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Combination drugs therapy delays diabetic induced cognitive impairment

Tenzin Tender[1]*, SR Mubeena[1]

[1] Al-Ameen College of Pharmacy, Rajiv Gandhi University of health Science, Bangalore, India

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ABSTRACT

Presently drugs that are available to treat the diabetic induced cognitive impairment presents adverse effect on long term used. This study offers evidence that old drugs can be combined and used. Existing and affordable drugs can be repurposed and can benefit patients .The study used a combination of drugs with proven safety for preventing/delaying the development of cognitive impairment in diabetes with a hope to improve the quality of life of diabetic patients.

In current study, diabetic rats were treated for eight weeks with combination of gamma linolenic ac-id (30mg/kg,p.o), alpha lipoic acid (30mg/kg,p.o), phloroglucinol (250mg/kg,p.o) l-thyroxine (1mg/kg, s.c) in one group and combination of of gamma linolenic acid (30mg/kg,p.o), alpha lipoic acid (30mg/kg,p.o), allantoin (200mg/kg,p.o), l-thyroxine (1mg/kg,s.c) in other group.The degree of preventative was determined by various parameters like body weight, measurement of neurotransmitter and calculating glycosylated haemoglobin and behavioural studies to check whether the combination therapy has positive impact on diabetic induced cognitive impairment.

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Keywords
Gamma linolenic acid,
Alpha lipoic acid,
Phloroglucino,
Allantoin,
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Corresponding to:
Tenzin Tender,
Al-Ameen College of Pharmacy,
Rajiv Gandhi University of health Science, Bangalore, India

Email:
tentenderla007@gmail.com

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INTRODUCTION

The number of diabetes patients increases year by year dramatically. Diabetes can induce many complications out of which cognitive impairment is one of the most deleterious and it accounts for 10-15% people with diabetes. Patients with diabetes can develop various complications at any stage of the disease. Generally, cognitive impairment due to diabetes is less acknowledge and lots of research is requiring to know correct pathogenesis and to develop ideal treatment for cognitive impairment. It has been reported that diabetes has close association with the reduced performance in numerous domains of cognitive function [1- 4]. One study suggested that diabetes patients have a 50-75% increased risk of developing Alzheimer's disease (AD) compared to non-diabetic patients [5]. It has been shown that impaired insulin signaling results in abnormal protein expression in the brain and cognitive decline [6, 7]. Unlike other complications due to diabetic, diabetic induced cognitive impairment can develop in any period. Unfortunately, the current treatment for diabetes induced cognitive impairment is symptomatic. Moreover, drugs use for the treatment has severe side effects. Hence, the need to develop drugs with minimum side effects and also able to prevent the diabetes induced cognitive impairment becomes essential. Therefore, in my present study effort was made to delay the diabetic induced cognitive impairment using combination of drugs. Although the exact pathogenesis for the diabetes induced cognitive impairment is not known but it is likely to be related with the defects in insulin signaling and functions in type I and II diabetes which leads to variety of abnormality in the body functions. Most probable the combination of drugs delays the diabetic induced cognitive impairment by inhibiting the initiation of polyol pathway, hexosamine pathway and formation of advanced glycation end product which in turn blocks increased in oxidative stress, pro-inflammatory gene expression, alter protein function and hence delayed or prevented the diabetic induced cognitive impairment in rats.

Levo -thyroxine is a synthetic thyroid hormone that is chemically identically to thyroxine (T_4) which is naturally secreted by the follicular cells of the thyroid gland. It has been reported that L-thyroxine provides neurotropic support and also has the action of regeneration of myelin sheath. In this experiment, racemic form of thyroxine is not used because dextro form is cardiotoxic in nature. Thyroid hormones (TH) [T4 (tetraiodothyronine) and T3 (triiodothyronine)], the only iodine containing compounds with biological activity, TH stimulate synthesis of Na+/K+ ATPase and also regulates metabolism by stimulating protein synthesis and increase the use of glucose and fatty acids for ATP production. They also increase lipolysis and enhance cholesterol excretion [8].

