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In–Vitro Drug Interaction between Tulsi and Glimepride

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Abstract: The study was designed to determine the interaction of *O. sanctum,* an herbal drug used in many herbal formulations for hypoglycemic activity with Glimepiride, a sulfonylurea (SU) derivative, widely used in the treatment of type-2 diabetes, in diabetic rats. Whole study was divided into four phases in which hypoglycaemic activity with single doses of *O. sanctum* and glimeperide was established in single day study and repeated dose treatment of *O. sanctum* (7 days) followed by a single dose of glimeperide on 8th day was also established. Further the interaction between the two drugs was studied in both single and repeated treatment, i.e., the effect of single and glimeperide repeated doses treatment on *O. sanctum.* All the results were analysed by one way ANOVA followed by Dunnett's't' test.

Rats were administered with streptozotocin for inducing diabetes and left for 2 weeks to stabilize blood glucose levels. Various doses of *O. sanctum* (100, 200 and 400 mg/kg) and glimeperide (½ TD, TD and 2 TD) upon single dose treatment of each in different groups of diabetic rats produced a dose dependent decrease in blood glucose levels. The maximum reduction in serum glucose was observed at 6 th h in all the groups. Single dose of *O. sanctum* prolonged the effect of glimepiride from 4th to 6th h without any hypoglycemic convulsions.

Keywords: Hypoglycemic activity, Glimeperide, *O. Sanctum*, In-Vitro Interaction, Type 2 diebetes

INTRODUCTION:-

Herbal products are becoming popular as alternative medicines worldwide. According to a 2011 update on the health risks of herbal remedies in *Clinical Pharmacology and Therapeutics*, that about one third of adults in the developed countries and more than 60% Asians use herbal medicines for health promotion or treatment of various diseases.

The World Health Organization (WHO) in 1991 estimated that there were some 11,000 species of herbal plants for medicinal use and about 500 species of them are commonly used in complementary medicine. WHO defines herbal medicines as finished, labelled medicinal products that contain active ingredients from aerial or underground parts of plants, or other plant material, or combinations thereof, whether crude state or as other preparations.".[1]

Herbal product are being used as a home remedies Worldwide in a variety of healthcare settings and are often promoted/known as "natural" and completely "safe" alternatives to conventional medicines.

WHO survey indicated that about 70–80% of the World populations rely on non-conventional medicine mainly of herbal sources in their primary healthcare, in recent years, in developed countries it was witnessed as an increase in the growth in popularity of over-the-counter (OTC) health foods, nutraceuticals and medicinal products from plants or other natural sources. This indirectly indicates the public's dissatisfaction with the orthodox type of

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medical (OM) treatment.[2] Herbs with anti-diabetic effect may interfere with anti-diabetic potential of the drugs by enhancing hypoglycemic effects. The dosage of herbs and drugs must be balanced carefully to control effectively the blood glucose level without causing hyper- or hypoglycemia. [14] Herbs with definite hypoglycemic effects include Trigonella foenum-graecum [15] , Ocimum sanctum [15,] Hemionitis arifolia,[16]Withania coagulans[17], Viscum album[18], Aloe vera [19], Aerva lanata,[20] Murraya koenigii [21], Lepidium sativum [22], Eugenia jambolana [23], Inula viscose [24], Vernonia colorata[25],Ginseng [26], Urtica pilulifera [27], Chamaemelum nobile [28], Momordica charantia [29],

Herbal medicines include dietary supplements that contain herbs, either single or in mixtures, also called botanicals, are plants or plant parts used for their scent, flavour, and/or therapeutic properties. Since herbal medicines are classified as dietary supplements, there is no Food and Drug Administration (FDA) regulations regarding accuracy of active ingredients content efficacy or safety of active ingredients.[4]

The popularity of herbal medicinal products (HMPs) necessitates to understand potential interactions between herbs and prescribed drugs. The likelihood of herb-drug interactions could be higher than drug-drug interactions, if only because drugs usually contain single chemical entities, while almost all HMPs (even single- herb products) contain mixtures of pharmacologically active constituents.[5]

