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Original Article

Effect of fucoxanthin on the pharmacodynamics and pharmacokinetics of gliclazide in animal models

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ABSTRACT

Objective: Type 2 diabetes may occur in patients suffering from obesity. Gliclazide is the most commonly used drug of choice for the treatment of type 2 diabetes and fucoxanthin is widely used for the management of obesity. As these two drugs intersect at a point, it is essential to investigate the effect of oral administration of fucoxanthin on the pharmacodynamics and pharmacokinetics of gliclazide in animal models and to further evaluate the safety and effectiveness of this combination. **Methods:** Influence of fucoxanthin on the activity of gliclazide was determined by conducting single and multiple dose interaction studies in rats (normal and diabetic) and rabbits. Blood samples collected at predetermined time intervals from experimental animals were used for the estimation of glucose and insulin levels by using automated clinical chemistry analyzer and radioimmunoassay method, respectively. The insulin resistance and β-cell function were determined by homeostasis model assessment. Additionally, serum gliclazide levels in rabbits were analyzed by high performance liquid chromatography. **Results:** Gliclazide showed peak percent reduction in blood glucose levels at 2 h and 8 h in rats and at 3 h in rabbits. This activity of gliclazide was not altered by single dose treatment with fucoxanthin. However, multiple dose interaction study of fucoxanthin with gliclazide resulted in significantly greater reduction in blood glucose levels ranging from 22.27 to 45.81% in normal rats, 28.45 to 44.68% in diabetic rats and 15.01 to 40.72% in rabbits. This observation was coupled with significantly increased insulin level, β-cell function and insulin resistance index in animal models. The pharmacokinetics of gliclazide in rabbits was significantly altered by repeated dose treatments of fucoxanthin and the percent increase in serum gliclazide concentration was found to be 13.93 % when compared to gliclazide control. **Conclusion:** From this study, it was concluded that the interaction of fucoxanthin with gliclazide upon multiple dose treatments is pharmacokinetic in nature. This combination when prescribed/taken for clinical use in obese patients requires dose adjustments and periodic monitoring of blood glucose levels.

KEY WORDS: Fucoxanthin; Gliclazide; Homeostasis model assessment; Obesity; Pharmacokinetics; Pharmacodynamics

INTRODUCTION

It is a common practice to use herbal medicines along with prescribed drugs to treat various chronic disorders like obesity, metabolic syndrome, diabetes etc. Combination may be therapeutic or toxic at prescribed doses and such interactions remain to be verified. Significant changes in the pharmacological or toxicological effects of either component may be observed when herbal medicines and drugs are given in combination. Therefore, herb-drug interaction is a crucial field which requires research to evaluate the safety of these combinations [1]. Obesity is recognized as a worldwide crisis and represents an area of increasing concern because of its predominant effects on mortality and economics. Clinical pathophysiological studies indicate that obesity leads to risk factors like diabetes, insulin resistance, hypertension, dyslipidemia and cardiac complications. Management of such conditions and complications requires prolonged treatment with combinations of drugs and/ or herbal medicines. Patients with obesity are prone to develop diabetes and maintenance of normal blood glucose level in these individuals is very critical for the prevention of undesirable complications associated with both hyperglycemia and hypoglycemia. Oral hypoglycemic agents are used in the treatment of type 2 diabetes, of which gliclazide (second generation sulfonylurea derivative) is the preferred choice of drug. It acts by selectively inhibiting pancreatic K^+ ATPase channels [2]. Further, gliclazide was reported for antioxidant properties and other hemobiological effects [3]. Gliclazide after ingestion is primarily metabolized by hepatic microsomal enzymes CYP2C9 and partly by CYP3A4 [2].

Fucoxanthin is a carotenoid present in edible brown seaweeds such as *Fucus vesiculosus* and *Undaria pinnatifida*. Fucoxanthin is reported to exhibit beneficial health effects, such as anti-cancer [4] and anti-inflammatory activities [5]. Fucoxanthin exhibits anti-obesity activity by increasing the expression UCP-1 (Uncoupling Protein or Thermogenin) in white adipose tissue. This transmembrane protein regulates reactive oxygen species (ROS) and decrease the proton gradient generated in oxidative phosphorylation. Fucoxanthin stimulates thermogenesis and suppresses lipid accumulation by increasing the expression of UCP-1 gene in the mitochondria of white adipose cells [6]. Fucoxanthin is reported to reduce abdominal white adipose tissue weights in wistar rats and KK-Ay mice [7]. Many studies have shown that fucoxanthin significantly decreased body weight [8],

