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PHARMACODYNAMIC HERB-DRUG INTERACTIONS: THE EFFECTS OF *AZADIRACHTA INDICA* LEAF EXTRACTS ON TWO COMMONLY USED SECOND GENERATION SULFONYLUREAS

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ABSTRACT

The co-administration of *A. indica* – an herb with reported hypoglycermic effect, with antidiabetic drugs is a common practice with little or no concern of possible herb-drug interactions that may affect patients' treatment outcomes. We Evaluated herb-drug interactions between *A. indica* and two second generation sulfonylureas- glibenclamide and glimepiride in streptozotocin induced diabetic animal model. Both immediate drug-extract interactions and 10 days administration of aqueous extract of *A. indica* prior to co-administration with the drugs were assessed. Diabetes was induced on the animals intraperitoneally using 70 mg/kg streptozotocin. Animals with fasting blood glucose > 160 mg/dl were considered diabetic and grouped for the study. The extract, glibenclamide and glimepiride produced progressive significant ($p < 0.05$) reductions in blood glucose when administered alone. Co-administration of the extract with glibenclamide produced antagonistic herb-drug interactions that started from 0.5 h of co-administration. Higher antagonistic interactions were recorded with 10 days pre-treatment with the extract prior the administration of the agents. Antagonistic interaction was also reproduced with the extract and glimepiride co-administration similar to that of glibenclamide. Result from this study established for the first time negative interactions between *A. indica* leave extract and the agents- glibenclamide and glimepiride and as such

provides scientific evidence to guide patients and health care providers. However, further investigation is required to elucidate possible mechanism(s) of interaction.

KEYWORDS: Antagonism; *Azadirachta indica* ; Glibenclamide; Glimepiride; Herb-drug interactions.

INTRODUCTION

There has been a growing trend in the use of herbs to combat several disease conditions both in developed and developing countries.[1] Due to the assumed safety of herbal products, it is a common practice especially among rural dwellers to often combine these agents with conventional drugs with resultant herb-drug interactions.[2] The fact that most patients do not disclose this practice of co-administration of drugs with herbs to their health care providers coupled with the fact that most of these health care providers are less informed of herbal-drug interactions and their potential risks raises lots of concerns.[3] Most times, the potential risks of drug-herbal interaction, even when known, are often ignored or underestimated.[4]

Glibenclamide and glimepiride are among the hypoglycermic agents of great interests in type 2 diabetes therapy belonging to the sulfonylurea family. They generally stimulate insulin release through inhibition of ATP-sensitive potassium channels in pancreatic beta cells and enhance beta cells sensitivity to glucose. They are highly protein bound (90-100 %) mainly to albumin and are completely metabolised by the liver making them vulnerable to drug interactions.[5] Meanwhile, most interaction studies involving glibenclamide and herbs revealed moderate to severe consequences.[2]

A. indica also known as neem tree is a tropical evergreen tree. Its numerous medicinal properties have made it a very important plant in the global context.[6] Numerous compounds have been isolated from various parts of the tree.[7, 8] Its antidiabetic potentials have been extensively studied and have been shown to ameliorate lesions of pancreatic islets and reduced hyperglycermia in streptozotocin induced diabetes.[9] Also inhibitions of α-amylase and α-glucosidase activities are part of its established mechanism of action.[10] As a result of its remarkable antidiabetic activity, it is usually combined with antidiabetic drugs with intention to boost efficacy.[11] Furthermore, opening of voltage-dependent Ca^{2+} channel and Ca^{2+} mobilisation represent a key step in sulfonylurea mediated hypoglycermic effect.[12] Therefore, alteration of this process by any agent co-administered with sulfonylureas would lead to pharmacodynamic interactions.

Despite reports of concurrent use of the aqueous leaf extract of *A. indica* with prescription oral hypoglycermic agents, there is little or no documented information regarding potential interactions which may arise from their combinations. To this end, we investigated the effects of co-administration of *A. indica* aqueous leaf extract on the hypoglycermic activities of two oral hypoglycermic agents, glibenclamide and glimepiride, using a diabetic animal model.