Phloroglucinol (1, 3, 5-trihydroxybenzene) is the monomeric building unit phlorotanins, phenolic compound known only brown algae (phaeophyceae). Phloroglucinol has been reported to have inhibitory activity in the formation of advanced glycation endproducts (ADEs) and also provides anti-hyperglycaemia and good anti-oxidant [9,10].

Allantoin (5-ureidohydantoin [2,5-dioxo-4-imidazolidinyl]urea): A urea hydantoin that is found in urine and plants. It is used in dermatological preparations. Allantoin have anti-diabetic effects by modulating antioxidant activities, lipid profile and by promoting release of glucagon like peptide (GLP-1), thereby improving the function of β-cells maintaining normal insulin and glucose level.

Allantoin is also found to increase nitric oxide (NO) levels [11].

α- lipoic acid is an organosulfur compound derived from octanoic acid. It is a cofactor essential in mitochondrial metabolism with anti-oxidant and anti- inflammatory activity. Lipoic acid has been shown to be effective in neuropathy pain treatment in patients with sciatica, carpal tunnel syndrome and diabetic neuropathy [12]. The mechanisms of action of ALA in experimental diabetic neuropathy include reduction of oxidative stress along with improvement in nerve blood flow, nerve conduction velocity, and several other measures of nerve function [13].

Gamma linolenic acid (GLA, all cis 6, 9, 12-Octadecatrienoic acid, C18:3, n-6), is produced in the body from linoleic acid (all cis 6,9-octadecadienoic acid), an essential fatty acid of omega-6 series by the enzyme delta-6-desaturase.It is found primarily in vegetable oils.

The most significant source of GLA for infants is breast milk. GLA is further metabolised to dihomogamma linlenic acid (DGLA) which undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoid. GLA and its metabolites also affect expression of various genes where by regulating the levels of gene products including matrix proteins. These gene products play a significant role in immune functions and also in cell death (apoptosis) [14].

MATERIALS AND METHODS

Animals

Experimental animals, adult male Wistar rats (n=5) weighing between 200-250 g were included for the study. All rats were maintained under standard housing condition at controlled temperature at 25°C ± 2°C with 12 hr light/dark cycle with food and water provided standard rat diet and water ad labitum. Animals which did not comply with above criteria, and which were found to be diseased were excluded from the study. After one-week adaptation period, the healthy animals were used for the study. All the protocols were approved by Institutional Animal Ethical Committee. IAEC NO: AACP/P-48, India.

Experimental design

Twenthy rats were randomly divided into four groups. Group I served as normal control group. Group II, Group III and IV were induced diabetic rats and included in the study as experimental rats. Group II served as diabetic control group whereas Group III received gamma linolenic acid, alpha lipoic acid, phloroglucinol, l-thyroxine and GroupIV received gamma linolenic acid, alpha lipoic acid,allantoin,l-thyroxine daily for eight weeks. Treatment was started after diabetic was confirmed in rats. Rats were also administered insulin (3IU/day, s.c.) [15] for the complete period of the study. After 8 weeks, initial and terminal body weight, behavioural and biochemical parameters were determined to evaluate the severity of diabetic induced cognitive impairment in treated group as compare to normal and diabetic control rats.

Assessment of body weight

To assess the general condition of animals, they were examined daily for clinical signs such as alopecia, piloerection or hind limb weakness and mortality. Body weight was measured using digital balance (Essae® DS-252). Loss of body weight was compared between body weight measured at the beginning and at the end of the study.

Behavioural parameters

Motor co-ordination test and Barnes maze test were done according to published papers [16,17].

Biochemical parameters

Measurement of glycosylated hemoglobin (GHb) [18], brain neurotransmitters like dopamine, noradrenaline, serotonin. Gamma amino butyric acid and acetylcholinesterase were done according to published papers [19-24].

