3H, s, H_3-27), 1.01 (3H, s, H_3-26), 0.97 (3H, s, H_3-28), 0.95 (3H, s, H_3-25), 0.93 (3H, s, H_3-30), 0.89 (3H, s, H_3-29), 0.82 (3H, s, H_3-23), 0.80 (3H, s, H_3-24).

β-Amyrin (**2b**): Colorless crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.31 (1H, t, *J* = 4.6 Hz, H-12), 3.23 (1H, dd, *J* = 10.2, 4.8 Hz, H-3), 1.27 (3H, s, H_3-27), 1.01 (3H, s, H_3-26), 0.97 (3H, s, H_3-28), 0.95 (3H, s, H_3-25), 0.91 (6H, s, H_3-29/H_3-30), 0.82 (3H, s, H_3-23), 0.80 (3H, s, H_3-24).

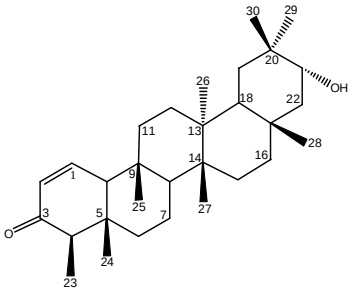
Daturadiol (**3**): Yellowish crystals: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.42 (1H, br. s, H-12), 4.58 (1H, br. s, H-6), 3.19 (1H, dd, *J* = 9.6, 6.0 Hz, H-3), 1.71 (1H, m, H-9), 1.32 (3H, s, H_3-25), 1.26 (3H, s, H_3-26), 1.24 (3H, s, H_3-24), 1.21 (3H, s, H_3-27), 1.10 (3H, s, H_3-23), 1.08 (3H, s, H_3-29), 0.98 (3H, s, H_3-30), 0.96 (3H, s, H_3-28).

Results and Discussion

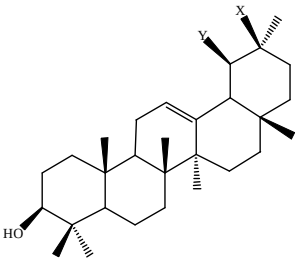
The *n*-hexane fraction of *T. bilabiatum* leaf extract was explored for separation of secondary metabolites from this plant species. Successive chromatographic techniques afforded four pentacyclic triterpenoid compounds *i.e.*, salacinin C, a mixture of α- and *β*-amyrin and daturadiol (Fig. 1).

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** (Fig. 1) exhibited eight signals for methyl protons at δ 0.91, 0.93, 0.96, 0.99, 1.02, 1.02, 1.17 and 1.23. The spectrum also displayed an oxymethine proton signal at δ 3.25 (1H, dd, *J* = 11.2, 4.8 Hz, H-21*β*), a double bond system at δ 6.83 (1H, dd, *J* = 15.6, 6.4 Hz, H-1) and 6.09 (1H, d, *J* = 11.5 Hz, H-2). According to the above spectral data, compound **1** was characterized as salacinin C or 21α-hydroxyfriedel-1-ene-3-one (Fig. 1). This identity was verified by its published values (Yu *et al*. 2014).

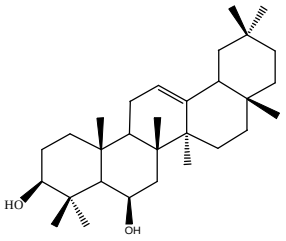
The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **2** exposed a mixture of two pentacyclic triterpenoids α-amyrin (**2a**) and *β*-amyrin (**2b**) with the ratio of 2:1 based on the ^{1}H NMR peaks. The spectrum exhibited two triplets at δ 5.28 and 5.31, suggesting the presence of olefinic proton, H-12 in α-amyrin (**2a**) and *β*-amyrin (**2b**), respectively.



