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EFFECT OF ABC JUICE (MIRACLE DRINK) ON ORAL BIOAVAILABILITY OF METFORMIN IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

An oral hypoglycemic medication called Metformin (biguanide) is frequently prescribed to treat type 2 diabetes mellitus. It is a medication from BCS class III with high solubility and limited permeability. In this study, we looked at how ABC (Apple, Beetroot, and Carrot) juice affected the oral bioavailability of metformin (50–60%) as well as serum levels of HDL, cholesterol, and triglycerides. There were three groups of three rats each. Group II received normal saline solution for one week along with oral Metformin (70 mg/kg) on the seventh day of the research, while Group I simply received normal saline solution. On the seventh day of the research, Group III received oral Metformin (70 mg/kg) along with ABC juice for a week. It was discovered that the highest reduction in glucose levels in both Groups (II and III) occurred during the 6th hour in diabetic rats. It was discovered to be 23.18% in rats given Metformin and around 20.3% in rats given ABC juice as a pretreatment. The higher HDL values in group III compared to group II suggest that ABC juice can raise levels of the good cholesterol. However, when compared to rats given with Metformin alone during the research period, a significant benefit could not be seen in rats pretreated with ABC juice.

Keywords: Metformin, Diabetes mellitus, ABC Juice, Triglycerides, Bioavailability

INTRODUCTION

A metabolic condition known as diabetes mellitus (DM) is characterized by hyperglycemia, glycosuria, hyperlipidaemia, a negative nitrogen balance, and occasionally ketonemia. The chance of numerous consequences, such as retinopathy, kidney failure, nerve damage, circulation issues, heart disease, and stroke, is raised **[1]**. It is a serious, protracted condition with a high risk of death in which insulin, a pancreatic hormone, inappropriately controls the equilibrium of carbohydrate and lipid metabolism, raising blood sugar levels **[2]**. According to projections from the World Health Organization (WHO), more than 220 million people worldwide have diabetes, and by 2030, this figure is expected to be doubled.

Diabetes mellitus is also one of the most common, expensive chronic diseases that considerably shortens life expectancy (the projected lifetime risk of having diabetes for people born in 2000 is 32.8% for men and 38.5% for women). There were 151 million people worldwide who had DM in 2000, and it is anticipated that this number will rise by 46% by the years 2010 and 2025, to 221 million and 300 million, respectively **[3]**. Diabetes prevalence in the USA, which comes in third behind China and India, is predicted to increase at a much slower rate from 13.9 million in 1995 to 21.9 million in 2025 **[4]**. Almost all guidelines and recommendations around the world list metformin as the current first-line pharmaceutical treatment. It belongs to the biguanide class of anti-diabetic medications pharmacologically, with a bioavailability of 50–60%. The primary mechanism of action of metformin was based on the improved transport of glucose through skeletal muscle cell membrane. The most crucial method for producing hypoglycemic action, according to studies, was lowering hepatic gluconeogenesis **[5]**.

Bioenhancers are chemical entities which promote and augment the bioavailability of the drugs which are mixed with them and do not exhibit synergistic effect with the drug **[6]**. ABC juice (Apple Beetroot Carrot juice) has been known as the "Miracle Drink" because of all the amazing things it can do for our body and brain. Chinese herbalists introduced this potent beverage centuries ago for the treatment of lung cancer and numerous other ailments, the strength of three fruits and vegetables, each rich in many antioxidants that can prolong life. Literature reveals that the active chemical constituents present in apple (quercetin, Naringin), beetroot (BHC1) and carrot (β-carotene) act as bioenhancers. The objective of the current study was to evaluate the impact of ABC juice (Miracle drink) on the oral bioavailability of metformin in diabetic rats.

MATERIALS AND METHODS

Streptozotocin (SRL. Pharma Pvt Ltd. Hyderabad, India.), Metformin (Status label, Hyderabad, India), Dextrose (Research- lab fine chem industries, Mumbai, India.), ether (SD fine-chem. Ltd., Mumbai, India), Apple, beetroot and carrot (Ratnadeep, Hyderabad, India), Glucose estimation kit (Arkray healthcare Pvt. Ltd. Gujarat, India.), Cholesterol kit (Proton Biologicals India Pvt. Ltd, Bangalore, India), Triglycerides kit (Proton Biologicals India Pvt. Ltd, Bangalore, India) were purchased from their respective representatives.