Statistical analysis

Statistical evaluations were done by ANOVA, expressed as mean ± S.E.M. followed by Tukey's multiple comparison test using Graph Pad Prism 5 computer program. $P<0.05$ was considered statistically significant.

RESULTS

Assessment of body weight

The percentage change in body weight of normal and diabetic rats at 8th week was found to be 17.72±1.66g and -24.53±2.706g. The body weight of diabetic rats was significantly reduced (P<0.001) as compared to normal control, similarly the change of body weight of diabetic treated with comb.1 and comb.2 was found to be 9.182±0.915g and -7.676±0.927g which significantly improved as compared to diabetic control rats (Fig. 1).

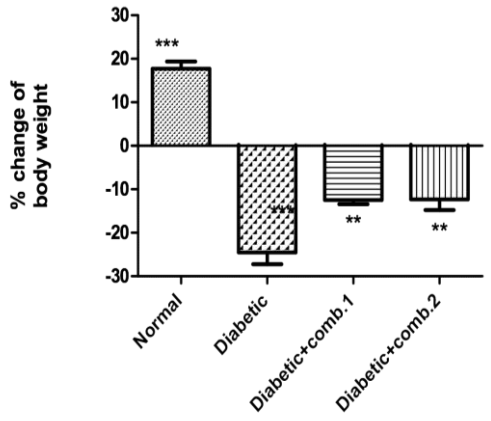

Figure 1. Combination therapy effect of the target drugs for eight weeks on % body weight change in diabetic rats. Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1,gamma linolenic acid,alpha lipoic acid, phloroglucinol, l-thyroxine; Comb.2,gamma linolenic acid,alpha lipoic acid,allantoin,l-thyroxine.

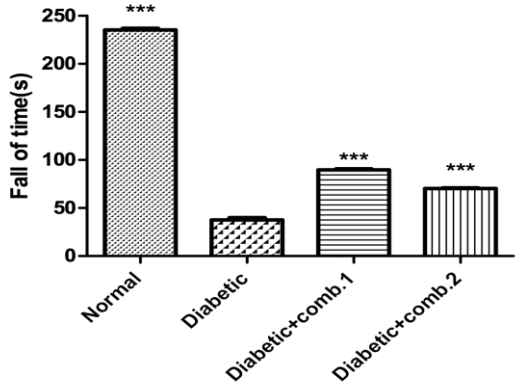

Figure 2. Combination therapy effect of the target drugs for eight weeks on muscle incoordination by rota rod performance in diabetic rats..Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group.Comb.1,gamma linolenic acid,alpha lipoic acid, phloroglucinol,l-thyroxine; Comb.2, gamma linolenic acid,alpha lipoic acid,allantoin, l-thyroxine.

Behavioural studies

Measurement of motor coordination using rota rod: Fall of time at 15 rpm of normal and diabetic rats was found to be 235.3±1.753 and 37.58±2.343 secs respectively, and the latency of diabetic rats was significantly reduced (P<0.001) as compared to normal control. Latency in diabetic rats treated with comb.1 and comb.2 was found to be 89.65±1.088 secs and 70.24±0.734secs and same was significantly P<0.001 improved when compared to diabetic control rats (Fig. 2).

Measurement of spatial memory and learning using Barnes maze: Time taken to enter escape cage by normal and diabetes rats was found to be 15.59±0.51 and 52.20±1.58 secs respectively, and the latency of diabetes rats was significantly increased (P<0.001) as compared to normal control. Latency in diabetic rats treated with comb.1 and comb.2 was found to be 24.50±0.77 secs and 28.61±1.34 secs and same was significantly P<0.001 improved when compared to diabetic control rats (Fig. 3).

Biochemical studies

Estimation of % glycosylated haemoglobin (GHB): The percentage GHb of normal and diabetic rats was found to be 1.667±0.1476 and 13.43±1.198 and same was significantly increased (P<0.001) as compared to normal control. The percentage GHb of diabetic rats treated with comb.1 and comb.2 was found to be 10.15±0.360 and 10.08±0.359 and same were significantly improved. P<0.001 when compared with diabetic control rats (Fig. 4).

