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INVESTIGATION OF BIOLOGICAL ACTIVITIES OF *XEROMPHIS ULIGINOSA* (RETZ.) ROOT EXTRACTS IN SWISS-ALBINO MICE MODEL, AN EXTINCTIVE MEDICINAL PLANT OF BANGLADESH

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Abstract

Xeromphis. uliginosa (Retz.) is an extinctive Bangladeshi medicinal plant that is locally used for the treatments of pain, diabetes, diarrhea, depressant, and other diseases. The present study was conducted to evaluate the peripheral analgesic activity (PAA), central analgesic activity (CAA), central nervous system antidepressant activity (CNS-AD), antidiarrheal activity (ADA), and hypoglycaemic activity (HGA) of methanolic root extract of *X. uliginosa* (MREXU) in a mice model.

The acetic acid-induced writhing inhibition and tail flick method were applied to determine the PAA and CAA of MREXU. The CNS-AD was measured using the phenobarbitone sodium-mediated sleeping method whereas, the castor oil-induced antidiarrheal method was used to determine the ADA of the crude extracts. To determine the HGA of MREXU crude extract, the tail tipping technique was conducted in a mice model.

The MREXU displayed potential PAA and CAA in mice models. The MREXU 200 and 400 mg/kg significantly inhibit the number of writings along with diclofenac sodium. On the other hand, MREXU both doses significantly inhibit thermal stimulus after 60 and 90 minutes respectively. In the CNS-AD study, crude extract of 200 and 400 mg/kg significantly increase the onset of sleep by decreasing the duration of sleep. Similarly, the dose of 200 mg/kg significantly reduced diarrheal faeces for the whole 4 hours of experiments. The heartiest outcome of MREXU was displayed in the HGA assay. Both doses of MREXU significantly reduced the blood sugar level for the entire 3 hours of the experiments.

In this study, it is revealed that the root of MREXU has extremely significant blood sugar-reducing activity, potential CNS-AD and mild to moderate nociceptive activity in the mice model.

Keywords: *Xeromphis uliginosa*, methanolic extract, analgesic, antidepressant, antidiarrheal, hypoglycaemic, mice model.

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1. Introduction

Awareness and knowledge in the field of public health are rising every day. So, the demand for traditional medicines is increasing for their safety and availability [1]. The use of ethnomedicine was begun the very early stage of earth discovery and it's still the first preference in the community [2]. However, the revolution and advancement of industrialization and prosperity of organic chemistry took a rapid step of using synthetic products in pharmacological aspects, application of natural drugs remains the primary choice for their limited adverse effects and cost-effectiveness [3]. World Health Organization (WHO) noticed that traditional drugs including, all trees, herbs, shrubs, grasses, and ferns are accomplishing more than 80 % of drug sources [4]. More recently, the field of natural drugs uplifted all over the world along with the Eastern and South Asian countries such as Bangladesh, China, and India. More recently, Latin Americans are investing in various research projects for the standardization and management of phytochemical products for the management of human diseases [3].

Ethnically, Bangladesh bearing a considerable amount of medicinal plants. These are organized and documented in The National Herbarium Database (NHD) according to their uses compared to modern medicines [5]. Therefore, a proper and scientific evaluation and documentation are required to examine the roles of unknown medicinal plants in various human diseases [6, 7]. Importantly, potential evaluation, investigation, and comparative advantages and disadvantages studies are required to enhance the application of natural drugs locally and globally [8].

The medicinal plant exerts its pharmacological activities in human diseases by their bioactive compound's containment [9]. The ample research evidence revealed that plant extracts are being anciently and regularly used to serve as peripheral analgesic activity (PAA), central analgesic activity (CAA), central nervous system antidepressant activity (CNS-AD), antidiarrheal activity (ADA), hypoglycaemic activity (HGA), anti-obesity, and anti-helminthic agents [9–13].

Xeromphis. uliginosa (Retz.) is an extinctive and underused Bangladeshi medicinal plant from the Rubiaceae family. It is a small to medium-sized tree, with an approximate height reached to 6 meters, having small thick branches, reddish-brown bark, and scaly in appearance. It has once a year flowering and fruits are ~6 cm in length [14]. The plant is widely distributed in the dried forest of south Asian countries such as Bangladesh, India, Pakistan, Sri Lanka, Thailand, and Nepal. The *X. uliginosa* is ethnically used for the treatment of various disorders including, diarrhea, cholera, pimples, diuretics, and as tonic [15]. The unripe fruits are well-established treatment options for cholera and diarrhea where fruit powder is used for intestinal worm treatment. Moreover, the fruit juice of *X. uliginosa* is locally used as a hair remedy, boiled fruits are used in migraine pain therapy and a mixture of *X. uliginosa* and *Bacopa monneiri* are traditionally used in treating cough [14–17].

