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RESEARCH ARTICLE

EVALUATION OF BIOMEDICAL IMPORTANCE OF CHLOROGENIC ACID IN THE TREATMENT OF DIABETES MELLITUS IN IN-VIVO ALLOXAN INDUCED DIABETIC RAT MODELS.

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Key words:-

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Abstract

Objective: The present study is designed to investigate the role of Chlorogenic (CGA) acid in the regulation of glucose metabolism in alloxanized diabetic rat models.

Method: For the current study, 30 albino rats were divided into five groups. Diabetes was induced by a single intraperitoneal (i.p.) injection of alloxan monohydrate (150mg/kg) in all groups except normal control group. Blood samples were collected at 0 and 28th day of study. Hypoglycemic potential of chlorogenic acid (CGA) was determined.

Results: Results of biochemical parameters showed that level of serum glucose and glycosylated haemoglobin was significantly ($p < 0.05$) decreased in CGA treated groups in dose dependent manner along with restored value of serum insulin. While level of serum glucose was observed to decrease significantly after the treatment of CGA. Concentration of serum LDL cholesterol, TC and TG was restored to normal range. While serum HDL cholesterol was raised that was comparable to normal control and glibenclamide treated group. Moreover, oxidative stress parameters and antioxidant profile have shown that CGA possess antihyperglycemic and hypolipidemic properties.

Conclusion: Dose dependent antidiabetic activity of CGA was observed.

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Introduction:-

Intracellular glucose and lipid homeostasis is very dynamic for sustaining the elementary life events of a cell. Intracellular glucose and phospholipid metabolic syndromes are the origin of different types of metabolic disorders. These metabolic disorders are meticulously linked with the incidence and development of diabetes mellitus and obesity (Shengxiet *al.*, 2013). Currently available therapeutic options for the treatment of diabetes mellitus are not only inadequate, but at the same time are also allied with undesirable side effects (Lincoffet *al.*, 2007). Treatments with innovative mechanisms of action to contest metabolic disorders would thus have noteworthy medical impacts. Treatment of diabetes mellitus includes use of medications that decrease glucose levels, together with insulin and oral hypoglycemic drugs. Most medications in existing use are utterly constrained by both their adverse effects and high cost of therapy. Due to these contests, people have resorted to inexpensive and readily available alternate sources, such as usage of medicinal plants and their phyto-constituents. The WHO approximates that 80% of the

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worlds' populations use plant based medicines for different ailments. The sustained use of plant based medicines is related to their cost effectiveness and minimal side effects (Moses *et al.*, 2016).

Chlorogenic acid (CGA), one of the furthermost ample polyphenol compounds in the diet of human beings, is a collection of phenolic metabolites formed by certain plants and a vital constituent of coffee. It has been stated that coffee has the maximum concentration of polyphenols (Olthof *et al.*, 2001). Amassing evidence has confirmed that CGA shows many biotic possessions, including antioxidant, anticarcinogenic and antibacterial activities, mainly hypoglycemic and lipid lowering effects (Bassoliet *et al.*, 2008). CGA has been newly claimed to control glucose and lipid metabolism (Zhanget *et al.*, 2011). Keeping in view the medicinal value of CGA, in the present investigation antihyperglycemic potential of chlorogenic acid has been explored in experimentally induced diabetic rat models.

Material And Methods:-

Animals:-

Thirty (30) healthy young albino rats weighing 180-200 g were used for study. Rats were kept at facility in animal room situated at Institute of Pharmacy, Physiology and Pharmacology. The animals were kept for two weeks as an acclimatization period prior to the start of experiment. They were housed 6/cage and received normal diet and water *ad libitum*.

Chemicals and Drugs:-

Alloxan monohydrate (AppliChem, Ottoweg-4, Germany) was used to induce experimental diabetes in rats. Tablet Glibenclamide (DAONIL, Sanofi-Aventis Pvt. Ltd) was administered orally at a dose of 10mg/kg orally to the respective group. Chlorogenic acid was obtained from Sigma-Aldrich Company, St. Louis, USA.