Salacinin C (**1**)



α-Amyrin (**2a**), when X = -H, Y= -CH_3
β-Amyrin (**2b**), when X = -CH_3; Y = -H



Daturadiol (**3**)

Fig. 1. Compounds isolated from *T. bilabiatum.*

The ^{1}H NMR spectrum of compound **2a** showed eight methyl singlets at δ 0.80 (3H, s, H_3-24), 0.82 (3H, s, H_3-23), 0.89 (3H, s, H_3-29), 0.93 (3H, s, H_3-30), 0.95 (3H, s, H_3-25), 0.97 (3H, s, H_3-28), 1.01 (3H, s, H_3-26) and 1.16 (3H, s, H_3-27). On the other hand, the methyl signals attributable to compound **2b** were observed at 0.80 (3H, s, H_3-24), 0.82 (3H, s, H_3-23), 0.91 (6H, s, H_3-29/H_3-30), 0.95 (3H, s, H_3-25), 0.97 (3H, s, H_3-28), 1.01 (3H, s, H_3-26) and 1.27 (3H, s, H_3-27). The ^{1}H NMR spectrum of compound **2** exhibited an oxymethine proton at δ 3.23 (1H, dd, *J* = 10.2, 4.8 Hz) that could be assigned to H-3 of both the isomeric pentacyclic triterpenoids. The above data facilitated the identification of compound **2** as a mixture of α-amyrin and *β*-amyrin which was further established by assessment of their published values (Virgilio *et al.* 2015; Rahman *et al*. 2018)

The ^{1}H NMR spectrum of compound **3** (Fig. 1) exhibited eight methyl protons in the range between δ 0.96-1.32. The spectrum displayed a double doublet at δ 3.19 (1H, *J* = 9.6, 6.0 Hz), characteristic of the carbinolic proton at C-3 of the pentacyclic triterpenoid nucleus. The spectrum also revealed a broad signal at δ 4.58 with integration corresponding to the existence of a second hydroxyl at C-6. The spectrum displayed a one proton broad singlet at δ 5.42 which is distinctive of the olefinic proton at C-12. The above data allowed identification of compound **3** as daturadiol or 3*β*,6*β*-dihydroxy-olean-12-ene (Fig. 1). The identity was confirmed by comparing with its reported values (Araujo and Helena 2005).

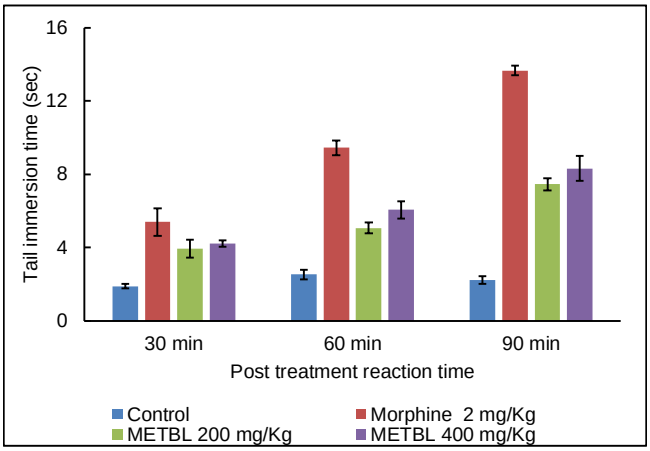


Fig. 2A. Effect of METBL on heat-induced pain response in mice.

The research on medicinal plants working as pain relieving agents is one of the productive ways to find out new drug molecules (Shakkeel *et al.* 2015). Therefore, the methanol extract of *T. bilabiatum* leaf was subjected to evaluation of analgesic potential by tail immersion assay and formalin-induced writhing test in mice model. Mouse tail immersion method is a thermal test employed for evaluating the analgesic properties of a compound. The examination is based on the observation that narcotic drugs, alpha-adrenergic compounds can induce the delayed onset of heat sensitivity upon mouse tail exposure to heat. Elongation of latency period specifies the extent of analgesia of a test compound (Shakkeel *et al.* 2015). As shown in Fig. 2A and 2B, the METBL augmented the reaction time of thermal nociception in a concentration-dependent manner. All these elongations in the reaction time were estimated by comparing with the equivalent control