It is reported that *X. uligiona* contains a variety of phytochemicals such as alkaloids, acetone, phenol, glycosides, saponins, tannins, and mannitol [14]. The GC-MS analysis of fruits identified about 10 chemical compounds from fatty acids and plasticizer family groups [18]. Besides, the study confirmed that *X. uliginosa* is a good source of antioxidants and may possess anticancer activity. Considering all these and the previous demonstration we therefore undertaken to investigate the PAA, CAA, CNS-AD, ADA, and HGA of methanolic root extract of *X. uliginosa* (MREXU) in mice model.

2. Materials and methods

2. 1. Collection and preparation of crude extract

The root of *X. uliginosa* was collected from Tangail, Bangladesh in the middle of August 2018. The specimen voucher is preserved in the NHD, Mirpur, Dhaka. After collection, the roots were washed thoroughly with clean water. The roasted part was then sun-dried for few days and followed by oven-dried for 48 hours at 40 °C to accelerate the crushing process. About 550 g of root powder was socked in 2.5 litters of methanol and an occasional stirring was performed for 2 weeks at room temperature to evaporate the mixture uniformly. Then, the remaining solution was filtered with a cotton plug followed by a Whatman filter paper. The final solution was concentrated with a manner of a rotary evaporator and applying pressure at controlled temperature (40–45 °C).

Then, the viscous and gummy methanolic crude extract was then aliquoted and considered for further biological screening.

2. 2. Experimental animals and ethical declarations

For the animal experiment, 4–5 weeks aged young Swiss-albino mice (22–25 g) from both sexes were previously ordered into the Animal resource branch (ARB) of the International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr, B). After collecting mice from icddr,B, they were kept in an appropriate polypropylene cage under controlled standard laboratory environments. The mice were given the icddr,B provided rodent food and water with a 12 hours dark and light cycle. Prior to the experiments, the mice were regularly observed and maintained for 2 weeks to adapt to the laboratory environments. The experiments were performed in accordance with the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences Ethical Practice on Animals (1995). Moreover, the Ethical Board of Pharmacy, State University of Bangladesh (SUB), Dhaka, Bangladesh was approved the experimental protocols (FHU/SUB2018-PG1407013010) to conduct our study.

2. 3. Drugs and chemicals

Diclofenac sodium, morphine sulfate, and loperamide hydrochloride were obtained from Square Pharmaceuticals Ltd, Bangladesh. Caffeine and glibenclamide were brought from Beximco Pharmaceuticals Ltd and Opsonin Pharma Ltd, Bangladesh. The remaining drugs, solvents, and chemicals were purchased from Sigma-Aldrich, Germany.

2. 4. Analgesic assay

The acetic acid-mediated writhing test was executed to determine the PAA of MREXU [19]. In this method, pain is induced by intraperitoneal (i.p) administration of acetic acid to the mice. As a result, abdominal pain is incited which can be characterized by counting abdominal constrictions or number writhes. A total of 24 experimental mice were selected for this study which was further sub-divided into four groups (six mice per group) consisting of negative control (NC), standard or positive control (PC), and two experimental groups of MREXU-I and MREXU-II. The mice were fasted for 12 hours and then various treatments were applied to the different groups. 1 % tween-80 in saline (dose of 10 ml/kg) and diclofenac sodium (dose of 50 mg/kg) was administered in NC and PC groups respectively. Whereas, MREXU-I (dose of 200 mg/kg) and MREXU-II (dose of 400 mg/kg) were orally treated with crude extracts. After application of treatments, 1.0 % glacial acetic acid (dose of 10 ml/kg) was given to all mice after 40 minutes to induce pain and followed by 10 minutes of rest. The number of writhing exerted by each mice were counted randomly for the next ten minutes, and acetic acid-mediated pain reduction percent (%) was calculated by using the following equation:

$$\text{Inhibition}(\%) = \frac{(\text{mean writhing of NC} - \text{mean writhing of test sample})}{\text{mean writhing of NC}} \times 100.$$

