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RESEARCH ARTICLE

A COMPARATIVE IN VIVO STUDY OF ANTIDIABETIC POTENTIALITY AND EVALUATION OF SAFETY PROFILE OF GYNURA PROCUMBENS, TERMINALIA CHEBULA AND FICUS RACEMOSA ON ALLOXAN-INDUCED DIABETIC RATS

Zahirul Islam[1], Md. Naeem Hossain Rony[2], Farhana Farin[3], Amatun Noor Prapty[1], Md. Mehedi Hasan Khandoker[1], Sanjida Jannath[1], Tahmina Sharif Nila[1], Jannatul Nayeem[4] and Fatema-Tuz-Zohra[1]

1. Department of Pharmacy, University of Asia Pacific, Farmgate, Dhaka, Bangladesh.
2. BRAC University, 66 Mohakhali, Dhaka-1212, Bangladesh.
3. Department of Pharmacy, East West University, Aftabnagar, Dhaka, Bangladesh.
4. National Medical College, Dhaka, Bangladesh.

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Abstract

Leaves of *Gynura procumbens*, seeds of *Terminalia chebula* and fruits of *Ficus racemosa* are the eminent plant sources that are very commonly used. These are consumed against diabetes for long while. Diabetes was induced in rats by intraperitoneal injection of alloxan at a dose of 150mg/kg bodyweight and ethanolic extract of leaves of *Gynura procumbens*, seeds of *Terminalia chebula* and fruits of *Ficus racemosa* has foraged to the rats at a dose of 750 mg/kg, 800 mg/kg and 1000 mg/kg respectively. We determined blood glucose level, and safety profile by determining SGOT, SGPT and creatinine levels on diabetic and non-diabetic rats before and after administration of the extract. After determining blood glucose level, it was detected that the hypoglycemic efficiency was analogous to that of metformin ($p > 0.05$) which was imparted at a dose of 500 mg/kg. The safety profile was probed by inspecting SGOT, SGPT and creatinine level. It was discerned that *Gynura procumbens* leaf extracts, seeds of *Terminalia chebula* and fruits of *Ficus racemosa* provide similar but to some extent lower effect than metformin with barren statistical significance. In addition to that, in healthy individual rats both metformin and leaf extract of *Gynura procumbens* did not substantially alter conventional physiological state. It can, hence, be conjectured that extract of leaves of *Gynura procumbens* could be exploited as a good alternative therapy to treat diabetes.

Introduction:-

Diabetes mellitus is a significant health problem in developed and developing countries. It is ranked seventh among the leading foremost of death, and third when its disastrous intricacies are taken into consideration[1]. Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities or as simple dietary adjuncts to available therapies. Herbal treatments are turning out to be progressively prevalent as the herbal preparations have no or slightest side effects[2].

Corresponding Author:- Zahirul Islam.
Address:- Department of Pharmacy, University of Asia Pacific, Farmgate, Dhaka, Bangladesh.

Gynura procumbens (GP) belonging to the family Asteraceae is commonly known as longevity spinach that grows extensively in India, Bangladesh, Southeast Asia, particularly in Indonesia, Malaysia and Thailand. Some of these traditional claims have been validated in scientific and pharmacological studies, including anti-herpes virus [3], anti-inflammatory [4, 5] and anti-hyperlipidemic and anti-hyperglycemic [6-11], anti-hypertensive activities.[12-16] Recently received particular attention of GP is in the pharmacology of anti-diabetic medicinal plants perhaps because of its empirical evidence and efficiency in the management of diabetes mellitus.

Terminalia chebula (TC) Retz., is a member of family Combretaceae. It is a native plant grows in India, Bangladesh and South-east Asia.[17] *Terminalia chebula* Retz., has been reported to possess numerous biological activities such as antidiabetic, antibacterial, anticancer, antioxidant, cardioprotective, hepatoprotective and antiulcerogenic activities.[17-23]

Another plant, *Ficus racemosa* (FR) belongs to family Moraceae, broadly distributed all over India, China, Australia and Southeast Asia. Many bioactive chemical constituents had been isolated from different parts of this plant [24]. Plant leaves consist of numerous types of alkaloids, flavonoids, tannins and sterols. Its leaves reported to possess hepatoprotective, anti-inflammatory, and antibacterial activities [25-27]. β-sitosterol and stigmasterol had been isolated from the bark of FR, had potent antidiabetic activity [24] and this compound is present in leaves as well.