Induction of diabetes mellitus in experimental animals:-

In overnight fasted rats, diabetes was induced by single i.p. injection of alloxan monohydrate (150mg/kg) 5% w/v in normal saline. Since, alloxan has capability of producing fatal hypoglycemia due to the enormous pancreatic release of insulin, so animals were given 20% glucose solution orally after 6 hrs. For next 24 hours, rats were fed 5% glucose solution to prevent hypoglycemic shock. Diabetes was confirmed in rats by determining glucose level with glucometer after 72 hours of alloxan injection. Experimental rats having blood glucose level above 300 mg/dl were believed to be diabetic and included in further studies.

Experimental Design:-

The rats were divided in 5 groups with 6 rats in each group. After the induction of diabetes, rats received following treatments by intra gastric route. Group I: Normal control (Routine diet + water *ad-libitum*); Group II: untreated control (alloxan monohydrate, 150mg/kg); Group III: glibenclamide treated (10mg/kg/day); Group IV: treated group I (chlorogenic acid, 5 mg/kg); Group V: treated group II (chlorogenic acid, 15 mg/kg).

Collection of blood samples:-

Blood samples were collected at 0 and 28th day. Collection was done in clot activator gel tubes. The serum was separated by centrifugation at 4000 rpm for 10 minutes.

Biochemical analysis:-

Biochemical analysis for serum insulin (Abcam's ab200011- Insulin Human Simple step ELISA® kit) and serum glucose (Flutiest® GLU- Analyticon diagnostic kit) was performed. Lipid profile for serum triglycerides, serum total cholesterol (Dia Sys; Diagnostic Systems USA reagent kit method), serum HDL and serum LDL (Randox; Randox Laboratories LTD, UK, reagent kit method) was done. Total oxidative status, total antioxidant capacity, (Mohammed *et al.*, 2009; Ohkawa *et al.*, 1979) superoxide dismutase, serum malondialdehyde levels were calculated (Pandey *et al.*, 2011).

Statistical analysis:-

The results were expressed as mean \pm SE. Statistical analysis was conducted by ANOVA followed by Duncan's multiple range test (DMR) at 5% level of significance ($P \leq 0.05$). Statistical Package for Social Sciences (SPSS) version 3.16 was used for statistical analysis.

Results:-

On 28th day level of serum glucose and glycosylated haemoglobin was significantly ($p < 0.05$) raised in alloxan monohydrate treated group while serum insulin level was lowered in comparison to the normal control. While

concentration of serum glucose and glycosylated haemoglobin was significantly decreased in CGA treated groups in dose dependent manner along with restored value of serum insulin that was comparable to the values of glibenclamide treated group (Table-1).

Table 1:- Effect of chlorogenic acid on serum glucose, serum insulin and glycosylated hemoglobin.

Groups	Serum glucose (mg/dl)	Serum Insulin (μ IU/ml)	Glycosylated Hemoglobin (% age)
Control	77.67 \pm 3.25 E	18.768 \pm 0.658 A	5.72 \pm 0.12 F
Alloxan monohydrate	341.17 \pm 5.08 A	6.283 \pm 0.817 E	13.92 \pm 0.70 A
Glibenclamide	92.83 \pm 2.94 DE	16.68 \pm 0.654 B	6.05 \pm 0.45 EF
Treated I	114.67 \pm 1.74 C	12.55 \pm 0.660 C	8.92 \pm 0.99 D
Treated II	99.00 \pm 1.81 D	15.1 \pm 0.815 B	6.62 \pm 0.27 EF

Each value is expressed as mean \pm SE. Varying alphabet in each column shows significant statistical difference ($p < 0.05$)

Figure-1 is graphical representation of blood glucose level in treated and untreated groups at 0, 7th, 14th and 28th day. After the administration of alloxan monohydrate at each weekly interval level of serum glucose was raised significantly ($p < 0.05$) while level of serum glucose after the treatment of CGA was observed to decrease significantly by the end of 4th week in both treated I and treated II groups dose dependently.

