https://doi.org/10.31925/farmacia.2021.4.16 **ORIGINAL ARTICLE**

UPLC/MS BASED PHYTOCHEMICAL SCREENING AND ANTIDIABETIC PROPERTIES OF *PICRORHIZA KURROA* IN MITIGATING GLUCOSE-INDUCED METABOLIC DYSREGULATION AND OXIDATIVE STRESS

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Abstract

Diabetes is a metabolic disorder increasing at an alarming rate in the current era. Medicinal plants and their supplements have shown protective effects against metabolic dysregulation and oxidative damage. The present study is aimed to explore the antidiabetic potential of hydroalcoholic extract of *Picrorhiza kurroa* (HAE-PK). The UPLC/MS analysis revealed 23 most abundant bioactive substances. HAE-PK exhibited significant α-amylase and α-glucosidase inhibitory activities with an IC_{50} value of 124.7 ± 0.59 and 135.03 ± 1.28, respectively, and paralleled with standard acarbose. It also contained a very high amount of phenols and flavonoids and exhibited potent free radical scavenging activity using the DPPH test. Further, HAE-PK reinstated the glucose-induced increase in hepatocytic enzymes, aldehyde dehydrogenase (ALDH) and hexokinase. Further, HAE-PK significantly improved the glucose-induced cytotoxicity in $HepG_2$ cells. Thus, we conclude that HAE-PK can be a considerable prospect in the management of hyperglycaemia, diabetes and related oxidative stress.

Rezumat

Diabetul este o tulburare metabolică ce evoluează alarmant în prezent. Plantele medicinale au arătat un efect protector împotriva dezechilibrelor metabolice și oxidative. Prezentul studiu are ca scop explorarea potențialului antidiabetic al extractului hidroalcoolic de *Picrorhiza kurroa* (HAE-PK). Analiza UPLC/MS a evidențiat prezența a 23 de substanțe bioactive. HAE-PK a prezentat activități semnificative de inhibare a α-amilazei și α-glucozidazei cu o valoare IC_{50} de 124.7 ± 0,59 și respectiv 135,03 ± 1,28, comparativ cu acarboza. De asemenea, extractul conține o cantitate mare de compuși fenolici și flavonoide și prezintă o activitate puternică de neutralizare a radicalilor liberi, evidențiată prin metoda DPPH. HAE-PK a echilibrat creșterea indusă de glucoză asupra enzimelor hepatice, aldehid dehidrogenaza (ALDH) și hexokinaza. HAE-PK a îmbunătățit semnificativ citotoxicitatea indusă de glucoză în celulele $HepG_2$. Astfel, se poate concluziona că HAE-PK prezintă perspective în managementul hiperglicemiei, diabetului și al stresului oxidativ.

Keywords: diabetes, *Picrorhiza kurroa*, UPLC/MS, antioxidant, α-amylase, α-glucosidase

Introduction

Diabetes is a serious disease and have contributed tremendously to the burden of human health and world economies. According to IDB (International Diabetic Federation), 693 million people will be living with diabetes in 2045 [1]. Despite a significant progress made in the treatment of diabetes, the result is still far from expected [2]. Albeit, high prevalence, variable pathogenesis, progressive process, and complications of diabetes warrant urgent need for its effective management.

Over the years, it has been generally noticed that the consumption of local herbs and vegetables improve the human health, in terms of prevention, and/or cure of diseases [3]. The World Health Organisation (WHO) recommended exploring diverse arrays of biologically active compounds to develop newer antidiabetic leads [4]. *Picrorhiza kurroa* Royle ex Benth. (*Scrophulariaceae* family), is a familiar herb in the indigenous system of medicine known for its varied pharmacological actions [5]. Kumar *et al*., 2017 reported that *P. kurroa* displayed β-cell regeneration with enhanced insulin production and antihyperglycaemic effects by increasing the insulin-mediated translocation of GLUT4 from cytosol to plasma membrane, which results in a better glucose uptake by skeletal muscle cells and an improved glycaemic control in diabetes [6].

Virtually, the entire spectrum of metabolic dysfunctions seen in patients with diabetes include abnormal liver enzymes activity and generation of reactive oxygen

species that reduces the defence system of the cells, eventually leading to diabetes [7]. Many drugs presently prescribed by physicians are either directly isolated from plants or artificially modified versions of natural products. However, the majority of the indigenous plants used as medicinal products scientifically validated for their safety and efficacy. Therefore, detailed and extended profiling of phytoconstituents is necessary to establish the quality of hydroalcoholic extract of *P. kurroa* and to explore the effects of glucose-induced hyperglycaemia in a hepatocellular carcinoma ($HepG_2$) cell line [8].