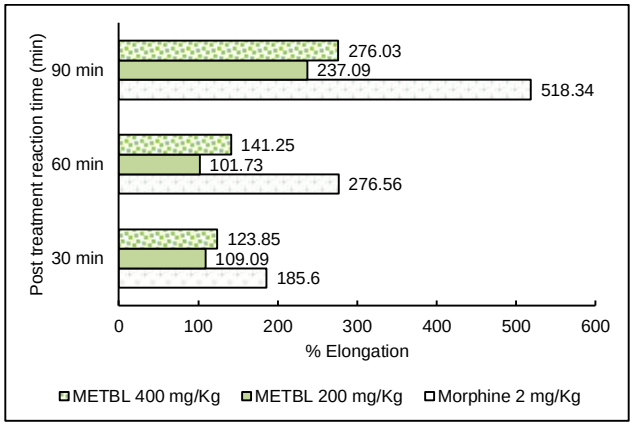


Fig. 2B. %Elongation of latency time by METBL.

groups. The maximum effect (276.03% elongation of reaction time) was observed at 90 min after treating the mice with 400 mg/kg of plant extract which was comparable to the standard morphine (518.34%). The present results (Fig. 2A and 2B) indicate that the analgesic action of METBL might be because of the occurrence of narcotic type chemicals in *T. bilabiatum* extract (Sofidiya *et al.* 2014).

The antinociceptive effect of the plant extract was further clarified by the formalin test, which is an advantageous model for screening the peripheral analgesic activity of test compounds (Kumar and Jain 2014). As shown in Fig. 3, METBL decreased the number of formalin-provoked writhing response in mice. The uppermost inhibition (69.39%) was detected by METBL (400 mg/kg), that was similar with aspirin (75.51%). The present findings clearly indicated that the test sample possessed both central (elongation of thermal reaction time) and peripheral (inhibition of writhing response) analgesic action in mice model. From these observations it may be concluded that *T. bilabiatum* could be considered as an auspicious source of analgesic molecules.

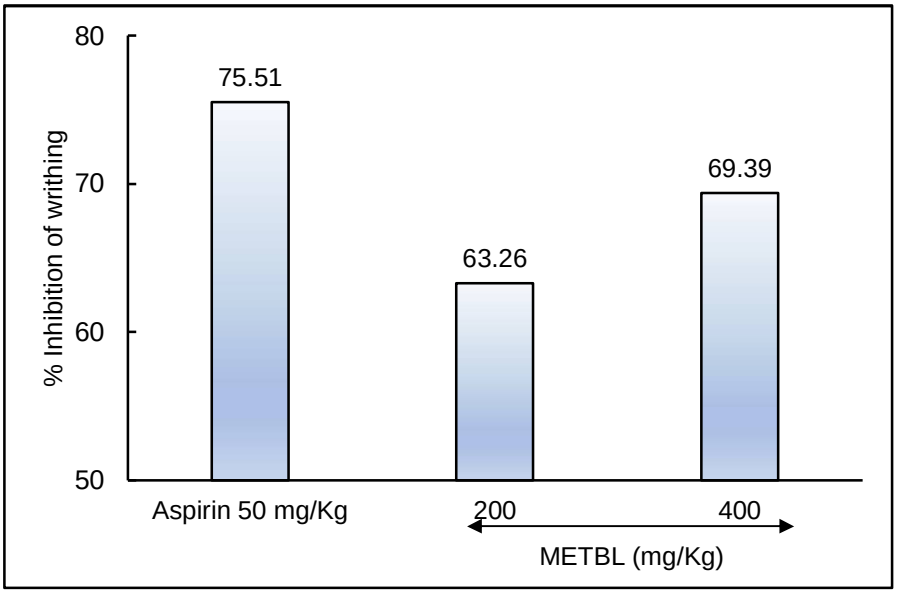


Fig. 3. Effect of METBL and aspirin on the licking induced by a formalin injection in mice.