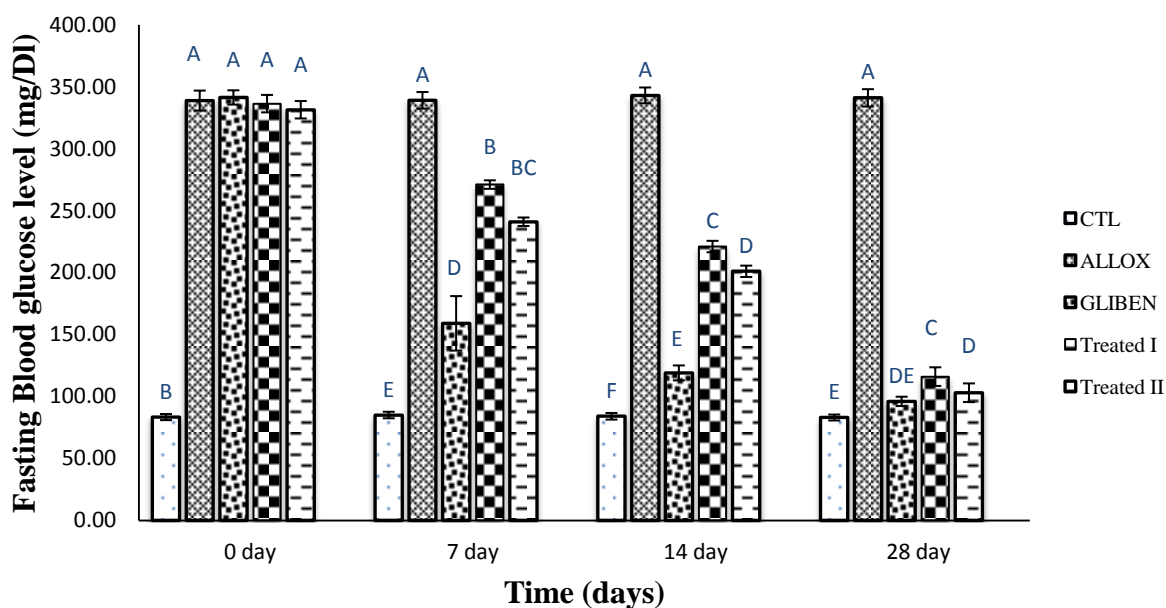


Fig # 1:- Fasting Blood Glucose level after administration of glibenclamide and chlorogenic acid in alloxanized hyperglycemic rats.

Oxidative health markers:-

Experimental diabetic rats showed a significant elevation in serum TOS level, while treatment with chlorogenic acid showed decrease in the level of total oxidant status exhibiting lowering of oxidative stress (Figure-2)

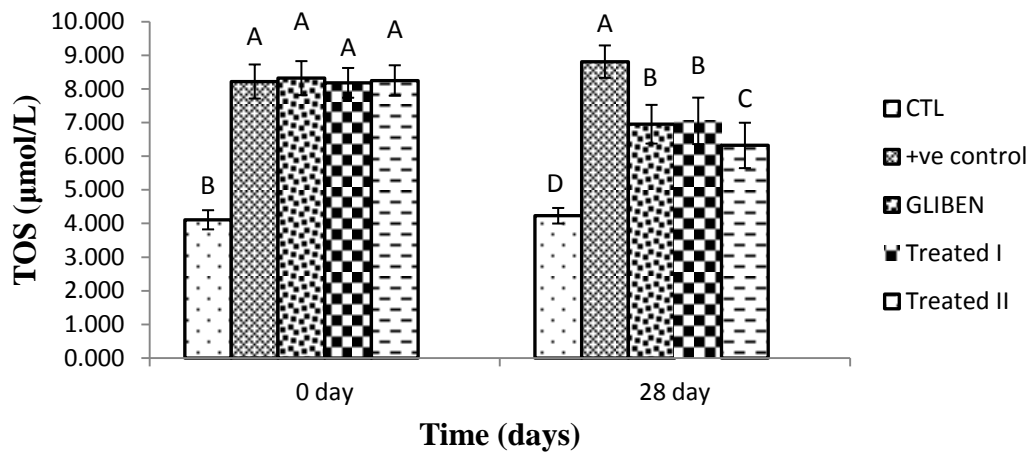


Fig # 2:- Serum TOS level after administration of glibenclamide and chlorogenic acid.