To the best of our knowledge, no previous scientific research has evaluated the efficacy of HAE-PK in glucose-induced hyperglycaemia and its cytotoxicity in hepatocytes. Therefore, the present research aims to the metabolomic profiling of HAE-PK and its potential in the glucose-induced hyperglycaemic in $HepG_2$ cells.

Materials and Methods

Plant material: The fresh rhizome of *P. kurroa* was obtained from Dabur India Limited, India, as gift sample. The voucher specimen has been retained in laboratory with the no BNPL/MI/2018/PK01.

Extraction procedure: 50 g of coarsely powdered *P. kurroa* rhizome were extracted using 400 mL of solvent, ethanol:water (1:1), by refluxing on a water bath at 60°C for 3 hours after macerating it for 12 hours at room temperature, with occasional shaking. The mixture was filtered using Whatman filter paper no. 1, and the residue was washed with fresh solvent. The filtrate and washing solution were pooled and evaporated to dryness on a Rotavapor below 60°C. The residue obtained was weighed and stored in well-closed containers at -20°C until used for analysis.

Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS) analysis of HAE-PK: Chromatographic analysis was performed on Acquity UPLC HSS C18 (50 × 2.1 mm, 1.8 m) column using an isocratic mobile phase consisting of 0.1% formic acid (A) and methanol (B) at a flow rate of 0.6 mL/ min. The separated metabolites were detected by MS. The separated compounds were identified based on their *m/z* value through literature database.

Estimation of total phenolic and flavonoid content: Total phenolic and flavonoid content were determined by the method described by Parveen *et al.* [9]. Gallic acid and quercetin were used as a reference standard for the calibration curve. All the experiments were performed in triplicate.

α-Amylase and α-glucosidase inhibition assay: Activity of α-amylase and α-glucosidase, respectively were carried out as in the previously described method [10]. The results were expressed as % inhibition of enzyme activity and calculated by the underneath equation:

$$\text{Percentage Inhibition} = (A_{control} - A_{sample}) \times 100/A_{control},$$

where, $A_{control}$ = absorbance of control sample and A_{sample} = absorbance of test sample.

DPPH radical scavenging assay: The DPPH radical scavenging activity was determined by the spectro-photometric method for the presence of DPPH as a free radical. All the experiments were performed in triplicate. The following equation has been used to calculate the percentage of DPPH scavenging activity [1]:

$$\text{Percentage scavenging activity} = (1\text{-}A_{sample}/A_{control}) \text{ x } 100,$$

where, $A_{control}$ = absorbance of control reaction containing methanol instead of sample and A_{sample} = absorbance of test sample.

Cell culture and treatment: $HepG_2$ cell lines were purchased from NCSS, India. The study was performed as *per* the defined protocol.

Cytotoxicity effect of glucose and HAE-PK on $HepG_2$: The assay was performed using MTT dissolved in culture medium followed by filtering and incubation for 2 - 4 h to assess the cytotoxicity [12]. Presence of viable cells was visualized by the development of purple colour recorded at 595 nm using UV spectro-photometer against blank.

Measurement of cellular reactive oxygen species (ROS)*: Intracellular antioxidant measurements were made as in the previously described method [13] with little modifications.

Assessment of aldehyde dehydrogenase and hexokinase: The aldehyde dehydrogenase (ALDH) activity was estimated based on the reduction of *β*-NAD (*β*-Nicotinamide adenine dinucleotide) while, the hexo-kinase activity was evaluated based on the reaction with glucose-6-phosphate dehydrogenase, respectively, as in the previously defined methods [14, 15].

Results and Discussion

UPLC/MS profiling of HAE-PK: HAE-PK was dissolved in methanol and UPLC/MS fingerprinting was performed for the identification of complete metabolites present in HAE-PK (Table I). Metabolites present in the HAE-PK are summarized with retention time, mass by charge (*m/z*) values, tentative name, nature of compound and chemical formula with respective literature IDs. A total of 23 most abundant metabolites were analysed and identified through *m/z* value and from a literature survey. Previous literature strongly revealed that, glycosides (iridoid glycosides) present in HAE-PK possess excellent antidiabetic potential [6]. Picroside was observed as the most abundant metabolite in HAE-PK. Thus, for the analysis of metabolite profiling, UPLC/MS method seems to be the best method of analysis.

Phenolic and flavonoid content of HAE-PK: The total phenolic and flavonoid content of the HAE-PK were determined using standard calibration curve of gallic acid ($r^2 = 0.996$) and quercetin ($r^2 = 0.992$), respectively. The total phenolic and flavonoid content were found

to be 37.26 ± 0.09 mg gallic acid equivalent/g dry wt. of HAE-PK and 28.65 ± 0.27 mg quercetin equivalent/g dry wt. of HAE-PK, respectively. Our results, showed that the extract is enriched with phenols and flavonoids which may be responsible for the therapeutic potential. Also, biologically and pharmacologically it is known that phenolic and flavonoid rich nutraceuticals/food reduce the risk of diabetes [16].