Materials and Methods:-

Chemicals

The Active Pharmaceutical Ingredient (API) Metformin was collected from Incepta Pharmaceuticals Ltd., Savar, Dhaka, Bangladesh and the leaves of the plant *Gynura procumbens*, seeds of *Terminalia chebula* and fruits of *Ficus racemosa* was collected from Mirpur Botanical Garden, Dhaka, Bangladesh. Alloxan monohydrate a diabetogenic agent was bought from Sigma Aldrich, Germany. Creatinine SGOT and SGPT measuring kits were purchased from Plasmatic Laboratory Product Ltd. Humalyzer 3000 (Semi-Automated Clinical Chemistry Analyzer originated from Medigroup Asia limited, Combodia) was used to measure the biochemical parameters and glucometer Alere GI of Alere Inc, USA was purchased from Shahbag, Dhaka, Bangladesh.

Extraction procedure

First, the leaves of the plant *Gynura procumbens* were dried in sunlight for 7 days. After that the dried leaves were crushed into powder, powdered leaves were soaked in ethanol for 7 days with occasional vigorous shaking. After the end of soaking, the extract was filtered and the filtered liquid was taken for the next step. The extracted solution was taken to the rotary evaporator machine to concentrate it. Then the dried extract was collected carefully.

Similarly, the seeds of *Terminalia chebula*, and fruits of *Ficus racemosa* were washed properly with water then dried under sunlight properly for 15 days and then dry seeds and fruits were crushed into powder. Afterward, the powder was shocked into ethanol for 15 days with continuous moderate shaking using a metabolic shaker. Then the extract was filtered and the liquid was collected using a rotary evaporator, the solution was made concentrated[28] .

Study design

Healthy adult male Wistar rats with bodyweight 130-150 gram were collected from the Department of Pharmacy of Jahangirnagar University, Savar, Dhaka, Bangladesh and the rats were kept under controlled temperature with 12±1h light/dark cycle at the Institute of Nutrition & Food Science, University of Dhaka. The animals were fed with standard pellet diet and water *ad libitum*. Before initiating the study, the rats were kept there for acclimatization. After that bodyweight of each rat was measured and were divided into 10 groups where an even distribution of rodent as per their body weight has been taken place and each group contained 5 rats.

1. Group 1: Normal control.
2. Group 2: Alloxan induced control.
3. Group 3: Alloxan induced animals receiving metformin 500 mg/kg of bodyweight
4. Group 4: Alloxan induced animals receiving the extract of *Gynura procumbens* 750 mg/kg
5. Group 5: Alloxan induced animals receiving the extract of *Terminalia chebula* 800 mg/kg
6. Group 6: Alloxan induced animals receiving the extract of *Ficus racemosa* 1000 mg/kg
7. Group 7: Non-diabetic rat receiving Metformin 500 mg/kg
8. Group 8: Non-diabetic rat receiving the extract of *Gynura procumbens* 750 mg/kg
9. Group 9: Non-diabetic rat receiving the extract of *Terminalia chebula* 800 mg/kg

10. Group 10: Non-diabetic rat receiving the extract of *Ficus racemosa* 1000 mg/kg

In day 1 the blood glucose levels were measured without inducing any extract or drug. Then on day 2, alloxan at a single dose of 150 mg/kg was injected via the intraperitoneal route in rats belonged to group 2,3,4,5,6. After 3 days blood glucose level was checked and diabetes was induced successfully[29-30].

Statistical analysis:-

Statistical analysis was performed by "One Way Anova T-test" of SPSS 16 software. Here all the data are expressed in Mean± Standard Deviation. We determined whether the intragroup variations are statistically significant or not. When the p value was found less than 0.05, the variation was considered as statistically significant.