The tail immersion method was performed to evaluate the CAA of MREXU [20]. In this method, the mice tail end (1 to 2 cm) is directly dipped into the constant radiant heat source (55±1 °C). The time is recorded for each mice to flick its tail from the heating source using a stopwatch which is referred to as pain reaction time (PRT) or latency period. To prevent heat-mediated tail damage, a cut-off period of 15 seconds was maintained. Similar to the PAA, the NC group received 1 % tween-80 in saline, PC received morphine sulfate (dose of 2 mg/kg), and the remaining two groups received MREXU. The PRC was recorded and determined by using the following equation:

$$\text{Elongation}(\%) = \frac{(\text{mean latency of test sample} - \text{mean latency of NC})}{\text{mean latency of NC}} \times 100.$$

2. 5. Phenobarbitone sodium-mediated sleeping assay

Phenobarbitone sodium-mediated sleeping test was conducted to assess the CNS-AD of MREXU in mice model [21]. Two experimental groups MREXU-I, MREXU-II, and NC were received different doses of respective treatments as mentioned earlier whereas the PC group intraperitoneally received caffeine (dose of 20 mg/kg). After a resting period of thirty minutes, all mice were intraperitoneally administered phenobarbitone sodium (dose of 50 mg/kg) to induce a forceful sleep. Afterward, the mice were carefully observed to record the onset of sleep and total sleeping time. The onset of sleep was calculated by counting the time between administration of phenobarbitone sodium to losing the righting reflex of mice, whereas total sleeping time was calculated by counting the time between losing to regaining of mice righting reflex.

2. 6. Castor oil-induced diarrheal assay

The method established by Shoba and colleagues and its partial modification by Sisay et al. protocols were followed to conduct the castor oil-induced ADA of MREXU crude drug in mice [22, 23]. Likewise, the previse experiments, the four groups of fasted mice were used where NC, MREXU-I, and MREXU-II groups received previously mentioned doses regiments. Alternatively, the PC group was orally administered standard antidiarrheal drugs loperamide hydrochloride (dose of 5 mg/kg). All mice were rested for an hour after receiving respective treatments, forceful diarrhea was induced by administering 1.0 ml of castor oil. The dry blotting paper was previously placed in new antidiarrheal cages and the floor of the lining was replaced every hour of the experiment. The dropping of both dry and wet diarrheic feces was recorded every 60 minutes of 4 hours. Furthermore, each group of data was compared with the NC group to determine the effectiveness of the treatments. The level of diarrheal reduction (%) was calculated by using the following formula:

$$\text{Diarrheal reduction}(\%) = \frac{(\text{mean of NC} - \text{mean of of test sample})}{\text{mean of NC}} \times 100.$$

2. 7. Tail tipping hypoglycaemic assay

The tail tipping technique developed by Durschlag and colleagues was used to evaluate the HGA of the test samples in Swiss-albino mice [24]. In this method, blood was taken from the tip of the tail, and glucose level was measured using a glucometer. Following the previous study, NC, PC groups orally administered 1 % tween-80 in saline and glibenclamide (dose of 5 mg/kg) whereas, the remaining two experimental groups received crude extract 200 mg/kg and 400 mg/kg respectively. After an hour, a dose of 2 g/kg of 10 % glucose solution was orally given to all mice to accelerate their glucose level. Then blood samples were withdrawn and the glucose levels were measured at the 1st, 2nd, and 3rd hour after glucose solution administration.

2. 8. Statistical analysis

The statistical level of significance was measured using Student's t-test. The values are expressed as mean of ± standard error of the median (S.E.M.) of six mice (n=6). The results were considered as statistically significant when P<0.05 compared to the NC group.

3. Results

3. 1. Peripheral analgesic study

The effect of MREXU as PAA and CAA are presented in **Tables 1, 2**. In acetic acid medicated PAA study, MREXU-I and MREXU-II displayed significant (P<0.05) inhibition of writhing numbers in a dose-dependent manner. The MREXU-I (200 mg/kg) and MREXU-II (400 mg/kg) shown 60.98 % and 63.41 % of writhing inhibition compared to the NC group. Along with, the PC group consisting of standard analgesic diclofenac sodium also significantly (P<0.05) reduced the number of writhing by 56.10 %. The PAA of MREXU was highly comparable with the PC group response (**Table 1**).