fat accumulation [9] in animal models. Fucoxanthin interaction with metabolising enzymes has been studied *in vitro* and is reported that fucoxanthin exerts its anti-cancer activity by inhibiting drug metabolic enzymes CYP1A2 and CYP3A4 in dose dependent manner which are essential for the activation of pro-carcinogens [10]. Although, *in vitro* investigations on fucoxanthin suggests that it might be an inhibitor of CYP enzymes, correlation of the same to its *in vivo* effects is hypothetical and opens an avenue for further investigation. There is no much *in vivo* data available for the potential interaction of fucoxanthin with drugs. Thus, the study was designed with the hypothesis that pretreatment of fucoxanthin with gliclazide will lead to significant herb-drug interactions in animal models.

MATERIALS AND METHODS

Drugs and chemicals

Gliclazide was obtained as a gift sample for Dr. Reddy's Laboratories, Bachupally, Hyderabad. Fucoxanthin commercially available as 10% was obtained from Best Naturals, USA. Alloxan monohydrate was purchased from LOBA Chemie (Mumbai, India). All reagents and chemicals used in the study were of analytical grade.

Experimental animals and husbandry

8 to 9 week old male albino rats weighing between 170-250 g were procured from Vivo Biotech, Hyderabad, India and 3 month old male albino rabbits weighing between 1-1.5 Kg were procured from Rabiroof, Hyderabad, India. They were maintained under standard laboratory husbandry conditions at 25 ± 2°C and 50 ± 15% relative humidity with a 12 h light/ dark cycle. Animals were fed with a commercially available pellet diet (Rayan's Boitechnologies Pvt Ltd, Hyderabad, India) and water was provided *ad libitum*. Animals were fasted for 10 h prior to the experiment and during the experiment they were withdrawn from food. The animal experiments were approved by Institutional Animal Ethics Committee (DLL/IAEC/2013/02/04) and the study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Study design

Fucoxanthin dose of 200 mg/kg and 100 mg/kg was calculated from human oral therapeutic dose based on body surface area for rats and rabbits, respectively[11]. From the results of gliclazide dose-effect relationship study conducted in normal rats and rabbits, the dose of 2 mg/kg and 4 mg/kg body weight were selected respectively, for administration in animals [12]. Oral dose for fucoxanthin was prepared by suspending in 0.5% Carboxymethylcellulose sodium. Gliclazide solution was prepared by dissolving in few drops of 0.1N Sodium hydroxide and final volume was made with water [13]. The design of the study is as follows.

Stage-1: Pharmacodynamic interaction in normal rats [2]

Stage-2: Pharmacodynamic interaction in diabetic rats [2]

Stage-3: Pharmacodynamic and pharmacokinetic interaction study in normal rabbits [13]

Pharmacodynamic interaction in normal rats

Six rats were selected for stage 1 experiment. These rats were given gliclazide via the oral route at 2 mg/kg body weight and their blood was collected at predetermined time points. With a week washout period between each experiments, similar procedure was performed with either orally administered fucoxanthin only or combination treatment with both fucoxanthin and gliclazide at the previously mentioned doses. After these single dose interaction study the same group of animals were given daily treatments with fucoxanthin for the next 20 days with regular feeding. On day 21, animals were fasted for 10 h before administering fucoxanthin. After 30 minutes, the animals were given gliclazide at 2 mg/kg body weight. Blood samples were collected at predetermined time intervals after each treatment with gliclazide alone, fucoxanthin alone or combination treatments (single and multiple).

Pharmacodynamic interaction in diabetic rats

For stage 2 experiment diabetes was induced in rats as previously described [14]. Briefly, diabetes was induced in rats by the administration of alloxan monohydrate in two divided doses, i.e. 100 mg/kg and 50 mg/kg body weight intraperitoneally for two consecutive days. After 72 h, blood samples were collected from surviving rats by retro- orbital puncture and blood glucose levels were measured using automated clinical chemistry analyzer. Rats with blood glucose levels ≥200 mg/dL were considered as diabetic and selected for the study. The same treatment procedures as described in stage 1 were tested in diabetic rats.