Estimation of dopamine: The dopamine content in brain of normal and diabetic rats was found to be 46.9±1.005 ng/dl and 71.7±0.88 ng/dl respectively and the dopamine level of diabetic rats was significantly increased (P<0.001) as compared to normal control rats. Dopamine level in diabetic rats treated with comb.1 and comb.2 was found to be 60±0.707

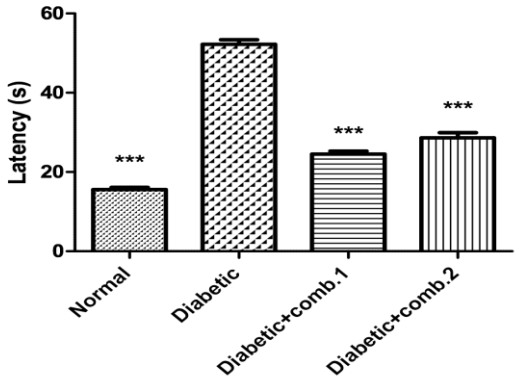

Figure 3. Combination therapy effect of the target drugs for eight weeks on spatial learning and memory by Barne maze in diabetic rats..Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1,gamma linolenic acid,alpha lipoic acid, phloroglucinol,l-thyroxine; Comb.2,gamma linolenic acid,alpha lipoic acid,allantoin,l-thyroxine.

ng/dl and 62.56±0.506 ng/dl and same was significantly increased (P<0.001) when compared to diabetes control rats (Fig. 5).

Estimation of nor-adrenaline: The nor-adrenaline level in brain of normal and diabetic rats was found to be 17.30±0.66 ng/dl and 5.060±0.31ng/dl respectively and the nor-adrenaline level of diabetic rats was significantly reduced (P<0.001) as compared to normal control rats. Nor-adrenaline level in diabetic rats treated with comb.1 and comb.2 was found to be 12.14±0.66 ng/dl and 12.76±0.522 ng/dl and same was significantly increased when compared to diabetes control rats (Fig. 6).

Estimation of serotonin: The serotonin content in brain of normal and diabetic rats was found to be 2.64±0.19 ng/dl and 8.02±0.33 ng/dl respectively and the serotonin level of diabetic rats was significantly increased (P<0.001) as compared to normal control rats. Serotonin level in diabetic rats treated with comb.1 and comb.2 was found to be 3.960±0.12 ng/dl and 3.8±0.158 ng/dl and same was significantly reduced/(P<0.001) when compared to diabetes control rats (Fig. 7).

Estimation of gamma amino butyric acid(GABA): The GABA level in brain of normal and diabetic rats was found to be 7.94±0.37 ng/dl and 2.84±0.143 ng/dl respectively and the GABA level of diabetic rats was significantly reduced (P<0.001) as compared to normal control rats. GABA level in diabetic rats treated with comb.1 and comb.2 was found to be 4.060±0.147 ng/dl and 4.6±0.216 ng/dl and same was significantly increased when compared to diabetes control rats (Fig. 8).

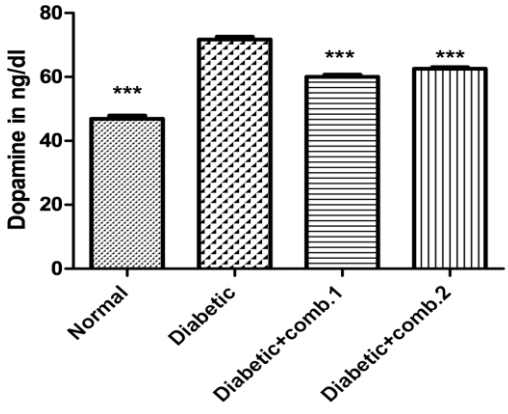

Figure 5. Combination therapy effect of the target drugs for eight weeks on brain dopamine level in diabetic rats.Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1,gamma linolenic acid,alpha lipoic acid, phloroglucinol,l-thyroxine; Comb.2,gamma linolenic acid,alpha lipoic acid,allantoin,l-thyroxine