Table I

Major metabolites detected in hydroalcoholic extract *Picrorhiza kurroa* (HAE-PK) by UPLC/MS

Metabolites (M)	Rt (min)	Name of metabolites	Class of compounds	References (Mass ID)	Molecular formula	(*m/z* value)
M1	0.850	D-(-)-quinic acid	Alkaloid	PubChem: 6508	$C_7H_{12}O_6$	192.16
M2	2.211	Kutkin	Glycoside	PubChem: 131750182	$C_{23}H_{28}O_{12}$	496.46
M3	3.028	Ergosine	Alkaloid	PubChem: 105137	$C_{30}H_{37}N_5O_5$	547.65
M4	3.198	Picroside-II	Glycoside	PubChem: 11944602	$C_{23}H_{28}O_{13}$	512.46
M5	3.674	(-)-epigallocatechin	Flavonoid	PubChem: 72277	$C_{15}H_{14}O_7$	306.27
M6	4.388	p-hydroxy benzoic acid	Phenol	PubChem: 135	$C_7H_6O_3$	138.12
M7	4.695	Caffeic acid	Phenol	PubChem: 689043	$C_9H_8O_4$	180.15
M8	5.307	Picroside IV	Glycoside	ChemBook: 61339879	$C_{24}H_{28}O_{12}$	508.17
M9	6.396	Pikuroside	Glycoside	PubChem: 132472101	$C_{23}H_{30}O_{14}$	530.47
M10	7.620	Isocorilagin	Phenol	PubChem: 10077799	$C_{27}H_{22}O_{18}$	634.45
M11	7.893	Cinchonine	Alkaloid	PubChem: 90454	$C_{19}H_{22}N_2O$	293.37
M12	8.879	Linoleic acid	Fatty acid	PubChem: 5280450	$C_{18}H_{32}O_2$	280.45
M13	9.287	Veronicoside	Glycoside	PubChem: 329824805	$C_{22}H_{26}O_{11}$	466.44
M14	10.410	Catalpol	Glycoside	PubChem: 91520	$C_{15}H_{22}O_{10}$	362.33
M15	10.954	3',6-dimethyl-flavone	Flavonoid	PubChem: 688822	$C_{17}H_{14}O_2$	250.29
M16	11.703	Ellagic acid	Phenol	PubChem: 5281855	$C_{14}H_6O_8$	302.19
M17	12.757	(-)-specioside	Glycoside	PubChem: 44566579	$C_{23}H_{28}O_{11}$	480.46
M18	13.030	Brevianamide A	Alkaloid	PubChem: 99771	$C_{21}H_{23}N_3O_3$	365.43
M18	13.710	Papaveraldine	Alkaloid	PubChem: 96932	$C_{20}H_{19}NO_5$	353.37
M19	14.425	6-ethoxy-3(4'-hydroxyphenyl)-4-methylcoumarin	Phenol	ChemSpider: 600190	$C_{18}H_{16}O_4$	296.31
M20	15.411	beta-hydrastine	Alkaloid	PubChem: 197835	$C_{21}H_{21}NO_6$	383.40
M21	17.214	2-coumaric acid	Phenol	PubChem: 637540	$C_9H_8O_3$	164.16
M22	17.384	Gelsenicine	Alkaloid	PubChem: 21123652	$C_{19}H_{22}N_2O_3$	326.39
M23	17.895	Aucubin	Glycoside	PubChem: 91458	$C_{15}H_{22}O_9$	346.33

α-Amylase and α-glucosidase inhibitory activities of HAE-PK: The IC_{50} values of HAE-PK showed potential inhibitory effects on α-amylase and α-glucosidase as compared to the standard antidiabetic compound, acarbose (Table II). The inhibitory potential of HAE-PK directly reduces the breakdown of starch to smaller molecules of oligosaccharides in to the gastro-intestinal tract and subsequently reduces the glucose absorption. This may also lead to a reduction in postprandial hyper-glycaemia levels. α-glucosidase, located in brush borders of small intestines, hydrolyses the oligosaccharides and cleave the glycoside bonds to liberate glucose from the non-reducing end of oligosaccharide and polysaccharide chains [17]. Hence, inhibition of α-amylase and α-glucosidase is a very effective way of delaying glucose absorption and lowering the postprandial blood glucose level, which can potentially suppress the progression of diabetes.