Pharmacodynamic and pharmacokinetic interaction study in normal rabbits

Six rabbits were selected for stage 3 experiment. These rabbits were given gliclazide via the oral route at 4 mg/kg body weight and their blood was collected at predetermined time points. With a week washout period between each experiments, similar procedure was performed with either orally administered fucoxanthin only or combination treatment with both fucoxanthin and gliclazide at the previously mentioned doses. After these single dose interaction study the same group of animals were given daily treatments with fucoxanthin for the next 20 days with regular feeding. On day 21, animals were fasted for 10 h before administering fucoxanthin. After 30 minutes the animals were given gliclazide at 4 mg/kg body weight. Blood samples were collected at predetermined time intervals after each treatment with gliclazide alone, fucoxanthin alone or combination treatments (single and multiple).

Collection of blood samples and estimation

Blood samples were collected from retro-orbital plexus [15]

of each rat at 0, 1, 2, 3, 4, 6, 8 and 12 h. Blood samples were withdrawn from the marginal ear vein of each rabbit at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h. The blood samples collected at all the intervals (except for 16 and 24 h in rabbits) were tested for blood glucose by using an automated clinical chemistry analyzer. Blood samples collected at 2 h and 8 h time intervals in rats (normal and diabetic) and at 3 h from rabbits were also used for the estimation of serum insulin by radioimmunoassay method [13]. These intervals were selected based on peak percent reduction in blood glucose levels observed rats and rabbits [13]. Additionally, blood samples collected from rabbits were used for the estimation of gliclazide concentration in serum by high performance liquid chromatography.

Determination of insulin resistance index and β-cell function

The insulin resistance index and β-cell function were assessed by homeostatic model assessment protocol and was calculated as follows [13, 16, 17].

Insulin resistance = (FSI*FSG)/22.5 and β-cell function = (20*FSI)/ (FSG-3.5)*100

Where FSI is fasting serum insulin (μIU/mL) and FSG is fasting serum glucose (mg/dL).

Pharmacokinetic analysis

One compartmental open model was used for estimation of pharmacokinetic parameters by using Kinetica 5.0 software. Pharmacokinetic parameters of gliclazide in rabbit serum such as peak serum concentration (C_{max}), peak time (T_{max}), area under the concentration time curve (AUC), area under first moment curve (AUMC), terminal half-life ($T_{1/2}$), elimination rate constant (K_{el}), mean residence time (MRT) and clearance (CL) were estimated.

Data and statistical analysis

Data are expressed as mean ± SD. The significance was determined by applying Student's paired t-test. $p < 0.05$ is considered statistically significant.

RESULTS

Pharmacodynamic interaction between fucoxanthin and gliclazide:

Gliclazide produced hypoglycemic activity in normal rats with maximum biphasic reduction of 40.82 ± 2.04 % and 38.54 ± 1.96 % (Fig 1) and antihyperglycemic activity in diabetic rats with peak biphasic reduction of 42.70 ± 0.66 % and 40.42 ± 1.27 % at 2 h and 8 h intervals, respectively (Fig 2). Peak hypoglycemic activity was observed with maximum reduction of 40.72 ± 1.84 % at 3 h in normal rabbits (Fig 3). Fucoxanthin alone and single dose treatment of fucoxanthin with gliclazide did not induce any significant change in pharmacodynamics of gliclazide. However, multiple dose combination of fucoxanthin with gliclazide produced significant increase in percent blood glucose reduction, insulin levels, insulin resistance and β-cell function in rats (normal and diabetic) and rabbits when compared to gliclazide control (Table 1, 2 and 3).

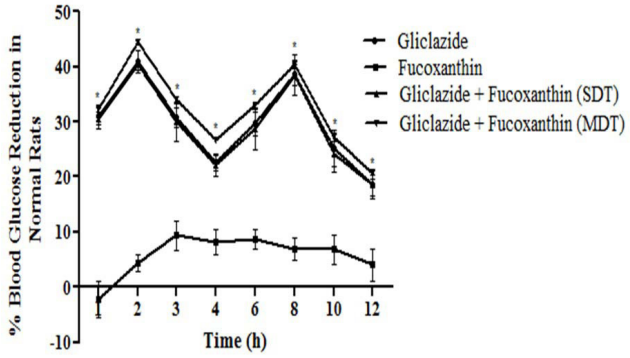

Fig 1. Mean percent blood glucose reduction with gliclazide in presence and absence of fucoxanthin in normal rats. SDT: Single Dose Treatment; MDT: Multiple Dose Treatment. *: Statistically significant when compared to gliclazide control.