Experimental

Maintenance of Experimental Animals

Male Wistar rats weighing between 180 and 250 g were bought from Mahaveera Enterprises in Hyderabad, India. Standard polypropylene cages were used to house the rats, which were kept in a room with a regulated temperature (20–22° C) and a 12-hour day/night cycle. Following a week of acclimation with unlimited access to food and water, animals were employed in investigations. The animal study protocol was reviewed and approved by Institutional Animal Ethics Committee (IAEC), Vaagdevi college of Pharmacy (1047/PO/Re/S/07/CPCSEA), Warangal, Telangana, India.

Experimental Induction of Diabetes Mellitus

There are numerous techniques documented for causing diabetes mellitus. Streptozotocin was selected among these to induce type 2 diabetes in the current investigation **[7]**. After a fast, diabetes was induced by injecting 55 mg/kg of STZ intraperitoneally (i.p.) in 0.1 M cold sodium citrate buffer (pH 4.5). The vehicle alone was given to the control rats. To reverse the drug-induced hypoglycemia, the animals were given access to 5% glucose solution to drink throughout the course of the night. After a week had passed since the onset of diabetes, rats with moderate diabetes who also had hyperglycemia (blood sugar levels above 250 mg/dl) and glycosuria were deemed diabetic and employed in the experiment.

Experimental Procedure

The rats were divided into three groups of three animals in each group (n=3) as follows.

Group I: Diabetic control rats

Group II: Administered with Metformin (50mg/kg b.w.) per orally suspended in normal saline, alone on 8^{th} day.

Group III: Pretreated with ABC juice perorally for seven days and on eight day the rats were administered with Metformin (50mg/kg b. w.) suspended in normal saline five min after administration of ABC juice.

Before the collection of blood samples, animals were fasted with water *ad libitum.* Blood samples were collected from retro-orbital vein puncture under diethyl ether anaesthesia at 0, 1, 2, 4, 6, 8, 10 and 12hrs.

Extraction of Serum Samples

100µl of serum was taken in a 2ml eppendroff's tube; to it added 100µl of acetonitrile to precipitate serum proteins and vortex mixed for 1min, centrifuged at 1300rpm for 15min.The supernatant was transferred into a clean, similarly labelled tube and was stored at -20ºC until use **[8]**.

RESULTS AND DISCUSSION

The serum glucose levels were estimated using GOD- POD method **[9].** The mean serum glucose levels of diabetic rats are shown in table 1. The data depicting the percentage reduction with respect to time are shown in fig.1. The data reveals that at 6th hr in diabetic rats, there is a maximum reduction of glucose levels in both Groups (II and III). It was found to be 23.18% in rats treated with Metformin and about 20.3% in rats pretreated with ABC juice. However, significant effect could not be observed amongst rats pretreated with ABC juice when compared to rats treated with Metformin alone during the study period.

Estimation of Body Weights

The rats in both the groups showed a gradual and significant($p<0.05$) increase in the body weights **(Table 2 and Figure2)**. The effect of ABC on the body weight of rats belonging to Group III was similar to that of Group II indicating that administration of ABC juice could not make a significant impact on the body weight.

Lipid Profile

The serum HDL levels were estimated using Phosphotungstate precipitation method **[10]** and Cholesterol by CHOD/PAP Trinder's method **[11]** and triglycerides by GPO-POP Trinder's method **[12]**. The levels of Cholesterol, Triglycerides and HDL levels are compared and given in **Table 3**. They were found to be normal in range in both the groups. However, HDL levels were found to be more in group III compared to group II indicating the ability of ABC juice to elevate good cholesterol levels.