DPPH antioxidant activity: 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay is widely used to determine the antioxidant activity of single compounds and plant extracts. Results of the present study revealed that HAE-PK shows significant inhibitory potential in dose-dependent manner on DPPH free radical at tested concentrations (25 - 250 µg/mL). IC_{50} values of HAE-PK and quercetin were recorded as 88.44 ± 0.42 and 71.54 ± 0.64 µg/mL (Table II). Recent evidence suggests that oxidative stress may contribute to the pathogenesis of diabetes. The diet, especially natural products or food supplements, contain a vast number of compounds with antioxidant activity, which may have cumulative/synergistic antioxidant effects [18].

Table II

Antidiabetic and antioxidant potential of HAE-PK

Antidiabetic Potential (IC_{50})				**Antioxidant Potential (IC_{50})**	
α-Amylase		α-Glucosidase		DPPH	
Acarbose	HAE-PK	Acarbose	HAE-PK	Quercetin	HAE-PK
164.7 + .81	124.7 + 0.59	164.7 + 1.09	135.03 + 1.28	71.54 + 0.64	88.44 + 0.42

Characterization of time course and dose-dependent toxicity of glucose and HAE-PK in HepG$_2$ cells: Results obtained from the cytotoxicity assay revealed that 5 and 25 mM glucose did not cause any significant toxicity to HepG$_2$ cells at 24 hrs and 48 hrs, and throughout the study, cell viability was maintained up to 90.5 ± 2.67% and 88.79 ± 1.70%, respectively. Further, treatment of HepG$_2$ cells with 50 mM glucose for 24 - 48 hrs significantly affected the viability of HepG$_2$ cells as compared to 5 and 25 mM of glucose and cell viability decreased to 71.36 ± 2.89% and 74.42 ± 3.24%, respectively (Figures 1a and 1b). In order to assess the nontoxic concentrations of HAE-PK for the cytoprotective studies, we carried out the MTT based cytotoxicity assay in HepG$_2$ cells for 24 and 48 hrs, respectively. HAE-PK did not cause any toxicity to the HepG$_2$ cells up to 500 µg/mL, and cell viability was maintained at about 92 - 97% throughout the study (Figures 2a and 2b). Further, treatment with HAE-PK (250 µg/mL) led to an enhanced cell viability in high glucose-induced HepG$_2$ cells for 24 - 48 hrs (Figures 3a and 3b). However, 50 mM glucose was used to replicate hyperglycaemia in HepG$_2$ cells.

Previous studies describe that high glucose induces oxidative stress and disturbs the normal physiology of the cell by damaging the cellular integrity. Protection of cellular physiology is of prime importance in order to counteract the hyperglycaemia-induced oxidative damage. Kong *et al.* described that cytotoxicity or the cytoprotective effect of the plant extracts is highly dependent on their concentration, bioavailability and complex interactions among phytochemicals [19]. However, current results strongly suggest that HAE-PK can maintain cell growth and physiological functions against glucose-induced hyperglycaemia and oxidative stress state and is only cytotoxic at very high concentrations.

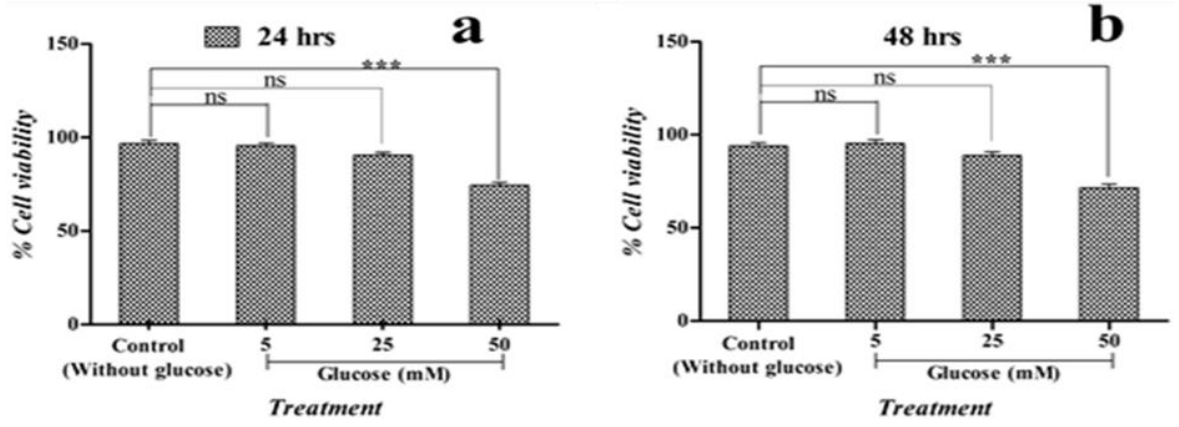

Figure 1.
Time and concentration dependent effects of glucose on viability of HepG$_2$ cells. The values are expressed as mean ± SD of triplicate tests as determined by ANOVA (*p < 0.05, **p < 0.01 and ***p < 0.001). p-value compared with untreated (control) HepG$_2$ cells