Results:-

Change in bodyweights

The bodyweight of rats was measured initially and again just before to sacrifice. The differences between the changes were observed. It has been observed that other than rats belonged to groups 2, 3 and 7 in all other groups bodyweight increased. The result was expressed in the Mean ± Standard deviation. The results are shown in below Figure 1.

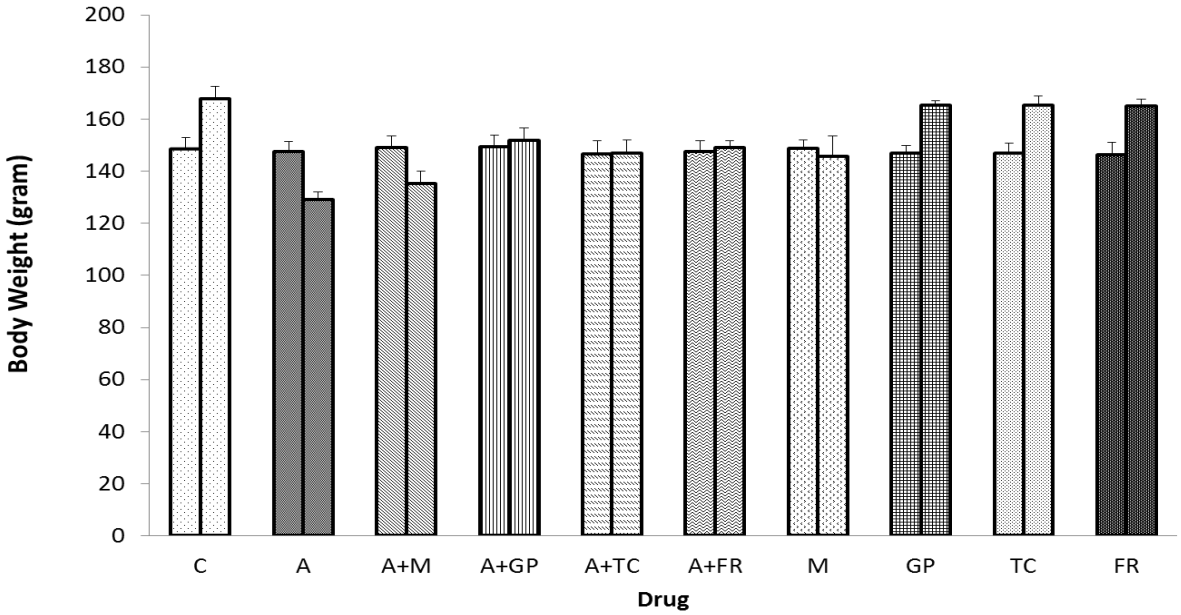

Figure 1:-Comparision between the average bodyweight (mean±standard deviation) of rats belong to ten groups at day one and day thirty five just before sacrifice. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+GP=Alloxan+*Gynura procumbens*, A+TC= Alloxan+*Terminalia chebula*, A+FR= Alloxan+*Ficus racemesa*, M=Metformin, GP= *Gynura procumbens*, TC=*Terminalia chebula*, FR= *Ficus racemesa*

Change in blood glucose level

Blood glucose levels of rats belonged to 10 groups were measured every week and the study. The study was conducted for 6 weeks. The blood glucose level of the different groups was observed. The results are shown in Figure 2.

