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IN VIVO STUDY OF ANTIDIABETIC POTENTIAL OF YAUNDENTINE HYDROCHLORIDE FROM *RINOREA YAUNDENSIS* ENGL. IN RATS

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ABSTRACT

The aerial parts of *Rinorea yaundensis* Engl. yields yaundentine hydrochloride. It was subjected to *in vivo* study to determine its antidiabetic potential. It significantly reduced the level of blood glucose compared with glibenclamide used as a control. It could also be validated from the significant lowering of serum lipid profile markers and rise in high-density lipoprotein cholesterol (HDL-C), to signify the protective effect. Histopathological evaluation further revealed the restoration of islet cells near to normal after treatment. This study revealed that yaundentine hydrochloride possesses antidiabetic potential which may due to the multitarget mode of action, which enables its utility as a complementary drug for diabetes and associated complications.

Key-words: *Rinorea yaundensis* Engl., monoterpene-indole alkaloid, yaundentine hydrochloride, *in vivo* study, antidiabetic potential

INTRODUCTION

Diabetes mellitus has now become a deadly disease after cancer. It is characterized by elevated glucose levels in blood due to insufficient insulin and insulin resistance, resulting in metabolic abnormalities in lipids, proteins and carbohydrates. It has become a chronic disease along with cancer, cardiovascular and cerebrovascular diseases for human health due to high mobility, prevalence and mortality. To avoid the complications of diabetic mellitus, patients need to regularly take different antidiabetics. However, such medicines have adverse side effects so there is a need to minimize the side effects by searching the alternatives. Naturopathy is the best option due to its lower toxicity compared to synthetic drugs (Chauhan *et al*., 2010).

Natural products play a vital role in drug development. Several new methods for drug development are available such as computer-based molecular modeling, synthetic chemistry and combinatorial chemistry. However, none of these has so far replaced natural products in drug development. In fact, most synthetic drugs are based on the skeleta of natural products (Veeresham, 2012).

There are several plant families which are reported to possess promising therapeutic effects including Violaceae, Rosaceae, Euphorbiaceae, Asteraceae, Lamiaceae, Liliaceae, Araliaceae, Cucurbitaceae and Moraceae. Studies on medicinal plants suggest the benefits of their hypoglycemic effect to control diabetic mellitus either by delaying diabetic complications or by improving metabolic disorder. Naturally occurring secondary metabolites which are usually derived from higher plants, animals and micro-organisms, generally display more efficacy than synthetic drugs. As a result, secondary metabolites including terpenes, steroids, phenols and alkaloids having a significant role in the cure of diseases including diabetes mellitus (Chauhan *et al*., 2010), as the synthetic drugs generally possess undesirable side effects (Rasouli *et al*., 2020). Over decades' alkaloids have been used as folk medicines due to their therapeutic potential. Indole alkaloids in particular have gained widespread interest because of their biological efficacy (Debnath *et al*., 2018).

Rinorea belongs to the family Violaceae which is one of the largest families. A variety of secondary metabolites have so far been reported from this family. Due to therapeutic potential, most of its isolates are used by the local population for the treatment of human ailments (Chandra *et al*., 2015). *Rinorea* is a large genus mostly represented in Cameroon and neighboring African countries. Two isoflavones namely alpinumisoflavone and di-*O*-methylalpinumisoflavone have been reported from the stem bark of *R. welwitschii* (Stewart *et al*., 2000). Essential oil from *R. subintrigrifolia* was reported to comprise seven simple aromatic compounds and caryophyllene (Agnaniet *et al*., 2003). The bark of the root exhibited antiradical / antioxidant activity besides being used for the

treatment of heart diseases, cough, oedema, constipation, stomach / rheumatismal aches and fever. The coruleoellagic acid derivatives namely 3,4′,5,5′-tetramethylcoru-leoellagic acid and 3′,4,4′,5,5′-pentamethylcoruleoellagic acid along with friedelane-type triterpene, have been reported from *Rinorea oblongifolia* (Munvera *et al*., 2020).