An increase in number of Indians are using herbal products for preventive and therapeutic purposes. Many patients take herbal products in combination with prescribed allopathic drugs without the consent of their doctors. The concomittant administration of herbal preparations with synthetic drugs may raise the potential of herb-drug interactions. Recent medical literature has recorded an increase in herb- drug interactions although many studies are from case reports and limited clinical observations. However, herb-drug interactions may be significantly under-reported and under-estimated.[7]

Materials and Method:-

S. No.	Materials
1	Albino Rats: Obtained from National Center for Laboratory Animal Science, C/O Shri. Venkateswara Enterprises Bangaluru, India.
2	*Ocimum Sanctum* leaves (Surajbala Exports Pvt. Ltd. Delhi, India)
3	Auto analyser (ERBA mannheim, CHEM- 5 plus v2, Transasia Bio-Medicals Ltd., Germany).
4	Citric acid monohydrate (S.d.fine chemicals limited, Mumbai)

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5	Glimepiride (Dr.Reddys Labs, Hyderabad)
6	Glucose kit (Erba Mannheim, Transasia Bio-Medicals Ltd., Baddi, H. P.)
7	Digital pH meter MK VI (Systronic, Ahmedabad)
8	Streptozotocin (Alexis Biochemicals,U.S.A)
9	Trisodium citrate (Karnataka fine chem., Banglore)
10	NaOH (S.d.fine chemicals limited, Mumbai)
11.	Spirit, low voltage table lamp, 1ml pipettes, micropipette, 10ml centrifuge tubes, 10 ml test tubes, thin aluminium foil, incubator etc. (ERBA mannheim Transasia Bio-Medicals Ltd., Baddi, HP and Remi equepments, Bombay)

Table no. 1

Preparation of aqueous extract of *O. Sanctum*:-

About 100 g of *O. Sanctum* of leaf powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 24 h with shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract (AQELOS) and then it was concentrated on a water bath maintained at 50^{o}C.

These extracts were stored in airtight containers in a refrigerator below 10^{o}C. The two extracts were examined for their colour and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Method for oral administration of drug:

An 18 –gauge needle was suitably covered with flexible polythene tubing, where the edge was made blunt; the needle was fixed to 1ml tuberculin syringe. The rat was held firmly in left hand, the tubing was moistened with glycerine and inserted right into the esophagus and gently pressing plunger for drug administration, and this was followed by 0.2 ml of distilled water to ensure administration of correct dose of the drug.

Method for collection of blood sample:

The rat was placed into the rat holder, such that the tail was pulled out and was deplitated for collection of blood sample. Tail vein was dilated by focusing a low voltage electric lamp. The tip of the tail was thin sliced (0.05 mm) using a sharp scissors. The blood drops were collected though the walls of 0.5 ml of centrifuge tube (to avoid haemolysis of the blood sample). The tail was gently pressed with fingers to enhance the blood flow and allowed to clot in centrifuge tube. Later dry cotton was applied for 5 min to stop the blood flow and the tail was sterilized by spirit.

Method of collection of Serum:

The serum was obtained by centrifuging the blood samples for 20 min at 3000 rpm supernatant fluid was decanted into the clean dry test tube.

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Experimental:

The whole study is divided into 3 phases. In the phase I, preparation of aqueous extract of *O. Sanctum* (AQELOS). In phase II, determination of LD_{50} and selection of low, medium and high doses from (AQELOS). In phase III, Study of herb-drug interaction between AQELOS and glimepiride in the streptozotocin induced diabetic rats and serum glucose levels were estimated (Phase III is divided into 5 stages.)

Determination of LD_{50} of AQELOS:

The acute toxicity of AQELOS was determined by using albino mice of either sex (20-25 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment. Animals were administered with single dose of AQELOS and observed for its mortality upto 48 h study period (short term toxicity) and further observed for a period at 14 day (Long term toxicity). Based on the short-term toxicity profile, the next dose was determined as per OECD guidelines No 425. From the LD_{50} dose $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ doses were selected and considered as low, medium and high dose respectively and are used in the entire study.

Dose selection of Glimepiride.