MATERIALS AND METHOD

Plant extract

Fresh leaves of *Azadirachta indica* A. Juss were collected from Agulu, Anambra State, Nigeria and were authenticated by Mr. Paulinus Ugwuozo of the Botany Department, Nnamdi Azikiwe University, Awka, Nigeria. The plant name has been checked with www.the plantlist.org and was found to be accepted name of a species in the genus *Azadirachta* (Family Meliaceae). This record was derived from WCSP (in review) (data supplied 0n 2012-03-23) which reported it as an accepted name with original publication details: Mem.Mus.Hist.19:221 1830. The leaf is generally known as Neem in English and as Dogo yaro in Nigeria. The leaves were dried at room temperature, pulverized and cold macerated with distilled water for 24hr. The extract was filtered with muslin cloth and freeze dried using lyophilizer.

Animals

Swiss albino rats (150 – 160 g) were obtained from the animal house of the Department of Pharmacology/Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka and were maintained in standard laboratory animal conditions. The animals were allowed free access to food and water *ad libitum* and were only deprived of food 12 hr prior to drug administration and during blood glucose monitoring. All animal experiments were conducted in line with the National Institutes of Health (NIH) Guide for Care and use of Laboratory Animals (Pub. No. 85. Revised 1985).

Induction of diabetes

After 12 hr fast, the animals were weighed and basal fasting blood glucose level determined from the tail vein using Accu-check® Glucometer. Diabetes was induced by intraperitoneal injection of 70 mg/kg streptozotocin dissolved in 0.1M sodium citrate buffer (pH 4.5). After 72 hours, animals with fasting blood glucose levels > 160 mg/dl were confirmed diabetic and selected for the study.

Experimental design

Seven groups of 5 diabetic rats each were used for the study. Groups 1 and 2 received single doses of 500 mg/kg of the extract and 5 mg/kg of glibenclamide respectively while group 3 received single co-administration of 500 mg/kg of the extract and 5 mg/kg of glibenclamide. Group 4 received 500 mg/kg of the extract for 10 days and co-administration of the extract and glibenclamide at the above stated doses on the 11^{th} day. Groups 5, 6 and 7 were treated as in groups 2, 3 and 4 respectively but, with replacement of glibenclamide with 2 mg/kg of glimepiride. After each treatment, blood glucose level was obtained at time intervals of 0.5, 1, 2 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours. Percentage blood glucose reduction was calculated as follows

$$\text{Percentage blood glucose reduction (\%)} = \frac{G_0 - G_t}{G_0}$$

Where G_0 and G_t are blood glucose levels at times 0 and t respectively.

Drug-herbal interaction was then determined from mean percentage blood glucose reduction using the equation

$$\frac{A}{AB} + \frac{B}{AB} = X$$

Where A represents drug, B represents extract and AB represents drug-extract combination.

When X = 1 ------ additive interaction

X > 1 ------antagonistic /negative interaction

X < 1 -------synergistic/positive interaction

Statistical analysis: Data obtained from the blood glucose concentration were subjected to one way ANOVA using SPSS 18.0. Post-hoc mean comparism using Turkey's test was also conducted using the same statistical tool. P values < 0.05 were considered significant.

RESULTS

Table 1 showed antihyperglycermic effects of various treatments against streptozotocin induced diabetic rats. Single dose administration of the extract (500 mg/kg) showed significant ($p<0.05$) progressive decrease in blood glucose from 30 minutes while glibenclamide treated rats started showing significant ($p<0.05$) reduction from 1 hour. Similarly, single dose of the extract with glibenclamide and the extract administered for 10

days followed by glibenclamide and the extract on the 11 th day also showed a progressive decrease in blood glucose from 30 minutes.

Table 1: Effect of extract, glibenclamide and their co-administration on blood glucose of diabetic rats.