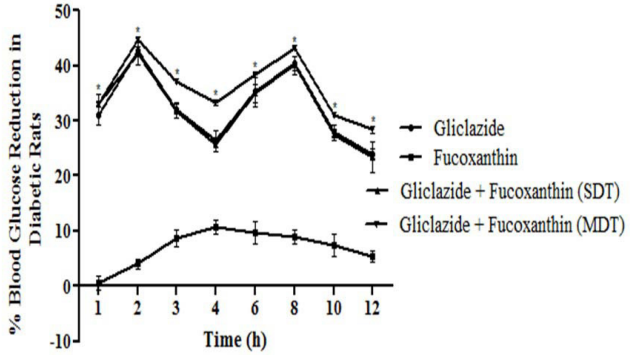

Fig 2. Mean percent blood glucose reduction with gliclazide in presence and absence of fucoxanthin in diabetic rats. SDT: Single Dose Treatment; MDT: Multiple Dose Treatment. *: Statistically significant when compared to gliclazide control.

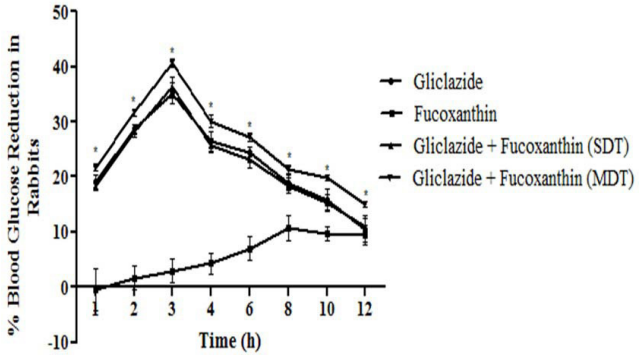

Fig 3. Mean percent blood glucose reduction with gliclazide in presence and absence of fucoxanthin in rabbits. SDT: Single Dose Treatment; MDT: Multiple Dose Treatment. *: Statistically significant when compared to gliclazide control.

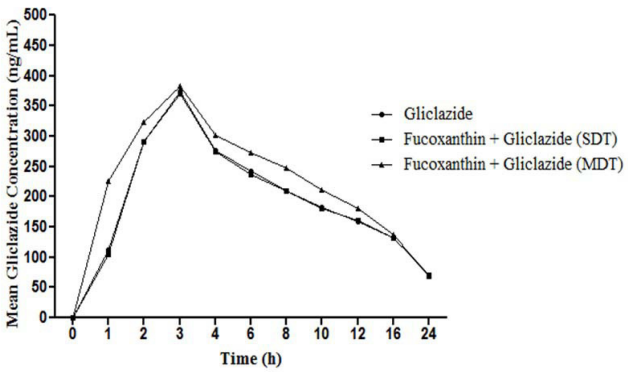

Fig 4. Mean serum gliclazide concentration (ng/mL) before and after treatment with fucoxanthin in rabbits. SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Pharmacokinetic interaction between fucoxanthin and gliclazide:

The mean concentration versus time curve after oral administration of gliclazide in presence of fucoxanthin is shown in Fig 4. The pharmacokinetic parameters of gliclazide alone and in the presence of fucoxanthin following single and multiple dose administrations are given in Table 4. Single dose treatment of fucoxanthin with gliclazide did not alter the pharmacokinetics of gliclazide in rabbits. However, in multiple dose treatments of fucoxanthin significantly increased the serum gliclazide levels and pharmacokinetic parameters such as C_{max}, AUC and AUMC.

Table 1. Effect of fucoxanthin on glucose insulin homeostasis of gliclazide in normal rats (n=6)

Parameter	Insulin		Insulin resistance		β-cell function	
	2 h	8 h	2 h	8 h	2 h	8 h
Gliclazide	12.17 ± 0.81	12.09 ± 0.63	30.71 ± 2.05	31.67 ± 1.51	458.19 ± 46.27	436.43 ± 34.91
Fucoxanthin	7.12 ± 0.43	6.66 ± 0.49	29.91 ± 1.86	27.21 ± 2.14	156.57 ± 10.09	150.44 ± 11.16
Gliclazide + Fucoxanthin (SDT)	12.21 ± 0.66	11.38 ± 0.97	31.36 ± 1.24	30.12 ± 2.39	450.14 ± 34.15	406.86 ± 48.48
Gliclazide + Fucoxanthin (MDT)	15.78 ± 0.53*	15.20 ± 0.67*	36.90 ± 0.71*	36.81 ± 1.82*	642.98 ± 42.23*	596.11 ± 27.84*