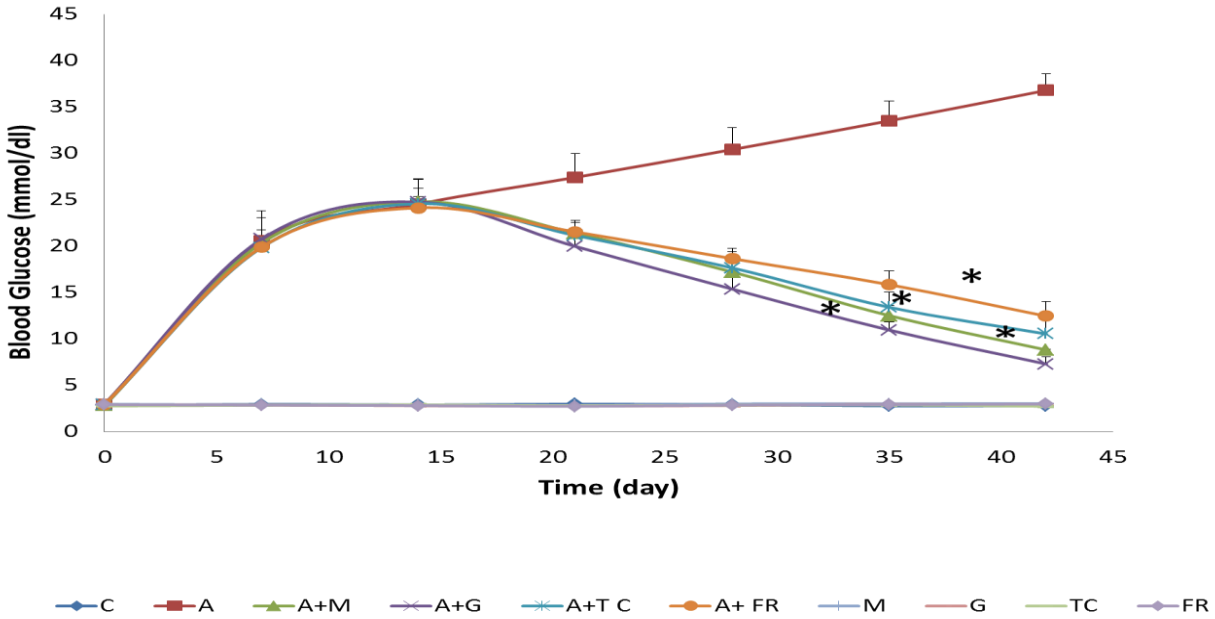

Figure 2:-Blood glucose level of ten groups from day zero to day thirty five. The data were **e**xpressed as mean± standard deviation. * Expresses the significant change.

Safety Profile Study (Liver Functioning Test)

The assessment of SGOT and SGPT levels was done after sacrificing to assess the function of the liver as a safety profile test. The results of SGOT levels are shown in Figure 3.