Table 1

Acetic acid-mediated PAA assay

Groups	Dose	Writhing number	Percent of writhing inhibition
NC	1 % tween 80 in saline	10.25±1.31	–
PC	50 mg/kg	4.50±0.29*	56.10
MREXU-I	200 mg/kg	4.00±0.41*	60.98
MREXU-II	400 mg/kg	3.75±0.48*	63.41

*Note: values are means of ±S.E.M. of six mice (n=6). *P<0.05 compared with the NC group (Student's t-test).*

3. 2. Central analgesic study

In the tail immersion or hot plate study, the MREXU shown both dose-dependent and independent pain threshold at 30 min, 60 min, and 90 min of the experiments. In the present study, let's found that MREXU shown a delayed onset of action compared to morphine sulphate. Even so, the MREXU duration of action was much higher than the PC group. After 30 minutes of the experiment, no significant pain threshold was displayed by MREXU however PC shown a significant (P<0.01) pain threshold of 82.55 %. The dose independent PRT was shown in 60 minutes and 90 minutes of the study. MREXU-I shown significantly (P<0.05) higher PRT of 114.31 % and 330.35 % whereas MREXU-II also displayed a significant increase in PRT 90.82 % and 326.20 % compared to the NC group. Moreover, the PC group shown significant (P<0.05) PRT 100.33 % and 72.11 % respectively in 60 minutes and 90 minutes respectively (**Table 2**).

Table 2

Tail flick method to assess CAA

Groups	Dose	Latency period			Percent of elongation		
		30 min	60 min	90 min	30 min	60 min	90 min
NC	1 % tween-80 in saline	4.49±0.47	3.02±0.56	1.93±0.06	–	–	–
PC	2 mg/kg	8.19±0.76**	6.06±0.31*	3.32±0.10**	82.55	100.33	72.11
MREXU-I	200 mg/kg	4.09±0.13	6.48±0.28*	8.30±0.87*	−8.86	114.31	330.35
MREXU-II	400 mg/kg	4.55±0.25	5.77±0.10*	8.59±0.53**	1.51	90.82	326.20

*Note: values are means of ±S.E.M. of six mice (n=6). *P<0.05, **P<0.01 compared with the NC group (Student's t-test).*

3. 3. Central nervous system antidepressant study

The phenobarbitone sodium mediated CNS-AD of MREXU is shown in **Table 3**. In our study, both doses of MREXU shown significantly increased latent period sleep and reduced total sleeping time in a dose-dependent manner. MREXU-II significantly (P<0.05) increased the latent sleeping time by 249.23 % while it decreased the total sleeping time by 22.04 %. However, MREXU-I did not show any significant latent sleep and total sleeping activity. Along with, the standard drug caffeine significantly (P<0.01) increased latent sleeping time 389.23 % and decreased total sleeping time 34.42 %.

Table 3

Phenobarbitone sodium-mediated sleeping assay

Groups	Dose	Latent period of sleep (min)	% increase of latent sleep (min)	Total sleeping time (min)	% decrease of total sleeping time (min)
NC	1 % tween-80 in saline	16.25±0.85	–	183.75±3.22	–
PC	20 mg/kg	79.50±4.37**	389.23	120.5±3.12**	34.42
MREXU-I	200 mg/kg	50.50±3.97	210.77	149.50±3.12	16.64
MREXU-II	400 mg/kg	56.75±1.93*	249.23	143.25±5.33**	22.04

*Note: values are means of ± S.E.M. of six mice (n=6). *P<0.05, **P<0.01 compared with the NC group (Student's t-test).*

3. 4. Antidiarrheal study

The castor oil-induced ADA of MREXU is shown in **Table 4**. The MREXU onset of action and duration of action was very comparable with standard drug loperamide hydrochloride. Dose-dependent antidiarrheal responses were shown in the 1st hour, 3rd hour, and 4th hour of the study while a dose-independent response was shown in the 2nd hour of the study. The crude extract dose of 200 mg/kg significantly (P<0.01) reduced diarrheal faces at the 2nd hour although it showed potential ADA in the whole period of the experiment. Apart from that, MREXU-II displayed significant (P<0.05 for 1st hour, P<0.01 for 2nd hour, P<0.05 for 3rd hour, and P<0.05 for 4th hour) diarrheal reduction in each hour of the experiment. Equally, a constant and significant (P<0.05, P<0.01) diarrheal reduction was shown by the PC group for the whole study.