The human dose (4 mg/kg, p.o.) of Glimepiride was extrapolated to rats based on body surface area and weight. The dose effect relationship is established using ½ TD, TD and 2 TD. The human dose (4 mg/kg, p.o.) of Glimepiride extended to rat as ½ TD (0.036 mg/200 g, p.o.), TD (0.072 mg/200 g, p.o.) and 2 TD (0.144 mg/ 200 g, p.o.).

Estimation of Serum glucose

There are several methods used in practice for estimating serum glucose levels and few of them are mentioned below [132-137].

1.Nelson Somogyi method
2.End point 0-Toludine method
3.GOD/POD method

The older methods were based on reducing property of glucose. But these methods do not measure true glucose because of interferences. Subsequently other chemical and enzymatic methods were involved to overcome this problem. The GOD/POD method is one such evolved method which is simple, single stepped, rapid, reliable, safe and precise. Hence, in the present study GOD/POD method was adopted. This method utilizes two enzymes Glucose Oxidase (GOD) and Peroxidase (POD) along with chomogen 4- amino antipyrine and phenol. This method is intended for *in-vitro* quantitative determination of glucose in serum, plasma and CSF. There is no interference due to the creatinine, fructose, galactose, reduced glutathione, ascorbic acid and xylose. Haemoglobin or bilirubin upto 10 mg% does not affect the test.

Citrate buffer (pH4.4, 0.1M):

Citric acid monohydrate 0.6306 g was dissolved in 50 ml of distilled water. Trisodium citrate 0.7352 g was dissolved in 25 ml of distilled water 28 ml of Citric acid monohydrate and 22 ml solution were taken and mixed together. It is made upto a volume of 1000 ml with distilled water. The pH of the solution was adjusted to pH4.4. One week after STZ treatment the serum samples were collected and analysed for serum glucose levels. Rats with serum glucose levels more than 200 mg/dl were included in the experiment. The experimental diabetes was characterized by weight loss and hyperglycemia and these animals were used for antidiabetic study.

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Experimental study in diabetic rats:

41 groups of Wister albino rats of either sex weighing 180- 220 g, selected for the study were kept in colony cages at ambient temperature of 28±2°C and relative humidity of 45 to 55% with a 12:12 h light/dark cycle. The animals were fasted for 18 h before commencing the experiment with water *ad libitum*. The fasting was continued till completion of the experiment. The '0' h blood samples were collected for the estimation of fasting serum glucose.

Stage I: One Day Treatment

Group–1 Animals were administered with 0.1NaOH only.(one day)

Group–2 Animals were administered with single dose of AQELOS (low dose) in diabetic rats.

Group–3 Animals were administered with single dose of AQELOS *(*medium dose) in diabetic rats.

Group–4 Animals were administered with single dose of AQELOS (high dose) in diabetic rats.

Group–5 Animals were administered with single dose of glimepiride 1/2 TD in diabetic rats

Group–6 Animals were administered with single dose of glimepiride TD in diabetic rats.

Group–7 Animals were administered with single dose of glimepiride 2TD in diabetic rats.

Stage II: One Week Treatment:

Group–8 Animals were administered with 0.1NaOH only.(one day)

Group–9 Animals were administered with repeated dose treatment of AQELOS (low dose) for 7 days.

Group–10 Animals were administered with repeated dose treatment of AQELOS (medium dose) for 7 days.

Group–11 Animals were administered with repeated dose treatment of AQELOS (high dose) for 7 days.

Group–12 Animals were administered with repeated dose treatment of glimepiride 1/2 TD for 7 days.

Group–13 Animals were administered with repeated dose treatment of glimepiride TD for 7 days.

Group–14 Animals were administered with repeated dose treatment of glimepiride 2 TD for 7 days.

Stage III: One Day Interaction

Group–15 Animals were administered with single dose of AQELOS (low dose), 30 min later glimepiride 1/2 TD was administered orally.

Group–16 Animals were administered with single dose of AQELOS (medium dose), 30 min later glimepiride 1/2 TD was administered orally.

Group–17 Animals were administered with single dose of AQELOS (high dose), 30 min later glimepiride 1/2 TD was administered orally.