Serum total antioxidant capacity (TAC) was decreased in alloxan treated rats due to over production of free radicals that increases oxidative stress markers. Whereas level of TAC in CGA and glibenclamide groups was significantly increased and that was comparable to normal control (Figure-3).

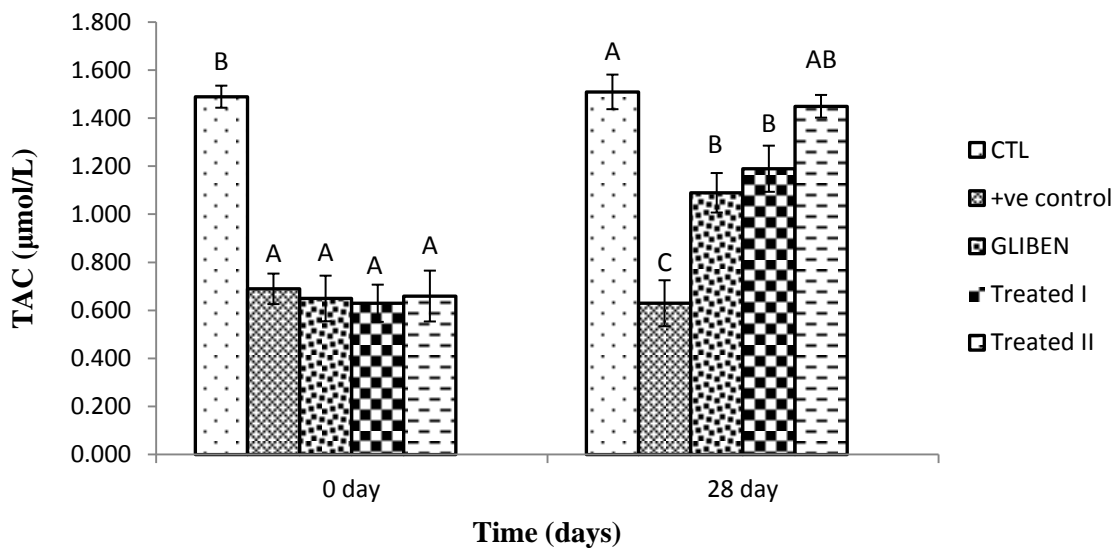


Fig.3:- Serum TAC level after administration of glibenclamide and chlorogenic acid

Experimental diabetic rats showed a significant decrease in serum SOD level, while treatment with chlorogenic acid and glibenclamide showed increase in the level of serum super oxide dismutase(Figure-4).