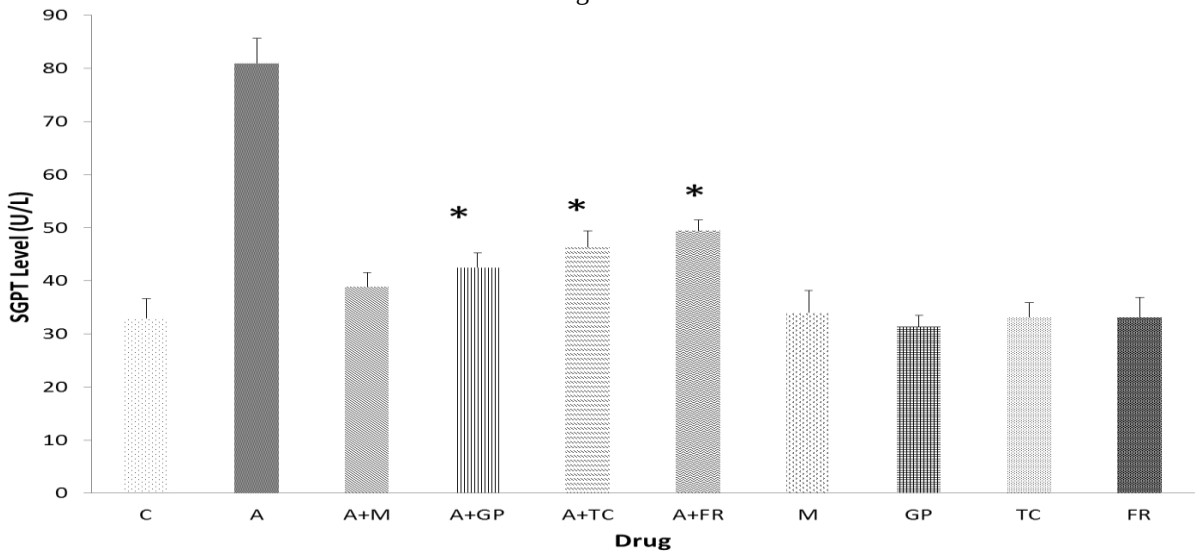

Figure 3:-Comparision between the SGPT Levels (mean±standard deviation) of rats belong to different groups at day one and day thirty five just before sacrifice. C= Control, A= Alloxan, A+M= Alloxan+Metformin, A+GP=Alloxan+*Gynura procumbens*, A+TC= Alloxan+*Terminalia chebula*, A+FR= Alloxan+*Ficus racemesa* M=Metformin,GP= *Gunura procumbens*, TC= *Terminalia chebula*, FR= *Ficus racemesa*

Similarly, the results of SGPT levels are shown in Figure 4.

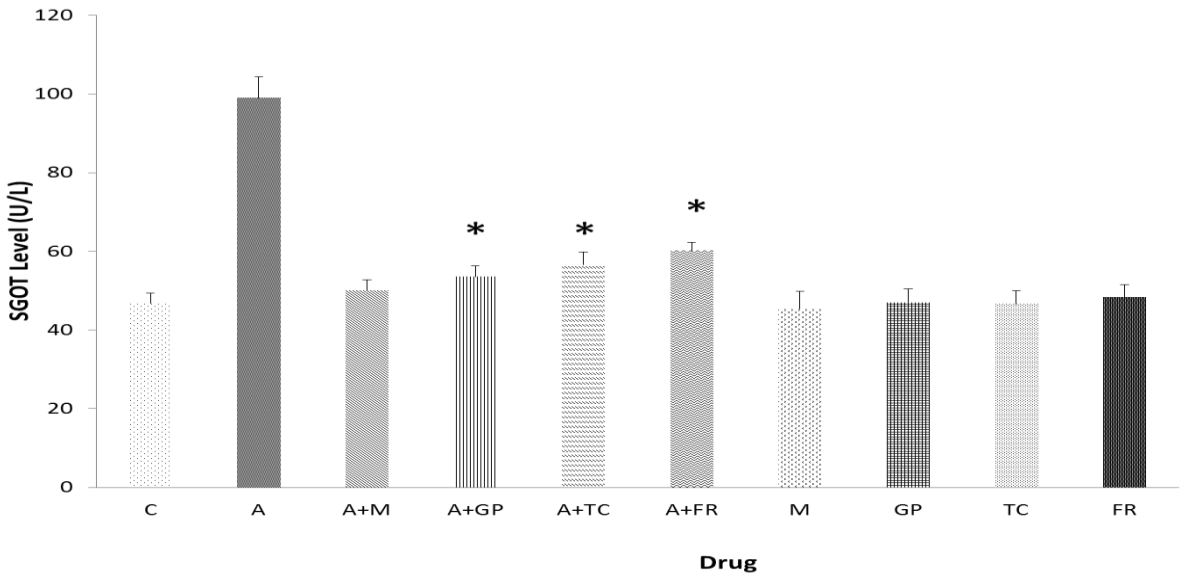

Figure 4:-Comparision between the SGOT Levels (mean±standard deviation) of rats belong to different groups at day one and day thirty five just before sacrifice. C=Control, A=Alloxan, A+M=Alloxan+Metformin, A+GP=Alloxan+*Gynura procumbens*, A+TC=Alloxan+*Terminalia chebula*, A+FR=Alloxan+*Ficus racemesa* M=Metformin, GP=*Gunura procumbens*, TC=*Terminalia chebula*, FR=*Ficus racemesa*

Safety Profile Study (Kidney Functioning Test)

Creatinine levels of rats were measured to assess whether the kidneys were functioning properly or not. The results are shown in Figure 5.