Group–18 Animals were administered with single dose of AQELOS (low dose), 30 min later glimepiride TD was administered orally.

Group–19 Animals were administered with single dose of AQELOS (medium dose), 30 min later glimepiride TD was administered orally.

Group–20 Animals were administered with single dose of AQELOS (high dose), 30 min later glimepiride TD was administered orally.

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Group–21 Animals were administered with single dose of AQELOS (low dose), 30 min later glimepiride 2TD was administered orally.
Group–22 Animals were administered with single dose of AQELOS (medium dose), 30 min later glimepiride 2TD was administered orally.
Group–23 Animals were administered with single dose of AQELOS (high dose), 30 min later glimepiride 2TD was administered orally.

Stage IV: One Week Interaction

Group–24 Animals were administered with repeated dose treatment of AQELOS (low dose) before treatment with single dose of glimepiride 1/2 TD.
Group–25 Animals were administered with repeated dose treatment of AQELOS (medium dose) before treatment with single dose of glimepiride 1/2 TD.
Group–26 Animals were administered with repeated dose treatment of AQELOS (high dose) before treatment with single dose of glimepiride 1/2 TD.
Group–27 Animals were administered with repeated dose treatment of AQELOS (low dose) before treatment with single dose of glimepiride TD.
Group–28 Animals were administered with repeated dose treatment of AQELOS (medium dose) before treatment with single dose of glimepiride TD.
Group–29 Animals were administered with repeated dose treatment of AQELOS (high dose) before treatment with single dose of glimepiride TD.
Group–30 Animals were administered with repeated dose treatment of AQELOS (low dose) before treatment with single dose of glimepiride 2TD.
Group–31 Animals were administered with repeated dose treatment of AQELOS (medium dose) before treatment with single dose of glimepiride 2TD.
Group–32 Animals were administered with repeated dose treatment of AQELOS (high dose) before treatment with single dose of glimepiride 2TD.

Stage V: One Week Interaction:

Group-33 Animals were administered with single dose treatment of AQELOS (low dose) after treatment with repeated dose of glimepiride 1/2 TD.
Group–34 Animals were administered with single dose treatment of AQELOS (medium dose) after treatment with repeated dose of glimepiride 1/2 TD.
Group–35 Animals were administered with single dose treatment of AQELOS (high dose) after treatment with repeated dose of glimepiride 1/2 TD.
Group–36 Animals were administered with single dose treatment of AQELOS (low dose) after treatment with repeated dose of glimepiride TD.
Group–37 Animals were administered with single dose treatment of AQELOS (medium dose) after treatment with repeated dose of glimepiride TD.
Group–38 Animals were administered with single dose treatment of AQELOS (high dose) after treatment with repeated dose of glimepiride TD.
Group–39 Animals were administered with single dose treatment of AQELOS (low dose) after treatment with repeated dose of glimepiride 2TD.
Group–40 Animals were administered with single dose treatment of AQELOS (medium dose) after treatment with repeated dose of glimepiride 2TD.
Group–41 Animals were administered with single dose treatment of AQELOS (high dose) after treatment with repeated dose of glimepiride 2TD.

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Result:-

Preliminary phytochemical investigation:-

Phytoconstituents like alkaloids, cardiac glycosides, proteins, amino acids, phenolic compounds triterpenes, steroids, sterols, saponins and flavonoids were present in AQELOS.

Acute oral toxicity study

The aqueous extract of *O. sanctum* was administered orally to different groups of mice at different dose levels. It was found that even up to the dose level of 2000 mg/kg body weight, the extract showed no effect on the behavioural symptoms or produced mortality during the observation period of 48 h (short term toxicity) and further no mortality was observed up to 14 days of monitoring (long term toxicity).

In groups treated with vehicle NaOH single or repeated dose treatment has not produced any effect on serum glucose levels.

In STZ induced diabetic rats selected doses of AQELOS (low, medium and high) with single as well as repeated dose treatment has produced a significant and dose dependent reduction in serum glucose levels.