Table 4

Castor oil-induced ADA assay

Groups	Dose	Number of diarrheal faces				% of diarrheal reduction			
		1 hour	2 hour	3 hour	4 hour	1 hour	2 hour	3 hour	4 hour
NC	1 % tween-80 in saline	2.50±0.29	3.00±0.41	1.50±0.29	1.25±0.25	–	–	–	–
PC	5 mg/kg	0.50±0.65**	0.50±0.29**	0.25±0.25*	0.00±0.00*	80.00	83.33	83.33	100.00
MREXU-I	200 mg/kg	1.50±0.65	0.50±0.29**	0.50±0.29	0.25±0.25	40.00	83.33	66.67	80.00
MREXU-II	400 mg/kg	0.75±0.48*	0.75±0.25**	0.25±0.25*	0.00±0.00*	70.00	75.00	83.33	100.00

*Note: values are means of ± S.E.M. of six mice (n=6). *P<0.05, **P<0.01, compared with the NC group (Student's t-test).*

3. 5. Hypoglycaemic study

Table 5 represents the tail-tipping HGA of MREXU in mice. It was found to be a potential blood-glucose-lowering agent for the whole 3 hours of the experiment at doses 200 and 400 mg/kg respectively. After administration of 10 % glucose solution, MREXU-I significantly kept reducing blood glucose level (P<0.001 at 1st hour), (P<0.01 at 2nd hour), and (P<0.01 at 3rd hour) respectively compared to the NC group. Similarly, MREXU-II shown significant antihyperglycaemic activity (P<0.001 for 1 hour), (P<0.01 for 2 hours), and (P<0.01 for 3 hours) gradually compared to the control group. Furthermore, the standard drug glibenclamide was shown significant hypoglycaemic activity at 68.08 % (P<0.001), 60.55 % (P<0.001), and 53.64 % (P<0.001).

Table 5

Tail-tipping method to assess the HGA

Groups	Dose	Blood sugar level (mmol/L)				Glucose reduction (%)		
		0 hour	1 hour	2 hour	3 hour	1 hour	2 hour	3 hour
NC	1 % tween-80 in saline	6.03±0.24	10.65±0.15	7.23±0.25	5.50±0.13	–	–	–
PC	5 mg/kg	5.98±0.19	3.40±0.16***	2.85±0.06***	2.55±0.06***	68.08	60.55	53.64
MREXU-I	200 mg/kg	5.60±0.20	3.65±0.25***	2.75±0.23**	2.63±0.33**	65.73	61.94	52.27
MREXU-II	400 mg/kg	5.89±0.45	3.48±0.47***	3.05±0.36**	2.45±0.23**	67.37	57.79	55.45

*Note: values are means of ± S.E.M. of six mice (n=6). **P<0.01, ***P<0.001 compared with NC group (Student's t-test).*

4. Discussion

Globally, the most effective analgesics are opioids, non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, and antidepressants. They can minimize approximately 50 % of pain and which is on average 30 % of the total patients [16]. Researchers explored that, prolong use of commonly used analgesics has serious side effects such as, long-time use of Paracetamol/ Acetaminophen caused kidney and liver damages, opiates induced addiction and dependency, and NSAIDs caused gastrointestinal disorder [17]. There are numerous studies have been conducted to isolate peripheral or central analgesics from natural sources so far [25, 26]. As a result, many natural drugs are traditionally being using to serve as PAA and CAA agents. The findings of the current study revealed that MREXU consisting both peripheral and central analgesic effects in mice model by reducing the writhing numbers and increasing PRT respectively. The

results were comparable with the standard NSAIDs diclofenac sodium and opioids morphine sulfate therapy respectively.

The previous study shown that *X. uliginosa* contains various saponins compounds [14] which exhibits strong analgesic activity in animal model [27, 28]. Therefore, saponins from MREXU may be exhibited analgesic activity in mice for the present study. Moreover, acetic acid promotes the synthesis of PGE2, PGF2a, and increases the level of eicosanoids into peritoneal fluids [13, 29]. So that, it generates writhing by releasing the pain mediator in mice. The MREXU at both doses may potentially suppress the writhing number to reduce the pain or it may suppress the activity of pain receptors through neutralizing the visceral sensitivity of acetic acid.

Apart from this, the tail-flick test is a widely used model for CAA study. It extent the reaction time in hot plate via acting on spinal cord level [30]. The standard central analgesic opioid, morphine sulfate is commonly used as positive control where it exhibits CAA by activating several opioid receptors (μ, δ, and κ) that acts on the spinal cord supraspinally and peripherally [31]. In our study, MREXU has shown a dose-dependent CAA after 90 minutes. Although, MREXU 200 mg/kg has shown a significant pain threshold at 60 minutes compared to the NC group. A constant elongation of PRT was shown by the standard drug at 30, 60, and 90 minutes data. Our findings revealed that the MREXU may be used as a slow-acting CAA agent, it starts working potentially after 90 minutes of administration.