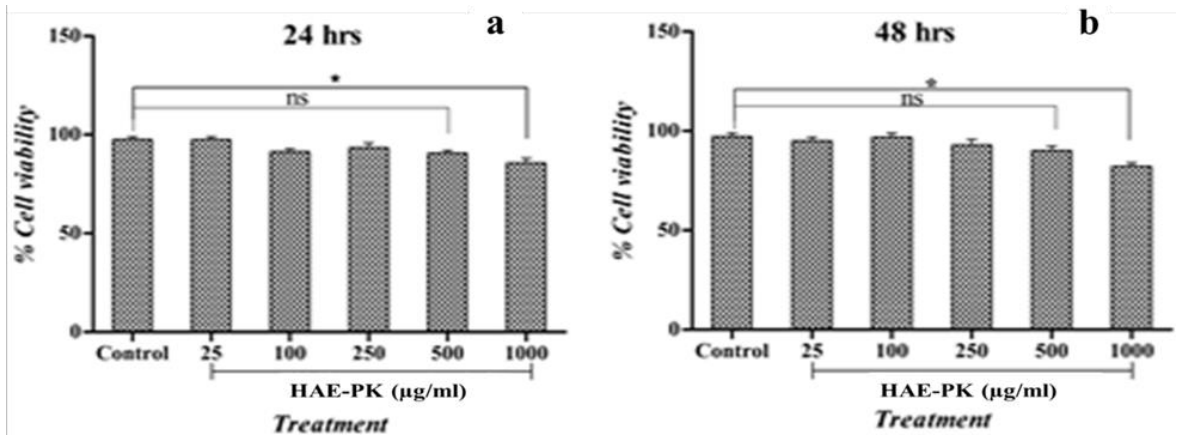

Figure 2.
Time and concentration dependent effects of HAE-PK on viability of HepG$_2$ cells. The values are expressed as mean ± SD of triplicate tests as determined by ANOVA (*p < 0.05, **p < 0.01 and ***p < 0.001). p-value compared with untreated (control) HepG$_2$ cells

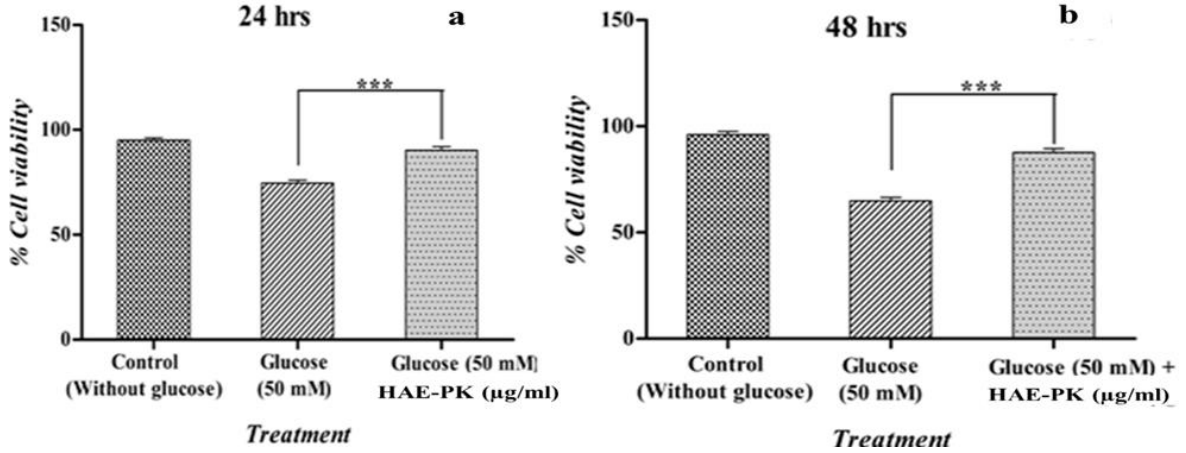

Figure 3.