A minimum of 20% reduction in serum glucose level has taken as onset of action recorded with both single and repeated doses treatment of of AQELOS is noted (low, medium and high) at 3-4 h, 2-3 h and 2-3 h and duration of action is noted as 6.5 h, 7.5 h and 9 h respectively.

Anti diabetic activity of AQELOS and Glimepiride in STZ induced diabetic rats.

All the groups i.e. treated with AQELOS low, med, high DOSES and glimeperide ½TD, TD, 2TD have shown a maximum reduction of 28.21, 32.74, 38.85% and 34.90, 40.46, 48.26% respectively in single doses treatment and 30.13,35.09, 40.35% and 38.49, 44.69, 53.07% in repeated dose treatment respectively at 6^{th} h and the results are represented graphically in Fig 5.1 and 5.2 respectively.

Influence of single dose treatment of AQELOS on antidiabetic activity of Glimepiride in STZ induced diabetic rats.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, AQELOS (100, 200 and 400 mg/kg, p.o.) single doses followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) group has shown a significant reduction in serum glucose levels at 4-10 h, 4-16 h and 2-16 h respectively.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, AQELOS (100 mg/kg, p.o.) single dose treated group followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has a significantly reduced serum glucose levels at 4-16 h, and AQELOS (200, 400 mg/kg, p.o) single doses followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) group has a significantly reduced serum glucose levels throughout the study.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, AQELOS (100 mg/kg, p.o.) single dose treated group followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 2-16 h, and AQELOS (200 and 400 mg/kg, p.o) single doses followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) group has significantly reduced serum glucose levels throughout the study.

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When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, AQELOS (100 mg/kg, p.o.) single dose treated group followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has not shown any significant reduction in serum glucose levels. But AQELOS (200 mg/kg, p.o) single dose treated group followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels only at 6 h. Also AQELOS (400 mg/kg, p.o) single dose treated group followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 4, 6, 8 and 10 h .

When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, AQELOS (100, 200 and 400 mg/kg, p.o.) single doses treated group followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 4-10 h, 3-16 h and 1-16 h. respectively.

When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group AQELOS (100 and 200 mg/kg, p.o.) single doses treated group followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 3-12 h. and 1-16 h . But AQELOS (400 mg/kg, p.o) single dose followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group has significantly reduced serum glucose levels throughout the study.

When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, AQELOS (100, 200 and 400 mg/kg, p.o.) single doses treated group followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has not shown any significant reduction in serum glucose levels.

When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, AQELOS (100 and 200 mg/kg, p.o.) single doses treated group followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has produced no significant reduction in serum glucose levels. But AQELOS (400 mg/kg, p.o) single dose treated group followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels only at 6 h.

When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, AQELOS (100 and 200 mg/kg, p.o.) single doses treated group followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) a significant reduction in serum glucose levels was observed at 4-10 h, 4-12 h. AQELOS (400 mg/kg, p.o) with single dose followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group has significantly reduced serum glucose levels throughout the study.

All the above groups have shown maximum reduction in serum glucose levels at 6^{th} h .

Influence of repeated dose treatment of AQELOS on antidiabetic activity of Glimepiride in STZ induced diabetic rats.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100, 200 and 400 mg/kg, p.o.) followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 6-10 h, 4-10 h and 2-16 h respectively.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100 and 200 mg/kg, p.o.) followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 2-16 h and 1-16 h respectively. The group treated with repeated doses of AQELOS (400 mg/kg, p.o) followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) group has