The antidiarrheal efficacy of *T. bilabiatum* extract was determined by castor oil-induced diarrheal method in mice. Ricinoleic acid is liberated from castor oil after oral administration by intestinal lipase. Consequently, it induces inflammation via elevated biosynthesis of prostaglandins, which in turn induces gastrointestinal motility in mice through hypersecretory response (Zewdie *et al.* 2020). Results in this experiment confirmed that *T. bilabiatum* leaf extract exhibited a dose-dependent antidiarrheal effect by decreasing the number of diarrheal feces in experimental mice. The average number and % inhibition of diarrheal feces have been shown in Fig. 4A and 4B, respectively. The average number of diarrheal feces for control and standard group are 7.33 ± 0.57 and 2.33 ± 0.57, respectively. For 200-and 400- mg/kg of METBL, the average number of diarrheal feces were 5.33 ± 1 and 4.33 ± 0.57, respectively. Highest 40.93% inhibition was exhibited by METBL (400 mg/kg), whereas loperamide (10 mg/kg, p.o.) demonstrated 68.18% drop in diarrheal feces. The antidiarrheal effect could be mediated by the bioactive phytochemicals present in *T. bilabiatum* (Birru *et al*. 2016).

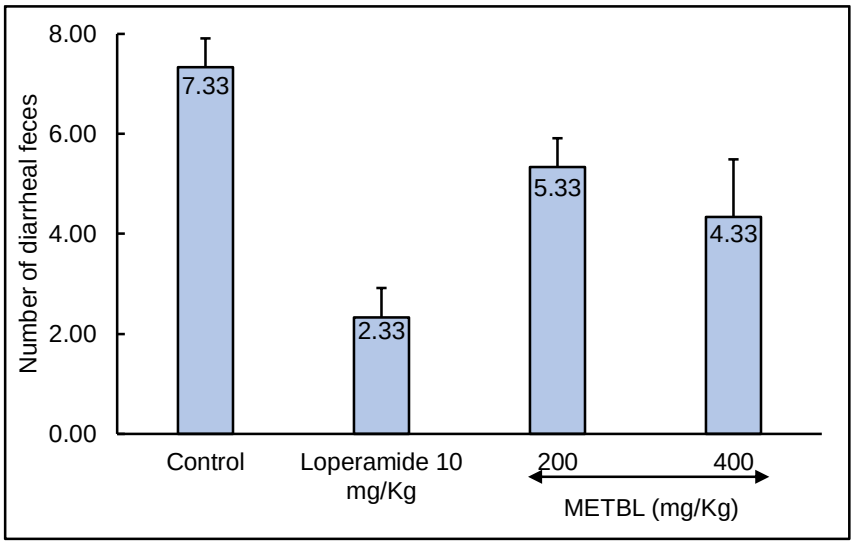


Fig. 4A. Effect of METBL and loperamide on the average number of castor oil-induced diarrheal feces in mice.