Table 1: Mean serum glucose levels of Group I, Group II and Group III

Time (hrs)	Group I	Group II	Group III
0	402±11.2	427±7.54	418±8.2
1	399±8.57	384±14.1*	367±12.5
2	396±13.12	353±17.6	348±14*
4	397±9.05	340±13.2**	339±10
6	391±10.01	328±9.07	333±6.5
8	389±7.65	349±11.7	352±11.4*
10	397±12.06	359±6.65*	364±16.7
12	395±8.32	356±7	378±9.16

All values expressed as Mean ±S.D., n=3

*** p< 0.05 significant, ** p<0.01 highly significant, *** p<0.001 very highly significant**

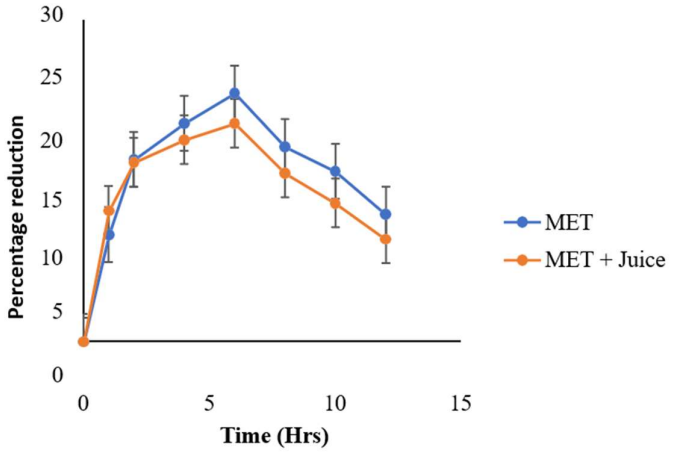

Figure 1: Percentage reduction of serum glucose levels

Table 2: Comparison of body weights

	Initial (gms)	Final (gms)
Metformin (Group II)	203±5.77	221±12.5*
Metformin + ABC juice (Group III)	176±25	185±25*

All values expressed as Mean ±S.D., n=3
*** p< 0.05 significant, ** p<0.01 highly significant, *** p<0.001 very highly significant**

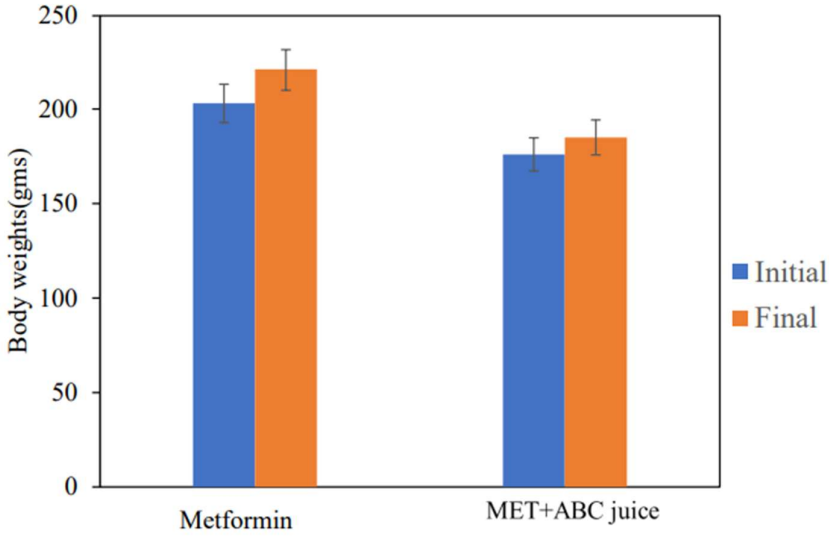

Figure 2: Comparison of body weights

Table 3: Comparison of Triglycerides, Cholesterol and HDL levels

	GROUP II	GROUP III
Cholesterol(mg/dL)	206± 2.2	196± 4.7*
Triglycerides(mg/dL)	131.6± 3.6	116.2± 2.8*
HDL (mg/dL)	40± 2.5	52± 2.5*

All values expressed as Mean ±S.D., n=3
*** p< 0.05 significant, ** p<0.01 highly significant, *** p<0.001 very highly significant**

CONCLUSION

ABC juice administration for a period of 1week did not affect the bioavailability of Metformin. There was no significant impact on the blood glucose levels and body weights in Streptozotocin induced diabetic rats and Serum Cholesterol, Triglycerides and HDL levels were found to be in normal range in all the animals. However, this work may be extended further by increasing the study period to understand better about the effect of the miracle drink.

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