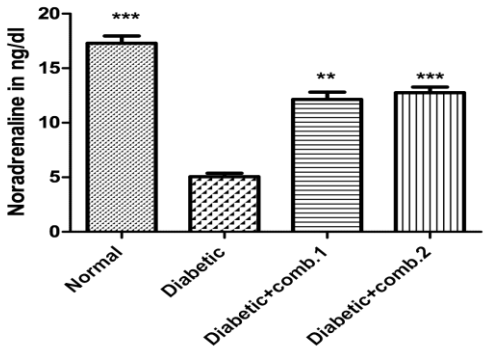

Figure 6. Combination therapy effect of the target drugs for eight weeks on brain nor-adrenaline level in diabetic rats.Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001,**P<0.01 Vs diabetic control group. Comb.1,gamma linolenic acid,alpha lipoic acid, phloroglucinol; l-thyroxine; Comb.2,gamma linolenic acid,alpha lipoic acid,allantoin,l-thyroxine.

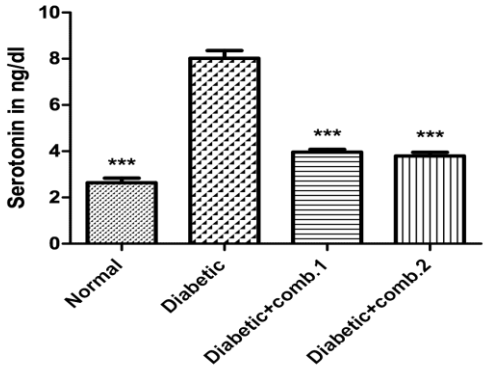

Figure 7. Combination therapy effect of the target drugs for eight weeks on brain serotonin level in diabetic rats. Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group.Comb.1,gamma linolenic acid,alpha lipoic acid, phloroglucinol,l-thyroxine; Comb.2,gamma linolenic acid,alpha lipoic acid,allantoin, l-thyroxine.

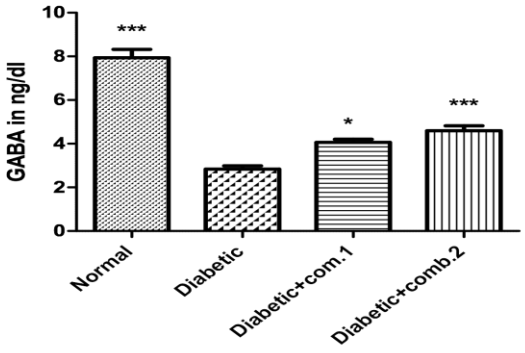

Figure 8. Combination therapy effect of the target drugs for eight weeks on brain GABA level in diabetic rats. Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001,*P<0.05Vs diabetic control group.Comb.1,gamma linolenic acid,alpha lipoic acid, phloroglucinol,l-thyroxine; Comb.2,gamma linolenic acid,alpha lipoic acid,allantoin,l-thyroxine.

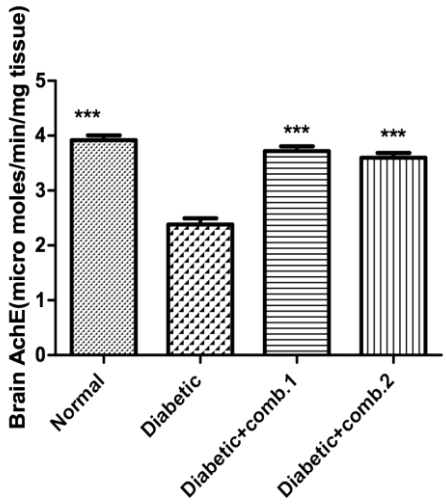

Figure 9. Combination therapy effect of the target drugs for eight weeks on brain AchE level in diabetic rats. Values are represented as mean ± SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001, P<0.05Vs diabetic control group. Comb.1, gammalinolenic acid, alpha lipoic acid, phloroglucinol, l-thyroxine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, l-thyroxine.

