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Evaluation of oral hypoglycemic potency of *Medicago polymorpha* and *Zygophyllum simplex*: A Drug – Drug interaction study

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Abstract
Objective: To identify a natural source of oral hypoglycemic agents outlining the herbal importance in modern day medicinal practice.
Materials and Methods: '*Medicago Polymorpha*' and '*Zygophyllum Simplex'* were selected as crude drugs from which the extracts shall be prepared and tested in male albino mice to determine the oral hypoglycemic potency.
Statistical Analysis used: Descriptive statistics were obtained through ANOVA and employed *Unpaired t test* as post-test, n=5 animals per group and results were expressed as Mean ± SEM, P* < 0.05 is considered as significant in the results
Results: the chosen agents failed to produce the intended effect in mice which were prior treated with alloxan as diabetes inducing agent. Event their combination to study the drug – drug interaction also failed to impress through results.
Discussion: neither individually nor in combination the drugs have got any marked medicinal importance. The in the future use of this medication may be justified less potent in the management of oral hypoglycemia through the natural agents.
Conclusion: the study identified the drugs to be less potent in the management of laboratory induced hyperglycemia in mice. If at all these agents are preferred as additives in the management of the hyperglycemia, a thorough study must be carried out before the usage of the crude drugs a therapeutic agents.

Keywords: Medicago Polymorpha, Zygophyllum Simplex, Hypoglycemia, Drug – Drug Interactions

Introduction
Diabetes Mellitus (DM) is a chronic disorder associated with increased blood glucose levels resulting in persistent hyperglycemia, if the hyperglycemia goes uncontrolled it may lead to secondary complications like diabetic foot, nephropathy, neuropathy, retinopathy etc [1]. The prevalence rate of DM is increasing throughout the world day by day, highly depending on the facts like changes as more of a sedentary lifestyle, different food habits including fast foods, and on the other hand the financial expenses required to manage the medical condition makes it as one of the worst nightmare of the 21st century globally [1].
The latest estimates show that 382 million people were identified to be suffering from DM globally in 2013, and this figure is expected to rise to 592 million by 2035 equally increasing physiological, psychological and economical burden on the mankind with life challenging consequences [2].
In case of Saudi Arabia, in the year 2000 an independent research published by *Al-Nozha et al*. Explains that about 23.7% out of 17,232 study participants were identified to be suffering with DM, out of which 26.2% were males and 21.5% were females [3]. later, in the year 2009, a cross sectional study performed by *Khalid A et al*. explored that 30% of the study participants out of 6024 subjects were identified to be suffering with DM, and also the study explains that there has been a steep increase in the prevalence of DM in males and females with 34.1% and 27.6%. and the reason of concern was identified to obesity which was more in females (87.7%) than males (83.1%) [4].
Saudi Arabia has a huge landscape approximately 90 – 95% of which is covered with desert. Many medicinally important plants might have missed or gone extinct before validated documentation of their medicinal importance or on the other hand plants with potential oral hypoglycemic strength is awaiting to be discovered in the far lands out of reach of common man. As per the study published by '*Yassin et.al.'* nearly 165 (97%) out of 170 of the recorded plants of Saudi Arabia possesses medicinal or economic benefits [5].

Hence, two such species namely '*Medicago polymorpha*' and '*Zygophyllum simplex'* have been selected for screening oral hypoglycemic potency against alloxan-induced DM in experimental animals and their possible drug – drug interactions with the following objectives;

- Planning to find a pharmacologically active natural product which can be used as a safe drug with little side effects for controlling elevated blood glucose levels
- Investigation of pharmacological active medicinal plants commonly grows in KSA.
- Comparative study of pharmacological activity between the above mentioned different medicinal plants.
- To highlight the importance of herbal medicinal use in the 21st century.

Materials and Methods

Plant Material

Both the plants '*Medicago Polymorpha*' and '*Zygophyllum Simplex'* was collected from their natural habitat grown around the outskirts of Jeddah, KSA, The specimen authentification of the plant material was done by Dr Samah Shabana, Pharmacognosist and a sample was preserved in the Department of Pharmacy of Batterjee Medical College, North Obhur, Jeddah, KSA for future reference.

Preparation of extracts

The whole plant material was shade dried with a view to preventing any possible loss of active ingredients under sunlight. Crude extracts were made by macerating 60g of each powder in 70% methanol and the macerated samples were filtered and the filtered samples were concentrated to dry in a rotary evaporator under reduced pressure, then the methanolic extracts were weighed and dissolved in 70% of ethanol in water to make conc. of 10mg/ml and 5mg/ml and followed by Ethanolic extracts were diluted to get the concentration of 1 mg/ml by using distilled water.