Time dependent effects of HAE-PK on viability of $HepG_2$ cells against glucose induce hyperglycaemia. The values are expressed as mean ± SD of triplicate tests as determined by ANOVA (*p < 0.05, **p < 0.01 and ***p < 0.001). p-value compared with glucose-induced hyperglycaemia on $HepG_2$ cells

Effect of HAE-PK on glucose-induced ALDH and hexokinase expression in $HepG_2$ cells: A significant increase in ALDH activity was found in hyperglycaemic treatment group in $HepG_2$ cells for 24 and 48 hrs, respectively as compared to normal. While, after treatment with HAE-PK (250 µg/mL), the level of ALDH was (***p < 0.001 and **p < 0.01) restored to normal (Figures 4a and 4b). Liver is a metabolic crossroad, crucial for glucose metabolism in animals, for the regulation of the systemic fuel and cellular functions. Increased sugar consumption is considered to be one of contributors to the worldwide epidemics of obesity and diabetes and their associated cardiometabolic risks. Because of its unique metabolic properties, the glucose and fructose component of sugar may be particularly harmful for the normal metabolic function of the liver. Altered metabolism of glucose or fructose in liver are the backbone of triglyceride synthesis [20].

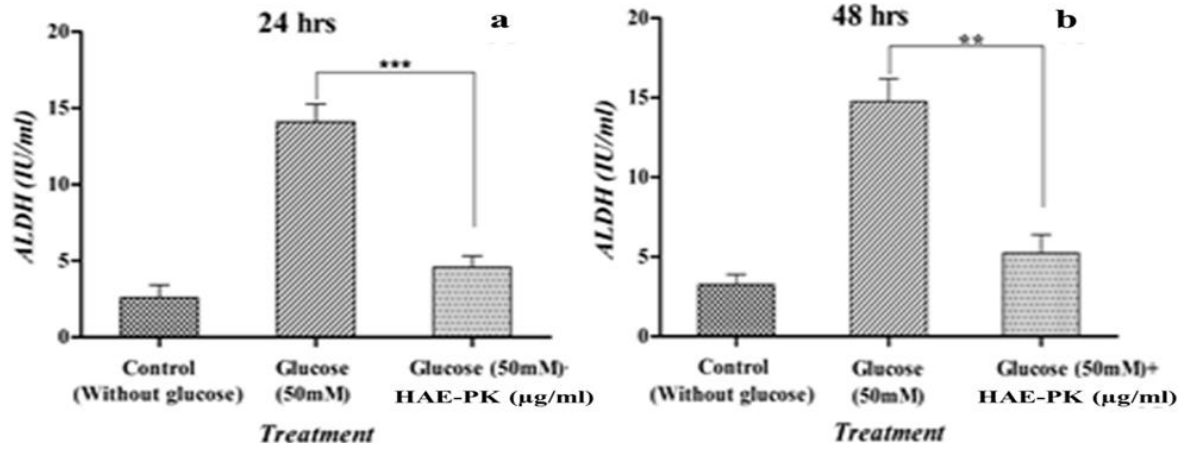

Figure 4.

Time dependent effects of HAE-PK (250 µg/mL) on level of ALDH in hyperglycaemia (glucose 50 mM) induced $HepG_2$ cells for **a)** 24 hrs and **b)** 48 hrs. The values are expressed as mean ± SD of triplicate tests as determined by ANOVA (*p < 0.05, **p < 0.01 and ***p < 0.001). p-value compared with group hyperglycaemia (glucose 50 mM) $HepG_2$ cells

In case of hexokinase activity, the level of hexokinase sharply blunted in hyperglycaemic groups for 24 - 48 hrs, as compared to control (Figures 5a and 5b). After treatment with HAE-PK (250 µg/mL), the hexokinase level was significantly (**p < 0.01 and *p < 0.05) brought to normal. Hexokinase is the first key enzyme which catalyses the first committed step in glucose metabolism by phosphorylating glucose to glucose-6-phosphate (G6P) in the glycolytic pathway. Recent studies have indicated that aberrant hexokinase activity is associated with many human diseases, such as diabetes, muscular dystrophy and cancer [21]. A study by Rabbani and Thornalley, described that in hyperglycaemic state, increased level of hexokinase and G6P initiate metabolic dysfunction leading to tissue damage. The hypothesis of hexokinase linked glycolytic

overload provides an explanation of pathogenic mechanisms and experimental observations associated with the development of vascular complications of diabetes and prediabetes, diabetic embryopathy and tissue progenitor cell dysfunction in diabetes. It may be postulated that hexokinase receptor is a therapeutically competent target to maintain the physiological function of the cells and inhibit the disease progression due to high concentrations of glucose [22].