One of the species of *Rinorea* is *R. yaundensis* Engl. which is native to Cameroon. The literature survey showed that no pharmacochemical studies have so far been carried out. The ethno-pharmacological and chemotaxonomic importance of this genus prompted us to carry out pharmacochemical studies on *R. yaundensis*. As a result of these studies, we previously reported a new monoterpene-indole alkaloid named as yaundentine together with three other alkaloids, two triterpenes, two steroids, a xanthone and benzoic acid from the aerial parts of this plant (Khatoon *et al*., 2020). Following further studies, we herein report the isolation of yaundentine hydrochloride, reported for the first time as a natural product. *In vivo* study of yaundentine hydrochloride revealed significant antidiabetic potential.

MATERIAL AND METHODS

The chromatographic and spectroscopic protocols, details of plant material, extraction, isolation and characterization of yaundentine hydrochloride were the same as previously reported by Khatoon *et al*. (2020).

Animals

Twenty-four, adult males (age: 8-10 weeks) albino Wistar rats (190 ± 10 g) were taken for this experiment which were kept under required environment. The animals were retained on lab. pellet diet and water *ad libitium*.

Dose Preparation

The sample was dissolved in dimethyl sulfoxide- water (0.5: 95.5) for the study.

Induction of diabetes

This was induced by injecting streptozotocin (STZ) (55 mg/kg) made in 0.1 molar sodium citrate buffer (pH 4.5). A solution of 5 % glucose was given to animals for a period of twelve hours to protect from hypoglycaemia. On day 4 after the administration of STZ, level of glucose in blood was estimated by a glucometer (UCheck blood glucose test meter). Those rats with glucose level above 250 mg/dL in blood were considered as diabetic-rats (Gjadosik *et al*., 1999).

Experimental Design

Altogether 24 rats (6 normal and 18 diabetes -induced) were distributed in four groups each having six animals:

Gp-1 Normal control rats (NC): Given vehicle only (normal saline; 2 mL/kg B.wt.)
Gp-2 Diabetes-induced rats: Received sample to be tested (5 mg/kg B.wt.)
Gp-3 Diabetic rats: Given sample to be tested (10 mg/kg B.wt.)
Gp-4 Positive control: Received glibenclamide (standard drug) (5 mg/kg B.wt.)

On day 4 after the induction of diabetes, only the diabetic rats (Gp-2 and 3) were treated orally for a period of 14 days. The body weight of the animals was taken initially and at the end of the experiment glucose level in blood was determined on 0, 3, 7 and 14^{th} day. The blood was taken from heart on 14^{th} day and the serum was separated from clotted blood at 4 °C by centrifuging. After giving mild anesthesia to the rats, the liver and pancreas were taken out, washed with chilled-saline and maintained at -20°C for detailed study (Nayak and Paltabiraman, 1981).

Estimation of Serum lipid profile

Serum lipid profile markers like total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), HDL-C, Very-low-density lipoprotein-cholesterol (VLDL-C), non-HDL-C and triglycerides (TG) were estimated spectroscopically with the aid of available kits in the market.

Histopathological Evaluation

The paraffin section of the liver and pancreas were stained after cleaning the paraffin using hematoxylin-eosin. The histopathological assessment was done under the optical microscope by a pathologist who was unaware with rats grouping. The histopathological appreciation was determined by assessing changes in the islets of Langerhans, presence of inflammatory infiltrate, fibrosis and vacuolization (Ferreira *et al*., 2010).

Ethical aspects

The Ethical Committee, Dow University of Health Sciences, approved the study. All the ethical aspects were taken seriously for the care of rats throughout the experiments.

Statistical analysis

This was executed using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., USA). All the results are expressed as mean ± SEM (n = 6) and statistical analysis was done by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The values of $p < 0.05$ were taken as statistically important.