Test animals

About 30 matured male albino mice weighing 20 - 30 gms were divided into six groups bearing five animals in each group. The animals selected for the study were acclimatized to the laboratory conditions for a week earlier to the beginning of the research. During this period the animals were given food and water adequately. Constant room temperature around 27 ± 1°C was maintained with 12/12 hrs dark and light cycle. The animal experiments were approved by the ethics committee of the institute before the beginning of the study.

Experimental part

Before the experiment, all the animals were deprived of rat food pellets for 18 hours where as water was allowed with no stoppage.

The sample size of the animals was calculated based on the "resource equation method", where [18]

E = [Number of groups – Number of animals]

E stands for required number of animals,

Number of groups are depended up on the study design, and

Number of animals are depended up on the study requirements.

Then the mice were randomly separated into six groups each group consists of five animals and the groups were named as: *Normal control*, *Diabetic control*, *Standard* (*Gliclazide*), *Medicago* extract treated, *Zygophyllum* extract treated, *Medicago* + *Zygophyllum* extract treated. All the groups except the *Normal control* were administered with *Alloxan* at a dose of 140mg/kg body weight in two divided doses and 28 days later the animals have shown increased blood glucose levels (Diabetic condition). Further, one group is separated and kept as it is and does not receive any treatment (Diabetic control) and out of five groups one group receives Standard (*Gliclazide*) and the four groups receive Ethanolic extracts as mentioned below in the schematic format [1, 6].

After the induction of diabetes, animals were administered with a calculated amount of drugs i.e. *Gliclazide* (6 mg/kg per oral), Ethanolic extract of *Medicago ethanolic extract* (25 mg/kg) *and Zygophyllum ethanolic extract* (25 mg/kg) respectively. Blood was withdrawn from the animals after the administration of the drug at particular intervals, and blood glucose was estimated by using advanced glucometer method [1, 6]. the doses of the extracts were calculated based on the results of the LD_{50} studies and the standard drug's dose was calculated basing up on the standard references [7] and the doses in detail are explained in the scheme of groups division.

The scheme of group division, drug administration with doses is given below,

Group I - Served as normal control and did not receive any treatment.

Group II - Alloxan; Diabetic control

Group III - *Alloxan* + *Gliclazide* (6 mg/kg, p.o.) and served as the Standard group.

Group IV - *Alloxan* + *Medicago ethanolic extract* (25 mg/kg, p.o.).

Group V - *Alloxan* + *Zygophyllum ethanolic extract* (25 mg/kg, p.o.).

Group VI - *Alloxan* +*Medicago ethanolic extract* + *Zygophyllum ethanolic extract* (25 mg/kg, p.o each.).

Results

The study was designed to evaluate the use of two medicinally important plants found in the Saudi Arabia. The plants were tested against the alloxan-induced diabetes in mice. Both the extracts treated groups failed to produce any action against the elevated blood glucose levels, even when the drugs were combined and administered the result was no different from the two other groups where the drugs were administered individually. The results with higher significance are considered when the test drug alters the test parameters (Blood glucose levels) to the lowest level throughout the diabetic state, and in all the groups this effect was lacking. The significance of the results was noticed when they were matched against the parameters of normal animals but not against the diseased ones. [Table 1]

The drugs were identified to be missing the pharmacokinetic and Pharmacodynamic action and interaction. However, the results of the study may not be beneficial for the clinical application, but may be employed in the prevention of possible medicinal misuse of the drugs in any case.

Table 1: The effect of drug's extracts on the mice's Blood glucose levels

Number of weeks	Normal Group	Diabetic Group	*Medicago* extract treated group	*Zygophyllum* extract treated group	*Medicago* + *Zygophyllum* extract group	P values	
						Against normal group	Against Diabetic group
1st	122±6.8	590±28.1	480±28.7	440±27.8	480±26.5	0.0026*	0.735
2nd	100±7.2	560±32.4	488±21.6	560±22.1	510±22.1	0.0019*	0.865
3rd	118±9.2	550±40.7	540±24.6	428±18.7	512±27.1	0.0015*	0.726
4th	119±10.2	520±27.6	420±20.7	510±15.2	498±27.1	0.0028*	0.728
Units	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl		

Descriptive statistics were obtained through ANOVA and employed *Unpaired t test* as post-test, n=5 animals per group and results were expressed as Mean ± SEM, P* < 0.05 is considered as significant in the results.