Data expressed as Mean ± SD. *: Significant increase when compared to gliclazide; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 2. Effect of fucoxanthin on glucose insulin homeostasis of gliclazide in diabetic rats (n=6)

Parameter	Insulin		Insulin resistance		β-cell function	
	2 h	8 h	2 h	8 h	2 h	8 h
Gliclazide	12.10 ± 0.62	11.63 ± 0.46	78.84 ± 3.70	78.82 ± 3.52	169.09 ± 9.86	156.11 ± 6.30
Fucoxanthin	7.21 ± 0.85	6.79 ± 0.50	79.09 ± 9.13	70.58 ± 4.73	59.30 ± 7.25	58.86 ± 4.82
Gliclazide + Fucoxanthin SDT)	11.80 ± 0.87	11.54 ± 0.84	80.47 ± 7.31	81.40 ± 6.01	157.41 ± 9.74	148.83 ± 11.26
Gliclazide + Fucoxanthin (MDT)	16.49 ± 1.00*	15.72 ± 0.91*	106.16 ± 6.55*	103.96 ± 6.19*	233.44 ± 14.52*	216.31 ± 12.69*

Data expressed as Mean ± SD. *: Significant increase when compared to gliclazide; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 3. Effect of fucoxanthin on glucose insulin homeostasis of gliclazide in rabbits (n=6)

Parameter	Insulin	Insulin resistance	β-cell function
	3 h	3 h	3 h
Gliclazide	20.34 ± 0.52	56.64 ± 2.44	688.20 ± 30.56
Fucoxanthin	8.49 ± 0.38	36.60 ± 1.82	181.58 ± 7.97
Gliclazide + Fucoxanthin (SDT)	19.40 ± 0.42	53.59 ± 1.31	661.29 ± 16.86
Gliclazide + Fucoxanthin (MDT)	24.19 ± 0.34*	63.59 ± 1.21*	869.67 ± 32.58*

Data expressed as Mean ± SD. *: Significant increase when compared to gliclazide; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 4. Mean pharmacokinetic parameters of gliclazide before and after administration of fucoxanthin in rabbits (n=6)

Pharmacokinetic parameters	Gliclazide	Gliclazide + Fucoxanthin (SDT)	Gliclazide + Fucoxanthin (MDT)
C_{max} (ng/mL)	373.40 ± 6.09	369.59 ± 3.54	433.84 ± 6.70*
T_{max} (h)	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
AUC_{last} (h*ng/mL)	4009.14 ± 23.62	3975.62 ± 22.24	4902.52 ± 29.99*
AUC_{inf} (h*ng/mL)	5047.21 ± 65.95	5025.43 ± 71.91	6046.16 ± 168.31*
$AUMC_{last}$ (h*h*ng/mL)	38505.18 ± 386.09	38334.94 ± 363.49	46983.97 ± 567.94*
$AUMC_{inf}$ (h*h*ng/mL)	78839.23 ± 2464.18	79293.14 ± 3025.93	90218.68 ± 7184.76*
$T_{1/2}$ (h)	10.29 ± 0.21	10.40 ± 0.27	10.16 ± 0.74
K_{el} (1/h)	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00
MRT (h)	9.26 ± 0.04	9.26 ± 0.03	9.45 ± 0.12
CL (L/h)	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00

Data expressed as Mean ± SD. *: Significant increase when compared to gliclazide control; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