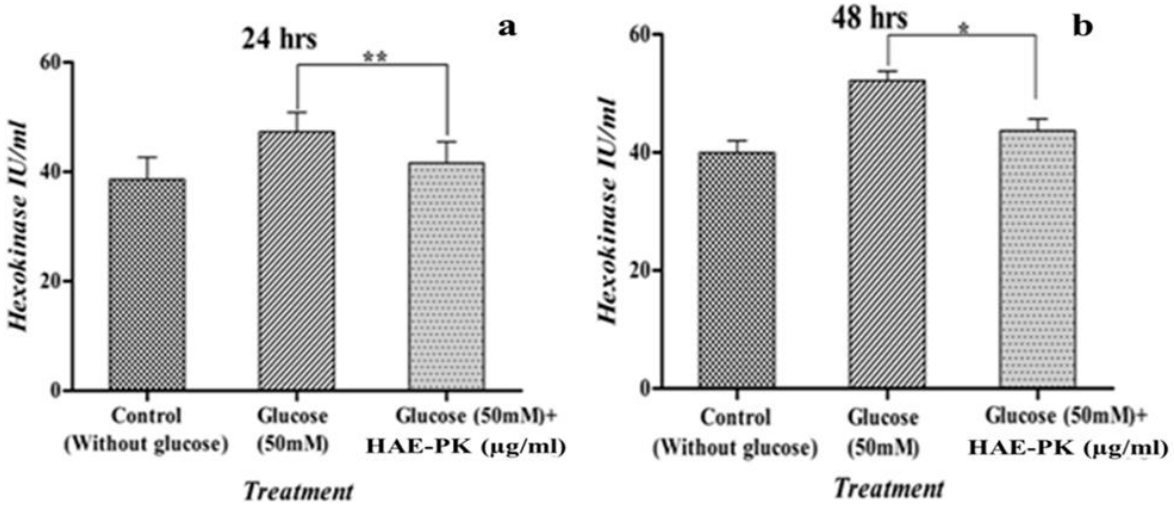

Figure 5.

Time dependent effects of HAE-PK (250 µg/mL) on level of hexokinase against hyperglycaemia (glucose 50 mM) induced in $HepG_2$ cells for **a)** 24 hrs and **b)** 48 hrs. The values are expressed as mean ± SD of triplicate tests as determined by ANOVA (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$). p-value compared with group hyperglycaemia (glucose 50 mM) $HepG_2$ cells

A significant increase in the level of ALDH and hexokinase in glucose-induced hyperglycaemia substantiated the hepatic damage/oxidative stress whereas, HAE-PK significantly reversed the level of ALDH and hexokinase against hyperglycaemia. The extract may regulate the activities of these enzymes in diabetes through either normalizing the glycolysis or increasing the utilization of glucose for energy production. In a previous investigation, coumarin and citral markedly reduced hyperglycaemia in diabetes by regulating the oxidative stress and hepatic metabolism [23]. This could be due to the activity of the extract in maintaining metabolic regulation, as evidenced by changes in the levels of biochemical parameters (ALDH and hexokinase) linked to different metabolic pathways. With such evidence, it is conceivable to propose that HAE-PK exhibits its antidiabetic effect in $HepG_2$ cell line by maintaining the hepatic key enzymes involved in the carbohydrate metabolism.

Conclusions

Our findings clearly demonstrated that HAE-PK inhibits the enzymes α-amylase and α-glucosidase as well as restores the level of the studied metabolic enzymes. ALDH and hexokinase, linked to diabetes, thus endorsing a great potential for its use in the prevention and treatment of diabetes. UPLC/MS profiling of HAE-PK showed the presence of different phytochemicals including alkaloids, glycosides, phenols and flavonoids etc. Hence, the observed antidiabetic activity may be the combined effect of all or either of these constituents. However, the future prospective use of HAE-PK in the treatment of diabetes warrants further exhaustive *in silico*, experimental and clinical studies to determine the exact mechanism of action of its bioactive principles. However, it can be attributed to an overall multi-component, multi-mechanistic synergistic action of flavonoids, phenols and iridoids.

Conflict of interest

The authors declare no conflict of interest.

References

1. International Diabetic Federation, 2017. Diabetes atlas. 8th edition.
2. Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, Del Cañizo-Gómez FJ, Update on the treatment of type 2 diabetes mellitus. *World J Diabetes*, 2016; 7(17): 354-395.
3. Uboh FE, Okon IE, Ekong MB, Effect of aqueous extract of *Psidium guajava* leaves on liver enzymes, histological integrity and hematological indices in rats. *Gastroenterology Res.*, 2010; 3(1): 32-38.
4. Wang Z, Wang J, Chan P, Treating type 2 diabetes mellitus with traditional Chinese and Indian medicinal herbs. *Evid Based Complement Alternat Med.*, 2013; 2013: 343594: 1-17.
5. Mallick MN, Singh M, Parveen R, Khan W, Ahmad S, Najm MZ, Husain SA, HPTLC analysis of bioactivity guided anticancer enriched fraction of hydroalcoholic