RESULTS

The level of glucose in blood was determined at 0, 3rd, 7th and 14th day of treatment in normal as well as experimental groups. STZ administration displayed a substantial promotion ($p < 0.05$) of glucose level when matched with the normal control group. After 14 days of treatment with yaundentine hydrochloride, a considerable decrease of glucose level in blood from 439 mg/dL to 205 mg/dL at a dose of 5 mg/kg and 372 mg/dL to 129 mg/dL at a dose of 10 mg/kg, respectively. The results signified the hypoglycemic effect of the isolated compound in experimental diabetes rats (Table 1).

Table 1. Effect of Yaundentine hydrochloride and Glibenclamide on Blood Glucose Level in STZ-induced Diabetic Rats.

	Dose	**Blood Glucose level (mg/dL)**			
Treatment	**(mg/kg)**	**Day 0**	**Day 3**	**Day 7**	**Day 14**
Control	-	85.83 ± 1.7	69.66 ± 0.88	84 ± 1.9	72.66 ± 2.3
Yaundentine hydrochloride	5 mg/kg	439.66 ± 2.8	333.16 ± 4.7*	256.3 ± 3.9*	205.5 ± 2.4*
Yaundentine hydrochloride	10 mg/kg	372.33 ± 4.4	322.66 ± 3.7*	245.16 ± 3.5*	129.16 ± 2.3*
Glibenclamide	5 mg/kg	344.83 ± 2.8	281.16 ± 2.0*	176.1 ± 2.5*	88.83 ± 0.4*

*Values are expressed as mean ± S.E.M (n = 6).

The STZ-induced diabetic rats were taken in the study to determine the anti-diabetic potential of tested compound. Streptozotocin was selected for the study because it causes hpoglycemia, hypoinsulinemia and hyperlipidemia conditions by making the pancreas swell and causes the degeneration of islets of Langerhans (Akbarzadeh *et al*., 2007). The STZ brings about the diabetic condition by the generation of free radicals and nitric oxide. These free radicals weaken the mitochondrial function of beta cells (Rakieten *et al*., 1963). The oral administration of compound to be tested dropped the enhanced glucose level of blood in STZ-induced diabetic rats. The possible mechanism might be due to the stimulation of insulin secretion from the remaining beta cells and promotion of glucose uptake metabolism in adipose tissues (Ali *et al*., 1993 and Gray *et al*., 2000).

The antidiabetic effect by yaundentine hydrochloride in serum was determined by assessing Lipid profile. The diabetic rats exhibited a substantial escalation in the TC, TG, LDL-C level, VLDL and Non-HDL as compared to the normal. The sample administration at a dose of 10 mg/kg, B.wt, brought back the level of lipid serum near to normal value which indicated the hypoglycemic effect of the sample (Table 2).

Table 2. Effect of Yaundentine hydrochloride and Glibenclamide on Serum Lipid Profile in STZ-induced Diabetic Rats.

Treatment	**TG**	**(TC)**	**HDL**	**LDL**	**VLDL**	**Non HDL**
Control	131± 2.2	48 ± 4.6	20 ± 1.4	5 ± 0.6	26.2 ± 0.14	28 ± 4.6
Yaundentine hydrochloride 5mg/kg	123 ± 2.0*	72 ± 2.2*	25 ± 0.6*	7 ± 0.6*	44.6 ± 0.14*	47 ± 1.4*
Yaundentine hydrochloride 10mg/kg	52 ± 0.89*	72 ± 1.7*	20 ± 1.4	7 ± 0.0*	10.4 ± 0.14*	52 ± 1.4*
Glibenclamide 5 mg/kg	89 ± 0.89*	70 ± 8.9*	19 ± 0.0	6 ± 0.89	27.3 ± 0.89*	29 ± 0.0

Values are expressed as mean ± S.E.M (n=6)

The secondary complication associated with diabetes is dyslipidemia (Zhang *et al*., 2008; Mooradian, 2009). In diabetes, usually the levels of TC, TG, LDL-C increase and the level of HDL-C decreases significantly (Arvind *et al*., 2002). The reason for abnormal concentration of serum lipids might be due to the disturbance in lipase hormone (Schofield *et al*., 2016). The administration of sample significantly showed the drop in the serum lipid profile markers and increase in HDL-C, also demonstrating the defensive effect of the isolated compound in diabetic-related issues.