Estimation of acetyl cholinesterase (AchE): The Ache level in brain of normal and diabetic rats was found to be 3.920±0.086µmoles/min/mg and 2.38±0.1 µmoles/min/mg respectively and the AchE level of diabetic rats was significantly reduced (P<0.001) as compared to normal control rats. AchE level in diabetic rats treated with comb.1 and comb.2 was found to be 3.720±0.086 µmoles/min/mg and 3.6±0.083 µmoles/min/mg and same was significantly increased when compared to diabetes control rats (Fig. 9).

DISCUSSION

Diabetic induced cognitive impairment is severe yet it is one of the most neglected complications of diabetes by researchers. Over the past years researchers tried to develop treatment for diabetes induced cognitive impairment but unfortunately they failed to develop the therapy for cognitive impairment.

The combination of drugs evaluated included combination 1 containing gamma linolenic acid, alpha lipoic acid, phloroglucinol, l-thyroxine and combination 2 containing gamma linolenic acid, alpha lipoic acid, allantoin, l-thyroxine were used and shown positive results.

Diabetes was induced by administration of STZ (52mg/kg,i.p).We have observed a significant reduction in the body weight of the diabetic rats as compare to the normal control group. Reduction in the body weight in type 1 diabetes can be due to muscle wasting and the process causes severe damage to the energy reserves in the rats and decreases the body weight as confirmed in our present study.

In the present study diabetic control rats showed a significant reduction in falling latency on rota rod apparatus. The severity of diabetic neuropathy has been associated with decreased muscle co-ordination and strength in both type 1 and type 2 diabetes and also weak grip strength in grip strength meter indicating poor motor/muscle activity. It was also seen that there was significantly increased in latency to locate hole in Barne maze apparatus in diabetic rats when compared to treated rats.

Marked increase in percentage of GHb has been reported in previous studies in diabetic rats. Increased GHb has been implicated in various diabetic microvascular complications like neuropathy, nephropathy, retinopathy etc. Previous studies revealed the role of glycosylation leading to the formation of oxygen-derived free radicals in diabetes mellitus and its level can be considered as one of the important marker of oxidative stress. Therefore, in the present study we have measured the level of GHb as a marker of diabetes induced oxidative stress. Our results showed that the level of GHb in diabetic rats was significantly higher as compared to the normal rats.

Proper levels of neurotransmitters are very crucial for proper functioning of brain. Impaired neurotransmitters levels in brain may result in inadequate functioning of brain which will give rise to several complications. In my present study, neurotransmitters levels were measured in whole part as well as some specific parts of rat's brain. Whole brain was isolated for the measurement of dopamine, nor-adrenaline and serotonin whereas cortex part of brain was isolated for the measurement of GABA and thalamus region of brain for the measurement of AchE. It was found that the levels of dopamine, serotonin were significantly increased in diabetic rats when compared with treated rats. However, the levels of nor-adrenaline, GABA and AchE were significantly reduced in diabetic rats when compared with treated rats. We can state that the combination therapy resulted in increased levels of nor-adrenaline, GABA and AchE whereas combina-

tion therapy caused reduction in levels of dopamine, serotonin in diabetic rats.

CONCLUSION

We can state that the combination therapy has shown protective effects in delaying the diabetic induced cognitive impairment and between the combination 1 and combination 2 there is no significant differences to protect the cognitive impairment in diabetic rats. However, we can conclude that the combination 2 which is gamma linolenic acid, alpha lipoic acid, allantoin, l-thyroxine proved to be better combination therapy for the cognitive impairment due to diabetics.

CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article

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