extract of *Picrorhiza kurroa*. *BioMed Res Int.*, 2015; 2015: 513875: 1-18.
6. Kumar S, Patial V, Soni S, Sharma S, Pratap K, Kumar D, Padwad Y, *Picrorhiza kurroa* enhances β-Cell mass proliferation and insulin secretion in streptozotocin evoked β-Cell damage in rats. *Front Pharmacol.*, 2017; 8: 537: 1-15.
7. Petersen MC, Shulman GI, Mechanisms of insulin action and insulin resistance. *Physiol Rev.*, 2018; 98(4): 2133-2223.
8. Sefried S, Häring H, Weigert C, Eckstein SS, Suitability of hepatocyte cell lines HepG2, AML12 and THLE-2 for investigation of insulin signalling and hepatokine gene expression. *Open Biol.*, 2018; 8(10): 18047: 1-11.
9. Parveen S, Ansari MHR, Parveen R, Khan W, Ahmad S, Husain SA, Chromatography based metabolomics and *in silico* screening of *Gymnema sylvestre* leaf extract for its antidiabetic potential. *Evid Based Complement Alternat Med.*, 2019; 2019: 7523159: 1-14.
10. Khan W, Parveen R, Chester K, Parveen S, Ahmad S, Hypoglycemic Potential of aqueous extract of *Moringa oleifera* leaf and *in vivo* GC-MS Metabolomics. *Front Pharmacol.*, 2017; 8: 577: 1-16.
11. Chester K, Paliwal S, Khan W, Ahmad S, UPLC-ESI-MS/MS and HPTLC method for quantitative estimation of cytotoxic glycosides and aglycone in bioactivity guided fractions of *Solanum nigrum* L. *Front Pharmacol.*, 2017; 8: 434: 1-15.
12. Senthilraja P, Kathiresan K, *In vitro* cytotoxicity MTT assay *in vero*, HepG2 and MCF-7 cell lines study of marine yeast. *J Appl Pharm Sci.*, 2015; 5(03): 80-84.
13. Subramaniyan SD, Natarajan AK, Citral, a monoterpene protects against high glucose induced oxidative injury in HepG2 cell *in vitro*-an experimental study. *J Clin Diagn Res.*, 2017; 11(8): BC10-BC15.
14. Bostian KA, Betts GF, Kinetics and reaction mechanism of potassium-activated aldehyde dehydrogenase from *Saccharomyces cerevisiae*. *Biochem J.*, 1978; 173(3), 787-798.
15. Brandstrup N, Kirk JE, Bruni C, The hexokinase and phosphoglucoisomerase activities of aortic and pulmonary artery tissue in individuals of various ages. *J Gerontol.*, 1957; 12(2): 166-171.
16. Testa R, Bonfigli AR, Genovese S, De Nigris V, Ceriello A, The possible role of flavonoids in the prevention of diabetic complications. *Nutrients*, 2016; 8(5): 310: 1-13.
17. Agatonovic-Kustrin S, Morton DW, High-performance thin-layer chromatography HPTLC-direct bioautography as a method of choice for alpha-amylase and antioxidant activity evaluation in marine algae. *J Chromatogr A.*, 2017; 29: 1530: 197-203.
18. Mancini FR, Affret A, Dow C, Balkau B, Bonnet F, Boutron-Ruault MC, Fagherazzi G, Dietary antioxidant capacity and risk of type 2 diabetes in the large prospective E3N-EPIC cohort. *Diabetologia*, 2018; 61(2): 308-316.
19. Kong KW, Mat-Junit S, Aminudin N, Hassan FA, Ismail A, Aziz AA, Protective effects of the extracts of *Barringtonia racemosa* shoots against oxidative damage in HepG2 cells. *PeerJ.*, 2016; 4: e1628: 1-20.
20. Hannou SA, Haslam DE, McKeown NM, Herman MA, Fructose metabolism and metabolic disease. *J Clin Invest.*, 2018; 128(2): 545-555.
21. DeWaal D, Nogueira V, Terry AR, Patra KC, Jeon M, Guzman G, Au J, Long CP, Antoniewicz MR, Hay N, Hexokinase-2 depletion inhibits glycolysis and induces oxidative phosphorylation in hepatocellular carcinoma and sensitizes to metformin. *Nat Commun.*, 2018; 9(1): 446: 1-14.
22. Rabbani N, Thornalley PJ, Hexokinase-2 glycolytic overload in diabetes and ischemia-reperfusion injury. *Trends Endocrinol Metab.*, 2019; 30(7): 419-431.
23. Yao Y, Zhao X, Xin J, Wu Y, Li H, Coumarins improved type 2 diabetes induced by high-fat diet and streptozotocin in mice *via* antioxidation. *Can J Physiol Pharmacol.*, 2018; 96(8): 765-771.