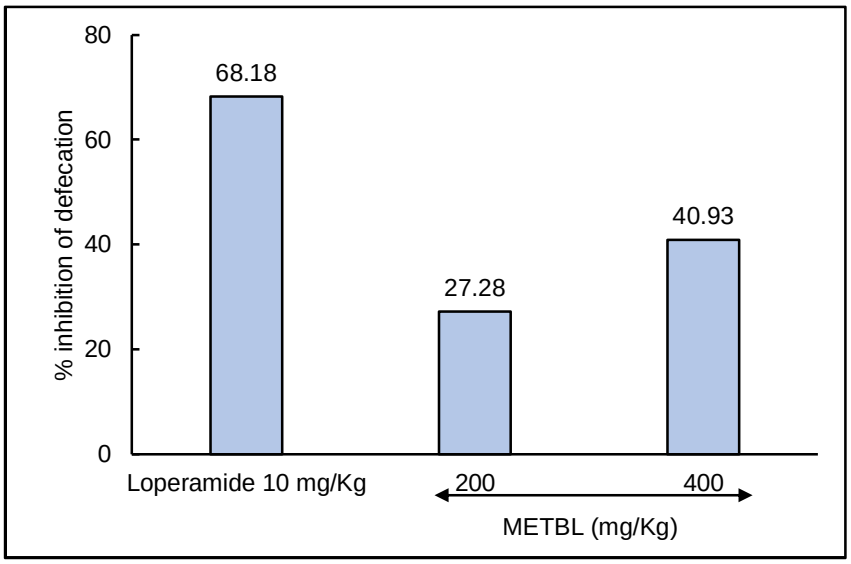


Fig. 4B. Effect of METBL and loperamide in castor oil-induced diarrhea in mice.

During hypoglycemic test, the blood glucose level was remarkably increased as compared to control after 30 min of glucose loading in mice. Both the doses of methanol extract of *T. bilabiatum* and standard glibenclamide significantly reduced the blood glucose level at all-time points in the experiment compared to the negative control (Fig. 5). The hypoglycemic potential of METBL might be due to the augmented secretion of insulin from beta cells of pancreas or by increasing the glucose uptake by liver and muscle tissues (Belayneh and Birru 2018). The

phytochemical study revealed that the crude extract of *T. bilabiatum* contains terpene type compounds which are reported to have several antidiabetic mechanisms such as inhibition of glucose metabolizing enzyme, prevention of development of insulin resistance etc. (Nazaruk and Borzym-Kluczyk 2015). Therefore, the blood glucose lowering activity of the METBL could be attributed due to the presence of triterpenoids.

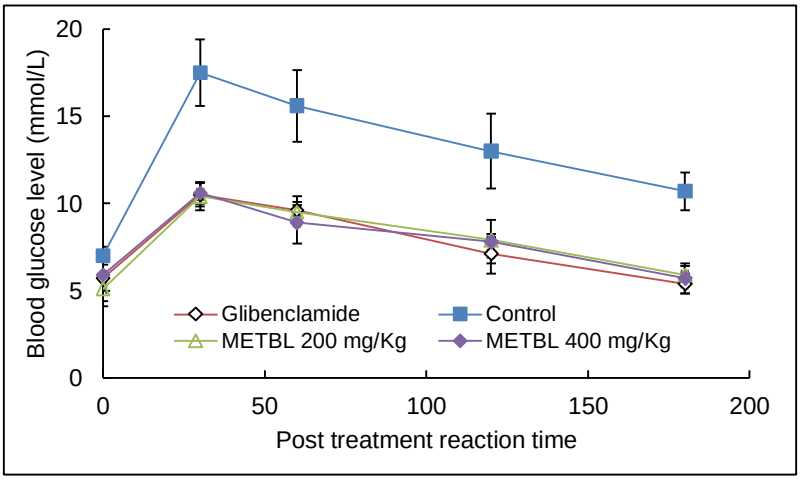


Fig. 5. Effect of METBL and glibenclamide on the blood glucose level in mice.

Phytochemical investigation of *n*-hexane fraction of *T. bilabiatum* leaf extract produced a total of four compounds which were characterized as salacinin C, daturadiol and a mixture of α-amyrin, *β*-amyrin by spectroscopic analysis. The current biological investigation suggests that *T. bilabiatum* leaf extract exhibit significant analgesic, anti-diarrheal and hypoglycemic activities in mice. *T. bilabiatum* extract revealed significant ($p < 0.05$) and dose-dependent analgesic and antidiarrheal action in mice model. Similarly, a remarkable blood glucose lowering effect was found after oral administration of 400 mg/kg bw *T. bilabiatum* extract. Thus, the present *in vivo* study unveiled the effectiveness of *T. bilabiatum* as an optimistic source of functional molecules. Nonetheless, further studies are imperative to determine the probable molecular mechanisms of these action.

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