Usually, the phenobarbitone sodium-induced sleeping method is used to evaluate the CNS-AD activity of drugs and plant extracts [32, 33]. The derivative of barbituric acid (phenobarbitone sodium) is a non-selective CNS depressant that acts on calcium channel regulated GABA-A receptor [34]. This short-acting barbiturate forcefully induced sleep in the animals through activating and allosterically modifying $GABA_A$ receptor's function [34, 35]. In the present study, the MREXU is significantly increased latent period of sleep followed by a reduction of total sleeping time in a dose-dependent manner compared to the control group. The onset of sleep increased to 210.77 % to 249.23 % against doses 200 to 400 mg/kg. Therefore, it is possible to assume that the MREXU might be a potential CNS-AD agent, acting on GABA-A receptor and may boost up the locomotor activity.

Besides, several mechanisms are associated with castor oil-regulated diarrheal effect including, active secretion of adenylate cyclase or cAMP, reduction of fluid absorption by suppressing the Na+/K+-ATPase activity, enhancing the synthesis of prostaglandin, and regulating platelet-activating factors (PAFs) and the level of nitric oxide [36, 37]. The castor oil contains nitric oxide which is a strong signaling molecule, powerful vasodilator, and has various physiological and pathological conditions, especially on diarrhea [39, 40]. Castor oil also contains ricinoleic acid that stimulates the hypersecretory response to induce immediate diarrhea [41]. In our current study, the 100 % diarrheal inhibition was observed after the fourth hour at a dose of 400 mg/kg with respect to its standard drug loperamide. The 83.33 % diarrheal inhibition was also observed by MREXU 400 mg/kg dose after the third hour of the experiment while a moderate activity was shown by 200 mg/kg dose. In second hours of our study, the both dose of MREXU has shown a significant reduction of diarreal faces and diarrheal reduction 83.33 % and 75.00 % respectively. Here, the MREXU may exhibit its ADA activity by following above mentioned pathways.

Importantly, very satisfying HGA by MREXU may be due to the presence of oleanolic acid in the plant [14, 42]. The previous reports have revealed that oleanolic acid has a significant role in reducing blood glucose levels and impacts glycogen synthesis to act as a preventive and therapeutic advantage in diabetes [43, 44]. The oleanolic acid promotes phosphorylation of AKT and AMPK to suppress the genes and proteins expression responsible for gluconeogenesis and glycolysis resulting a reduced hepatic glucose production [44]. A recent study has reported that *X. uliginosa* contains phytol [18]. Phytol or phytanic acid is a natural ligand for retinoid X receptor (RXR). It mimics the effect of conjugated linoleic acids and activates RXR to prevent diabetes [45]. Previously it was reported that phytol and its active metabolites RXR play a significant role in the management of diabetes and inhibiting insulin resistance [46].

In our tail tipping hypoglycaemic study, the extensive significant blood sugar-reducing activity was shown by MREXU. MREXU doses of 200 and 400 mg/kg have shown an extensive

impact on reducing blood glucose levels after the first, second, and third hours of administration. It immediately reduced the glucose levels after first hour to 65.73 % and 67.37 % at doses 200 and 400 mg/kg respectively where the standard of the experiment glibenclamide reduced 68.08 %. A constant reduction of blood glucose level has shown for the whole experimental time and MREXU has also shown similar blood glucose reducing profile with glibenclamide. Therefore, the MREXU may be a potential alternative to any synthetic drugs for the treatment of type-2 diabetes and other metabolic disorders.

5. Conclusions

Our present study revealed that like other medicinal plants, *X. uliginosa* root extracts 200 and 400 mg/kg have convincing bioactivities in the mice model. MREXU noteworthy potentials in blood sugar reducing activity, it has potential CNS-AD and ADA activities in mice model. In addition, it has also shown PAA and CAA. The recent trend of pharmaceutical industries is the isolation of novel bioactive compounds by different methods from plant sources that have traditional uses. Therefore, *X. uliginosa* would be a worthy candidate for future research in isolation of pure bioactive compounds and their implementation in various human diseases especially in the treatment of diabetes.

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Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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