DISCUSSION

Drug interactions studies are an important aspect of pharmacology research and such interactions are usually evaluated by conducting studies in animal models [18]. Although animal models can never replace the need for comprehensive studies in human subjects, their use can provide important information for understanding the mechanisms of drug interactions. The present study is designed to evaluate the effect of fucoxanthin on the activity of gliclazide in rats (normal and diabetic) and in rabbits. Normal rats were used to identify the interaction and diabetic rats aided to validate the interaction in the actually used condition of the drug. Rabbit model was used further to validate the same in dissimilar species [2, 19, 20]. Diabetes was induced with alloxan monohydrate, since it is more economical and most widely used toxicant to induce diabetes in animal models. Consistent with available literature, rats are known to be more sensitive to gliclazide treatment and produced a biphasic response at 2 h and 8h in rat model when administered alone, which may be due to biliary excretion and enterohepatic cycling [12, 13, 20]. The biphasic effect was not seen in rabbit model where maximum blood glucose reduction was observed at 3 h time interval. This might be due to absence of enterohepatic recycling in rabbits. Gliclazide is known for its hypoglycemic activity by blocking K^+ channels in the pancreatic β-cells thereby stimulating insulin secretion and antihyperglycemic activity by increasing tissue uptake of glucose in normal and diabetic rats, respectively [21, 22]. Insulin levels were estimated at time intervals where peak reduction in percent blood glucose levels were observed both in rats (2 h and 8 h intervals) and in rabbits (3 h interval). The study revealed the influence of fucoxanthin on the pharmacodynamic activity of gliclazide alone and in combination using single and multiple dose treatments in rats and rabbits. The end points were evaluated in terms of glucose levels (% reduction), insulin levels, β-cell function and insulin resistance using homeostatic model assessment and pharmacokinetics of gliclazide in rabbits.

In the present study, no significant changes were observed in pharmacodynamics and pharmacokinetics of gliclazide following single dose administration with fucoxanthin. Multiple dose treatments with fucoxanthin significantly altered the pharmacodynamics of gliclazide in terms of percent blood glucose reduction, insulin levels, insulin resistance and β-cell function in rats (normal and diabetic) and in rabbits. The elevated insulin levels accompanied with high glucose levels suggest an insulin resistant state. However, in present study, glucose levels were decreased due to combined effects of gliclazide and fucoxanthin. Therefore, subtle changes in insulin resistance can be considered biologically insignificant. The disposition of gliclazide after single oral dose was examined in rabbits with and without prior exposure to fucoxanthin. Mean C_{max} value of gliclazide obtained at 3 h time interval can be correlated with peak glucose reduction and maximum insulin level linking the consistency of pharmacokinetic data with pharmacodynamic results in rabbits. This consistency is not altered by single dose treatment of fucoxanthin in rabbits. The serum gliclazide levels obtained in the study is comparable to previous studies in rabbits with similar dose [2, 12]. It is previously reported *in vitro* that intake of fucoxanthin over a period of time significantly inhibited hepatic metabolic enzymes [10]. Enzyme inhibition has important clinical importance when decreased drug metabolism results in increased drug concentrations, which leads to increase in substrate efficacy. It is also reported that fucoxanthin attenuated rifampin induced CYP3A4 and pregnane X receptor (PXR), a xenobiotic sensor resulted in inhibition of xenobiotic detoxifying enzymes and transporters [23]. Fucoxanthin upon multiple treatments altered the pharmacokinetics of gliclazide in rabbits with increased C_{max}, AUC and AUMC. Further, fucoxanthin did not alter the T_{max}, MRT, K_{el}, $T_{1/2}$ and CL of gliclazide

implying no effect at excretion process. Hence, the increased bioavailability of gliclazide in the presence of fucoxanthin might be due to improved absorption. It is also proved that in high fat diet induced obese mice, oral administration of fucoxanthin displayed decrease in glucose levels due to promotion of β3-adrenergic receptors in white adipose tissue and glucose transporter 4 mRNA in skeletal muscles. Further, it is reported that fucoxanthin has the property to supress pro-inflammatory mediators such as monocyte chemotactic protein-1 (MCP-1) and proliferator-activated receptor γ (PPAR- γ) in adipocytes, thus having anti-diabetic property [24]. Decreased glucose levels in animal models may be attributed to increased uptake of glucose by skeletal muscles or anti-inflammatory or enzyme inhibition properties of fucoxanthin. Pharmacokinetic data suggests that fucoxanthin inhibits drug metabolising enzymes that are responsible for the metabolism of gliclazide there by increasing the concentration as well as the effects of gliclazide. The changes in blood glucose levels, insulin levels in rats (normal and diabetic) and in rabbits might be due to enhanced effects of gliclazide in presence of fucoxanthin. Thus, this study confirmed that the interaction of fucoxanthin with gliclazide is more of pharmacokinetic in nature upon multiple dose treatments. Since the interaction is observed in two dissimilar species, it is also likely to occur in humans. Hence, this combination needs attention in dose adjustment and periodic monitoring of glucose levels when administered for their clinical benefit in obese patients. However, further studies are required to correlate the significance of these interactions in humans and to determine the exact mechanism of action behind this interaction, if any.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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