Mean blood glucose (mg/dl)										
Groups	**0 h**	**0.5 h**	**2 h**	**4 h**	**6 h**	**8 h**	**10 h**	**16 h**	**20 h**	**24 h**
Extract	320.2 ± 2.4	*267.8 ± 2.5	*213.1 ± 1.9	*200.5 ± 4.2	*199.8 ± 5.6	*99.9 ± 1.7	*98.1 ± 2.6	*122.4 ± 3.3	*55.8 ± 1.1	*36.0 ± 0.8
Glibenclamide	289.8 ± 1.6	274.7 ± 2.8	*242.3 ± 3.1	*197.6 ± 2.8	*145.8 ± 2.4	*115.2 ± 2.2	*117.0 ± 1.9	*114.8 ± 1.5	*137.2 ± 1.8	*116.3 ± 1.6
Extract + Glibenclamide	336.6 ± 1.2	*274.1 ± 2.2	*253.8 ± 2.0	*231.1 ± 5.1	*176.8 ± 5.3	*69.3 ± 1.7	*112.5 ± 1.7	*157.3 ± 5.0	*62.1 ± 0.8	*76.7 ± 1.9
Extract 10 days + Glibenclamide	457.8 ± 1.0	*435.6 ± 1.1	*390.6 ± 0.7	*417.6 ± 0.7	*460.8 ± 2.8	*469.8 ± 2.9	*179.4 ± 1.3	*118.6 ± 0.9	*132.3 ± 1.7	*120.6 ± 2.4

** p < 0.05 compared with 0h*

However, antagonistic interactions were recorded in the extract-glibenclamide combination progressively from 30 minutes of administration in both immediate treatment and after 10 days treatment with the extract before co-administration therapy with glibenclamide (Table 2).

Table 2: Interactive effect of the extract on blood glucose reduction of glibenclamide

Blood glucose reduction (%)									
Groups	**0.5 h**	**2 h**	**4 h**	**6 h**	**8 h**	**10 h**	**16 h**	**20 h**	**24 h**
Extract	16.4	33.5	37.6	37.8	66.8	69.3	61.8	82.6	88.8
Glibenclamide	5.21	16.4	31.8	49.6	60.2	59.6	60.4	52.7	59.9
Extract + Glibenclamide	*18.6	*24.6	*31.3	*47.5	*79.4	*66.6	*53.3	*81.6	*77.2
Extract 10 days + glibenclamide	*4.8	*14.7	*8.78	*1.5	*0.6	*60.8	*74.1	*71.1	*73.7

** antagonistic effect/negative interaction*

The reduction in activity or negative interaction was more pronounced when the extract was given for 10 days before the combination with Glibenclamide on the 11th day. While the extract activity could be seen to wind-down after 12 hr, the glibenclimide alone and the single extract and glibenclamide co-administration had their activities wind-down after 8 hr each and the extract administered for 10 days followed by the drug on day 11 had maximal reduction activity at 3 hr. Just as seen with glibenclamide, glimepiride showed significant

(p<0.05) progressive reduction in blood glucose from 1 hour when compared with 0 hour (Table 3).

Table 3: Effect of extract, glimepiride and their co-administration on blood glucose of diabetic rats.

Mean blood glucose (mg/dl)										
Groups	**0 h**	**0.5 h**	**2 h**	**4 h**	**6 h**	**8 h**	**10 h**	**16 h**	**20 h**	**24 h**
Extract	320.2 ± 2.4	*267.8 ±2.5	*213.1 ±1.9	*200.5 ±4.2	*199.8 ± .6	*99.9 ± 1.7	*98.1 ± 2.6	*122.4 ±3.3	*55.8 ± 1.1	*36.0 ± 0.8
Glibenclamide	343.8 ± 2.7	348.8 ± 3.4	*296.3 ±2.9	*215.6 ±3.3	*192.2 ±3.2	*163.1 ±5.2	*165.6 ±3.3	*145.4 ±2.4	*118.4 ±1.2	*125.6 ± 4.4
Extract + Glibenclamide	279.9 ± 2.4	*319.9 ±3.1	*270.5 ±4.3	*265.5 ±5.3	*133.7 ±3.3	*113.4 ±2.8	*89.4 ± 2.4	*85.2 ± 2.6	*81.5 ± 1.7	*78.6 ± 2.1
Extract 10 days + Glibenclamide	363.6 ± 2.9	*394.2 ±1.7	*322.0 ±3.1	*298.8 ±2.6	*315.0 ±1.5	*334.8 ±1.8	*279.8 ±2.0	*182.8 ±2.4	*168.4 ±2.1	*157.3 ± 1.9

** p < 0.05 compared with 0h*

Though there was significant (p<0.05) reduction from 30 min in the extract glimepiride combination both after single dose combination therapy and 10 days administration of extract before combination, the interaction analysis revealed an antagonism in both combination therapy when compared with hypoglycermic effect produced separately by the extract and glimepiride (Table 4). This same effect was also observed for glibenclamide-extract combination therapies however, antagonistic effect started after 30 minutes of co-administration.. Similar trend of activity as seen with the glibenclamide was also observed with glimepiride. While glimepiride maximal reduction activity was seen at 8 hr, the extract and drug combination on day 11 had maximal activity at time 2 hr. However, unlike the former, the activity of the single combination activity had sustained activity until the 24th hour.