The liver has various functions which includes detoxification, production of bile as well a large role in metabolism. The liver is an important organ and its main function is to maintain blood glucose through glycogenesis and glycogenolysis. While pancreas works as an endocrine gland which produces important hormones including insulin and glucagon (Brzóska *et al*., 2003; Ruiz-Muñoz *et al*., 1997). Histopathological evaluation of the liver and pancreas of STZ induced diabetic rats further revealed that the lesser number of islet cells were repaired near to normal after the treatment with sample.

The results of the histological evaluation showed that the control group represented the normal morphology and texture of the liver tissue in the H & E staining while in the STZ group when compared to control animals, inflammatory infiltrations were observed due to degeneration and more cytoplasmic vacuoles were present in the hepatocytes (Fig. 1: non-stained spaces, indicated by arrows).

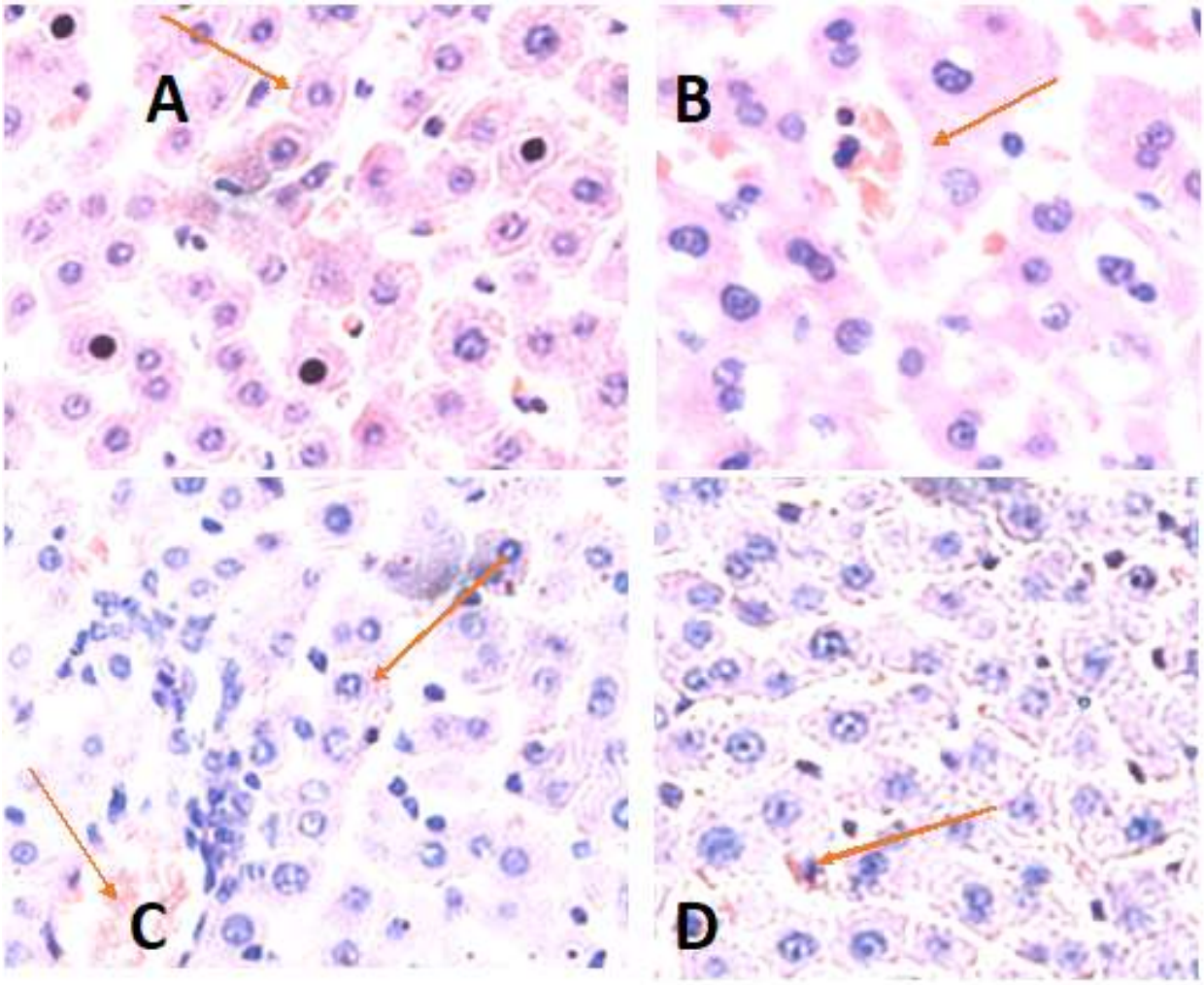

Fig. 1. Hematoxylin and eosin (H&E) staining of liver tissue. (A) Control group; normal histological appearance of liver in control rats. (B) STZ group; degeneration of hepatocytes in diabetic rats. (C) and (D) STZ yaundentine chloride at a dose of 5 and 10 mg/kg, b.wt; mild inflammatoryaggregation.

In pancreatic sections of diabetic rats, the islets were reduced in number with increased vacuolation as compared to the control. The group administered with sample at a dose of 5 mg/kg, B.wt, showed improvement in the number of islets together with vacuolation. The sample at a dose of 10 mg/g, B.wt, depicted same positive

changes of improvement comprising of an rise in the islet cell number with a reduction in the vacuolation (Fig. 2). Prior to these experiments, the cytotoxicity of yaundentine hydrochloride as well as crude extract against the rats was determined which showed very low LD_{50} values.