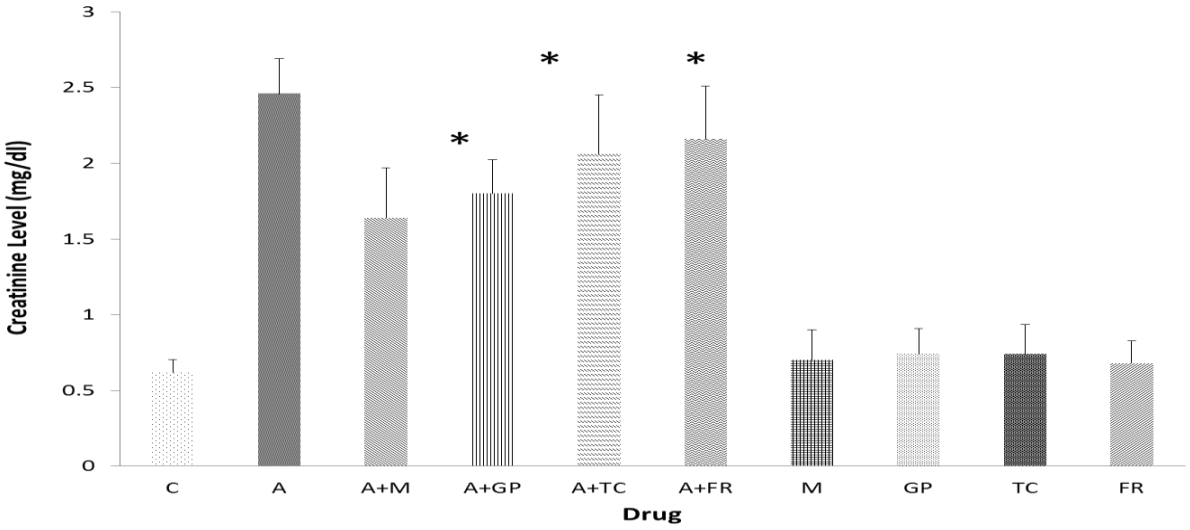

Figure 5:-Comparision between the Creatinine Levels (mean±standard deviation) of rats belong to different groups at day one and day thirty five just before sacrifice. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+GP=Alloxan+*Gynura procumbens*, A+T= Alloxan+*Terminalia chebula*, A+FR= Alloxan+*Ficus racemesa* M=Metformin, GP=*Gunura procumbens*, TC=*Terminalia chebula*, FR= *Ficus racemesa*

Discussion:-

In the conducted experiment, it was found that the rat's bodyweight raised in group 1. Whereas rats of the diabetic controlled and metformin-treated groups, we found a weight loss though they were fed properly and equivalently as a control group. In type 1 diabetes weight reduction is a physical feature and metformin itself can cause weight reduction. On the other hand, extract treated diabetic rats belonged to group 4, 5 and 6 gained weight but not like the control group. So, we can state that all the plant extract can revoke one symptom of the type 1 diabetes group. In addition, we found weight gain in test groups 7, 8, 9 & 10 were similar to the control group.

The blood glucose levels of rats were found normal in the control group when measured. In contrast, the outcome of beta-cell destruction and untreated condition, the blood glucose level appeared to be higher in groups 2, 3, 4, 5 and 6. In both alloxan injected metformin and alloxan injected extract-treated groups blood glucose level was reduced significantly ($p<0.05$). The reduction rate of blood glucose level in the metformin-treated group was a little high than extract-treated groups though it doesn't carry any statistical significance. Furthermore, blood glucose levels of rats belonged to groups 7, 8, 9, and 10 were similar to group 1.

When test groups 3, 4, 5, 6 were compared with rats of group 2, it was showed statistical significance in cases of SGOT, SGPT, and creatinine levels.

We also found that the SGOT, SGPT, creatinine and blood glucose levels of healthy rat that were treated with 3 extracts and metformin was almost identical with no statistical significance.

Conclusion:-

From the aforementioned results, it may be deduced that *Gynura procumbens* leaf extracts, seeds of *Terminalia chebula* and fruits of *Ficus racemosa* provide similar but to some extent lower effect than metformin with barren statistical significance. Notwithstanding, in diabetic rats, it ameliorated the conditions of pathological parameters like SGOT, SGPT and Creatinine along with imparting anti-diabetic effect. Additionally, these parameters are traced unchanged when non-diabetic rats were fed with respective with identical doses. We, consequently, infer that this herbal medicament can be amalgamated for disease management of type I diabetes mellitus.

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