Table 4: Interactive effect of the extract on blood glucose reduction of glimepiride

Blood glucose reduction (%)									
Groups	**0.5 h**	**2 h**	**4 h**	**6 h**	**8 h**	**10 h**	**16 h**	**20 h**	**24 h**
Extract	16.4	33.5	37.6	37.8	66.8	69.3	61.8	82.6	88.8
Glibenclamide	-	13.8	37.3	44.1	52.6	51.2	57.7	65.6	63.5
Extract + Glibenclamide	-	*3.36	*5.1	*52.2	*59.5	*68.1	*69.6	*70.9	*71.9
Extract 10 days + glibenclamide	-	*11.4	*17.8	*13.4	*7.9	*23.0	*49.7	*53.7	*56.7

** antagonistic effect/negative interaction*

DISCUSSION

Concomitant intake of drugs and herbs is a common practice particularly in the management of chronic disease conditions like diabetes.[13] Considering plethora of compounds present in herbal medicines, drug-herbal interactions frequently occur when drugs are co-administered with herbs.[14]

The result of our current study indicated that concurrent administration of *A. Indica* with the sulphonylureas may adversely affect treatment outcome especially with prolonged administration of the herb and this may impair patient safety. Drug-herbal antagonistic interaction recorded in this study when *A. indica* is co-administered with glibenclamide or glimepiride may have partly been contributed to by extract linked alteration in Ca^{2+} channel and Ca^{2+} mobilisation. Ferulic acid has been identified as an inhibitor of voltage-dependent calcium channel and as well exhibit strong inhibition on Ca^{2+} release from intracellular stores and extracellular Ca^{2+} influx.[15] Ferulic acid have been identified through HPLC analysis to be abundant in the leave extract of *A. indica*[16] and may have altered Ca^{2+} channel mediated mechanism of glibenclamide and glimepiride.

Induction in the intestinal and hepatic metabolic enzymes particularly the CYP enzyme family, transporters and efflux proteins may also contribute to the antagonistic interaction between these sulfonylureas and *A. indica*. Glibenclamide and glimepiride have been shown to be metabolised by cytochrome P450 enzymes, mainly by CYP 2C9.[17] The induction of this enzyme by the extract may have contributed to the reduction in activities of the two hypoglycaemia agents when combined with the extract which also has a hypoglycaemic activity when administered alone. The decrease in time of maximal activity especially when the extract was administered for 10 days, followed by the administration of the extract and agents were indicative of enzymes inductive activity. CYP 3A4 may also play a minor role in the metabolism of these sulfonylureas.[18] This may further explain the antagonistic effects seen with the combinations of the drugs and the *A. Indica* extract. Meanwhile, *A. indica* has been established to induce phase II enzymes in mice liver.[19, 20] Considering that both the extract and the sulfonylureas used in this study exhibited hypoglycermic effect, antagonistic interaction observed is contrary to most reported additive to synergistic antidiabetics herbal-sulfonylurea interactions suggesting that the interaction affected the potency of both the extract and drugs.[2] Considering that sulfonylureas have high affinity for proteins[5] coupled with the abundance of tripeptide proteins in the leaves of *A. indica*,[21] Complexation reaction

between these proteins in the extract and the drugs may have contributed to the observed antagonistic effect. Alteration in absorption and interference in distribution pattern of both agents may therefore represent an added mechanism of action.

CONCLUSION

This study revealed herbal-drug interactions between *A. indica* and glibenclamide or glimepiride. The nature of the interaction was demonstrated to be antagonistic. There was a strong indication of enzyme induction by the extract – a strong pointer to further drug interactions even with other drugs that patients might be on – a subject for further investigation. Further studies are also required to elucidate mechanism(s) of interaction.

DISCLOSURES

The authors declare no conflict of interest. The authors alone are responsible for the funding, content and writing of this article.

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