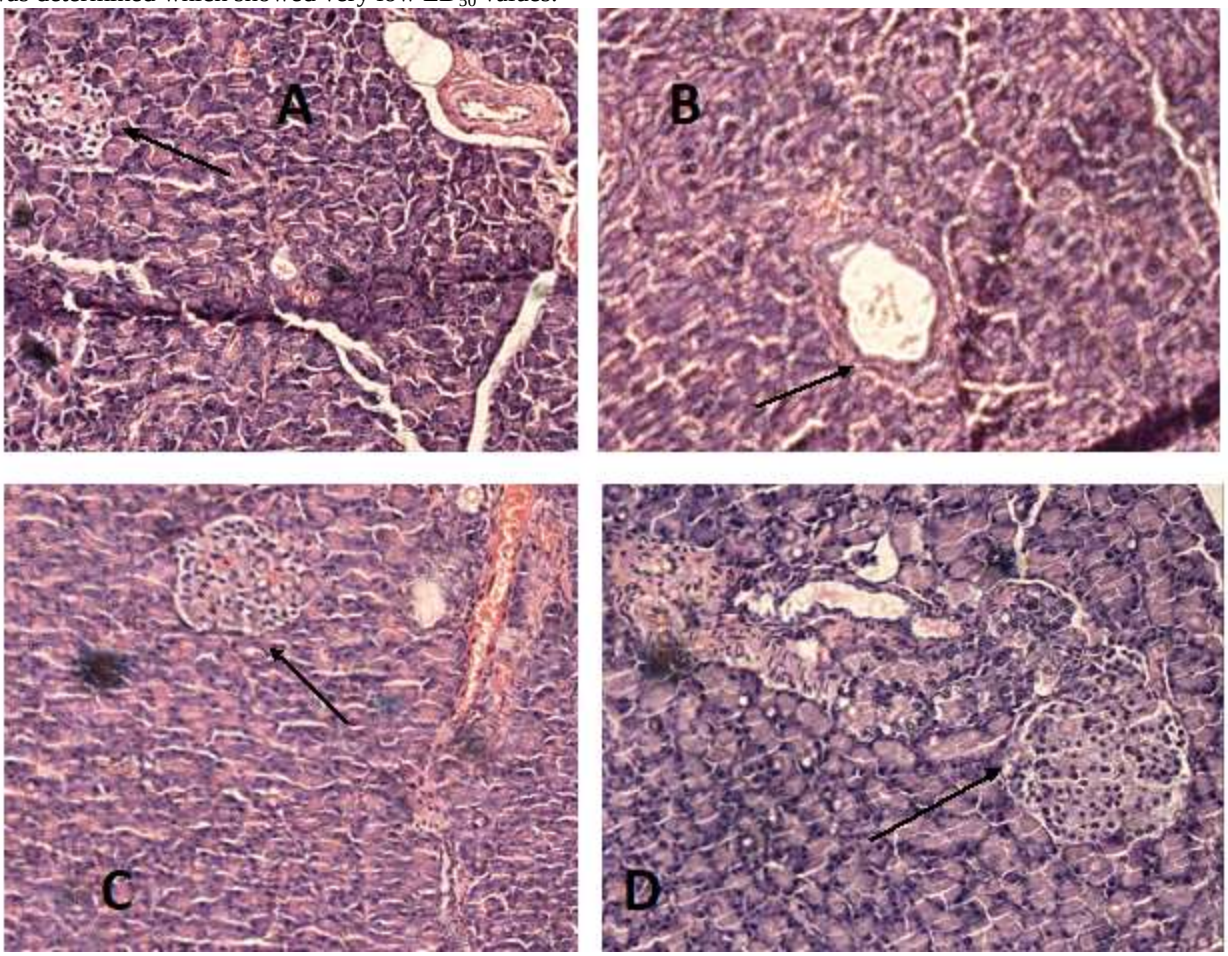

Fig. 2. H & E staining of pancreas (A) Normal Control: section shows normal sized islets of Langerhans indicating with black head arrow and normal blood vessels width and shape showed with white head arrow (B) STZ induced diabetic: H&E stained section shows decrease in size and number of pancreatic islets normal surrounding acini and bulggy blood vessels. (C) STZ+ yaundentine chloride (5 mg/kg b.wt) treated: H&E stained section shows islets with moderate increase innumber and normal acini. (D) STZ +compound 2 (10 mg/kg, b.wt): showed increase number of islets in normal acini.

DISCUSSION

Diabetes is a universal disease being a source of serious health concerns. The present antidiabetic drugs have adverse side effects and also suffer from high degree of tolerance. Therefore, there is extreme need of discovery of new and effective antidiabetic agents. Some kinds of natural products such as indole alkaloids are reported to have specific antidiabetic efficacies (Yuqian *et al*., 2021). Recently, some mono-terpene indole alkaloids have been reported to possess potential antidiabetic activity (Wang *et al*., 2021). This prompted us to determine the possible antidiabetic property of yaundentine hydrochloride, a major constituent in *Rinorea yaundensis*, as indicated in a previous study (Khatoon *et al*., 2020).

CONCLUSION

This study exhibits the evidence of antidiabetic potential of a previously reported natural monoterpene-indole alkaloid hydrochloride namely yaundentine hydrochloride in STZ-diabetic rats. In addition, the treatment of sample also showed an improved lipid profile that reveals its protective role in secondary complications associated with diabetes. Therefore, it can be concluded that yaundentine hydrochloride might be a candidate to be used in adjunct

therapy for the treatment of diabetes. Detailed research is needed to determine the exact mechanism of action through which yaundentine hydrochloride produces its antidiabetic effect. The results signified that the isolated compound has potential antidiabetic activity when compared to the reference drug (glibenclamide).

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