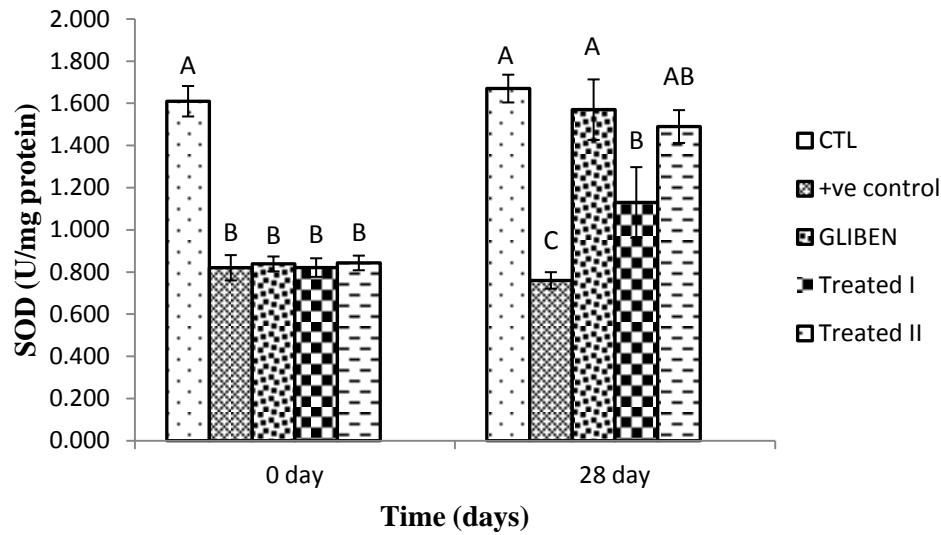


Fig. 4:- Serum SOD level after administration of glibenclamide and chlorogenic acid.

Serum MDA was increased in alloxan treated rats due to over production of free radicals that increases oxidative stress. Whereas level of MDA in CGA and glibenclamide groups was significantly decreased and that was comparable to normal control (Figure-5).

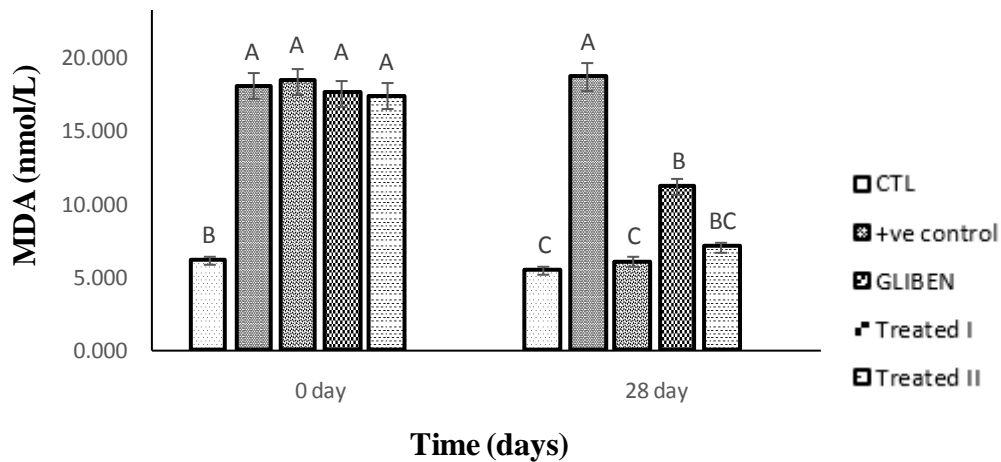


Fig.5:- Serum MDA level after administration of glibenclamide and chlorogenic acid.

When experimental rats were treated with alloxan monohydrate then concentration of total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL) cholesterol was significantly elevated along with a significant decrease in level of high density lipoprotein (HDL) cholesterol. After treatment with CGA level of serum LDL cholesterol, TC and TG was restored to normal range. While concentration of HDL cholesterol was raised that was comparable to normal control and glibenclamide treated group (Table-2).

Table 2:- Determination of lipid profile after treatment with chlorogenic acid

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)
Control	72.83±6.804 C	82.00±1.18 C	29.83±0.31 A	11.58±0.45 C
Alloxan monohydrate	108.67±3.471 A	137.67±9.45 A	16.33±0.33 C	28.25±0.92 A
Glibenclamide	74.45±4.945 C	89.00±3.76 C	27.00±0.86 A	12.08±0.68 C
Treated I	89.67±4.224 B	107.33±3.57 B	21.50±1.73 B	16.92±0.78 B
Treated II	77.17±5.518 C	92.00±2.50 C	25.98±0.62 A	14.02±0.69 C

Each value is expressed as mean ± SE. Varying alphabet in each column shows significant statistical difference (p <0.05)