Discussion

The Saudi Arabia bears a wide variety of natural flora which is yet to be explored for their potential against various disorders suffered by mankind. Though a good part of the work has been done on the variety of plants present in the region, yet many of the medicinally potent species might have missed from the research's eye, which is still waiting to be explored for the betterment of the mankind [8, 9]. On the other hand, the treatment of the diseases is becoming costly day by day throughout the world either because of the under availability or the misuse of the drug [10]. In this regards, there is an increasing demand for the development of the newer agents which are easily applicable in the management of chronic disorders with least economic and therapeutic burden. Hence, the current study was designed to evaluate the use of '*Medicago Polymorpha*' and '*Zygophyllum Simplex'* in alloxan induced diabetes in albino mice under laboratory conditions along with their *in-vivo* drug interaction study.

Basing up on the personnel experience, Alloxan at a dose of 140 mg/kg body weight was administered in two divided doses i.e. *100 mg/kg per oral* on the first day of the first week and *40 mg/kg per oral* on the first day of the second week, the pattern of the administration was chosen to avoid any fatality towards mice as alloxan at a dose of 140 mg/kg was identified to be producing fatal results in animals.

There is a strong relationship between obesity and development of the *Diabetes Mellitus* [12]. About this one of the primary challenges for the diabetic patient is to reduce the body weight. *Zygophyllum Simplex* is a natural remedy identified to be helpful in the weight reduction of the oversized animal, [9] and *Medicago Polymorpha* is a rich source of minerals [11] which shall be used as a supplemented in an individual who is willing to undergo the process of weight reduction without lowering the basic mineral content of the body and this was idea behind current the study i.e. if both the drugs are combined to manage the excess glucose levels without losing out the essential mineral contents in a diabetes-induced mice.

The individual results of the drugs expressed no effect on the hyperglycemic state of mice, even the combination of the two drugs also failed to produce the beneficial effect rather it worsens the condition of the mice. As the mice were treated with the extracts of the two plants and the hyperglycemia was not getting treated the change in the behavior patterns of the animals were identified like, decrease in the activity of the animal in the cage, social isolation, behaving aggressively sometimes, with this results it may be said that both the drugs either individually or in combination have got neither preventive nor protective effect on biochemical parameters during the hyperglycemic state resembling the one produced during the absence of insulin release. However, the research identifies no benefit of the plant products to produce the expected results rather it ended having neither pharmacokinetic nor pharmacodynamic interaction.

The concept of reporting the plants with no expected results is a good concept, and various manuscripts have been published like *Yuttana Sudjaroen et.al.* explains that *'Suaeda Maritima'* lacks anti-cancer and anti-microbial activity [13] in an another work published explores that seven of the plants *'Caesalpina Bahamensis, Hypericum Styphelioides, 'Hypericum limosum', 'Vaccinium Leonis', 'Vaccinium Ramonii', 'Agdestis Clematidea'* included in the study failed to express antimicrobial activity, [14] *'Mormodica Charantia'* has shown low potency as *α- Glucosidase and Xanthine Oxidase* inhibitor [15]. '*Medicago Polymorpha*' and '*Zygophyllum Simplex'* may also add on to the list as plants which lack oral hypoglycemic potency.

As in case, various pharmacognostic preparations have been reported to be unhealthy and toxic, [16] the combination of '*Medicago Polymorpha*' and '*Zygophyllum Simplex'* for getting the therapeutic benefits is not only inefficacious but also non-toxic, the use of such preparations is not recommended for clinical application.

Knowledge of plants lacking the therapeutic potency helps the prescriber and the user in many was as both mutually spend lot of time together during the diagnosis and prescribing [17]. various people throughout world have a common feeling that the natural products heal the ailments in a safer manner when compared to the synthetic one and using such agents without proper research support may cause fatal unwanted effects, as identified in the current research that both the plants employed in the research lacks oral hypo glycemic potency and consider if anyone still opts for using these plants in formulation of a product not only decreases the efficacy of the preparation but also may impose unnecessary economic burden on patient by forcing them to use spurious, adulterated and less efficacious preparations [10]. Such situations can be avoided by reporting the non-beneficial outcomes of the research.

Conclusion

The findings from the current study may explain that the usage of either the individual or the combination of '*Medicago Polymorpha*' and '*Zygophyllum Simplex'* lacks the potency as an oral hypoglycemic agent. Their use either individually or in combination may not be beneficial. However, a thorough research by separating the individual fractions may yield appropriate results in future.

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