Discussion:-

Diabetes mellitus is characterized by deficiency of insulin consequence in glucose intolerance and in aberrations of other metabolic fuels (Kolanaet *et al.*, 2017). It affects almost 3% of population world widely. Awareness and information about the risk factors, complications and management of diabetes mellitus are important features for better control and improved quality of life (Wild *et al.*, 2004). There are several restrictions for the use of oral hypoglycemics in the treatment of diabetes mellitus. As oral hypoglycemic agents are associated with severe side effects so focus is now diverted towards the use of natural origin based synthetic and semi-synthetic substances with more efficacy and less side effects for the treatment of diabetes mellitus. The trademark of both type 1 and 2 diabetes is the inability of the beta cell of pancreas to secrete the necessary amount of insulin to maintain the blood glucose homeostasis. The prediabetic state in type 2 diabetes mellitus is linked with the glucose intolerance and defective insulin homeostasis.

Alloxan monohydrate induced diabetes mellitus is a very useful investigational way of demonstrating the antihyperglycemic potential of newer agents. In the current study alloxan monohydrate (150 mg/kg b.w) was used to prepare diabetic models and serum glucose level was found to be meaningfully raised than in normal albino rats. Alloxan is a diabetogenic mediator which destroys β -cells of the islets of langerhans and causes increase in hyperglycemia (Bhaskaret *et al.*, 2016).

The mechanism involved in antihyperglycemic action of CGA might be the increased secretion of insulin from pancreatic beta cells. Previous data has revealed that CGA acts as insulin sensitizer and it works like metformin in increasing the insulin sensitivity on tissue site. At the same time CGA also reduces the phosphorylation of glucose by inhibiting the glucose-6-phosphatase activity. The hypoglycemic potential of CGA in alloxanized hyperglycemic rats was comparable to glibenclamide. Our findings are in agreement with previous research data (Ahrens and Thompson, 2013).

Our findings have demonstrated that after 28 days of treatment, chlorogenic acid (5 mg/kg and 15 mg/kg) showed significant dose related antihyperglycemic activity in in-vivo diabetic models which was comparable to glibenclamide. Previous research data has revealed that the mechanism involved in antidiabetic activity of chlorogenic acid might be its potentiation effect on insulin action (Pariet *et al.*, 2010). Chlorogenic acid also enhances the glucose uptake by adipose tissues and muscles (Shengxiet *et al.*, 2013).

Chlorogenic acid is a hypoglycemic agent and also maintains the lipid homeostasis. Results of current study have demonstrated that chlorogenic acid significantly reduced the serum cholesterol and triglyceride levels in dose dependent manner. These results are in accordance with previous research studies (Wang *et al.*, 2012).

The indicator of the oxidative stress is the total antioxidant capacity (TAC) (Young, 2001). TAC level was reduced considerably in group treated with alloxan in present study. TAC levels were markedly raised in rats treated with chlorogenic acid. Total oxidant status (TOS) acts as a determining factor of oxidative stress formed by reactive

oxygen species. Excess amount of reactive oxygen species are produced in the pathological conditions which upsets the balance between oxidants and antioxidants (Harmaa *et al.*, 2005). TOS level was increased markedly in alloxanized diabetic rats in the given study. However, TOS level was decreased by chlorogenic acid due to its ability to scavenge free radicals produced by alloxan. SOD display a cytoprotective effect (Lenzen, 2008). Our findings are related to increased level of SOD in chlorogenic acid treated rats.

In the given study, glycosylated haemoglobin level was markedly raised as compared to normal group, which is related to the high risk of retinopathy and renal complications. In contrast, administration of chlorogenic acid for 28 days, markedly reduced the level of glycosylated haemoglobin (Balamurugan *et al.*, 2014). These results are in agreement with previous research studies.

Conclusion:-

Current study was perceived with an opinion to deliver scientific and pharmacological suggestions for antihyperglycemic potential of CGA. The results attained in present study may demonstrate valuable in discovering holistic approach for diabetes mellitus. However, further research studies are also required to elucidate the mechanisms involved in hypoglycemic potential of CGA as well as the comprehensive investigation for side effects of CGA is also required for its safe use in the treatment of diabetes mellitus.

Declarations:-

Conflict of interest:-

The authors declare that they have no competing interests.

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