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shown a significant reduction in serum glucose levels through out the experimental study.
When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100 mg/kg, p.o.) followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at throughout the experimental study except at 1 h . The group treated with repeated doses of AQELOS (200 and 400 mg/kg, p.o) followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels throughout the experimental study.
When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100 mg/kg, p.o.) followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has not shown a significant reduction in serum glucose levels, the group treated with repeated doses of AQELOS (200, 400 mg/kg, p.o) followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 6 h only.
When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100 mg/kg, p.o.) followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 6 h only. The group treated with repeated doses of AQELOS (200 and 400 mg/kg, p.o) followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has shown a significant reduction at 2-16 h and 2-24 h respectively.
When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100 mg/kg, p.o.) followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 4-8 h . The group treated with and repeated doses of AQELOS (200, 400 mg/kg, p.o) followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) has shown a significant reduction throughout the experimental study.
When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100, 200 and 400 mg/kg, p.o.) followed by single dose of glimepiride ½ TD (0.036 mg/ 200g, p.o) has not shown any significant reduction in serum glucose levels.
When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100 mg/kg, p.o.) followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has not shown significant reduction in serum glucose levels. The group treated with repeated doses of AQELOS (200 and 400 mg/kg, p.o) followed by single dose of glimepiride TD (0. 072 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels only at 6 h and 6-8 h respectively.
When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of AQELOS (100, 200 and 400 mg/kg, p.o.) followed by single dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) has shown a significant reduction in serum glucose levels at 6-8, 4-10 and 1-12 h respectively.
All the groups have shown maximum reduction in serum glucose levels at 6^{th} h only.

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Influence of repeated dose treatment of Glimepiride on antidiabetic activity of AQELOS in STZ induced diabetic rats.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride ½ TD (0.036 mg/ 200g, p.o) followed by single dose of AQELOS (100 mg/kg, p.o.) has shown a significant reduction in serum glucose levels at 4, 6, 8 and 16 h . The group treated with repeated doses of glimepiride ½ TD (0.036 mg/ 200g, p.o) followed by single dose of AQELOS (200 mg/kg, p.o.) group has shown a significant reduction in serum glucose levels at 3, 4, 6, 8, and 16 h. The group treated with repeated doses of glimepiride ½ TD (0.036 mg/ 200g, p.o) followed by single dose of AQELOS (400 mg/kg, p.o) has significantly reduced serum glucose levels throughout the study.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride TD (0. 072 mg/ 200g, p.o) followed by single dose of AQELOS (100 mg/kg, p.o.) has shown a significant reduction in serum glucose levels at 1, 2, 3, 4, 6, 8, 10, 12 and 24 h . The group treated with repeated doses of glimepiride TD (0. 072 mg/ 200 g, p.o) followed by single dose of AQELOS (200 and 400 mg/kg, p.o) has significantly reduced serum glucose levels throughout the study.

When compared to glimepiride ½ TD (0.036 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride 2 TD (0. 144 mg/ 200g, p.o) followed by single dose of AQELOS (100, 200 and 400 mg/kg, p.o.) has shown a significant reduction throughout the experimental study.

When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride ½ TD (0.036 mg/ 200 g, p.o) followed by single dose of AQELOS (100, 200 and 400 mg/kg, p.o.) has shown a significant reduction in serum glucose levels at 6 h, 4-6 h and 3-16 h respectively.

When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, the group treated with repeated doses of TD (0. 072 mg/ 200g, p.o) followed by single dose of AQELOS (100 mg/kg, p.o.) has shown a significant reduction in serum glucose levels at 4-6 h . The group treated with repeated doses of TD (0. 072 mg/ 200g, p.o) followed by single dose of AQELOS (200 and 400 mg/kg, p.o) has shown a significant reduction throughout the experimental study.

When compared to glimepiride TD (0. 072 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride 2 TD (0. 144 mg/ 200g, p.o) followed by single dose of AQELOS (100 mg/kg, p.o.) has shown a significant reduction in serum glucose throughout the experimental study except at 2 h. The group treated with repeated dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) followed by single dose of AQELOS (200, 400 mg/kg, p.o) has shown a significant reduction throughout the experimental study.

When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride ½ TD (0.036 mg/ 200g, p.o) followed by single dose of AQELOS (100 and 200 mg/kg, p.o.) has not shown any significant reduction in serum glucose levels. But the group treated with repeated

doses of glimepiride ½ TD (0.036 mg/ 200g, p.o) followed by single dose of AQELOS (400 mg/kg, p.o) has shown a significant reduction in serum glucose levels at 4 and 6 h respectively.

When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride TD (0. 072 mg/ 200g, p.o) followed by single dose of

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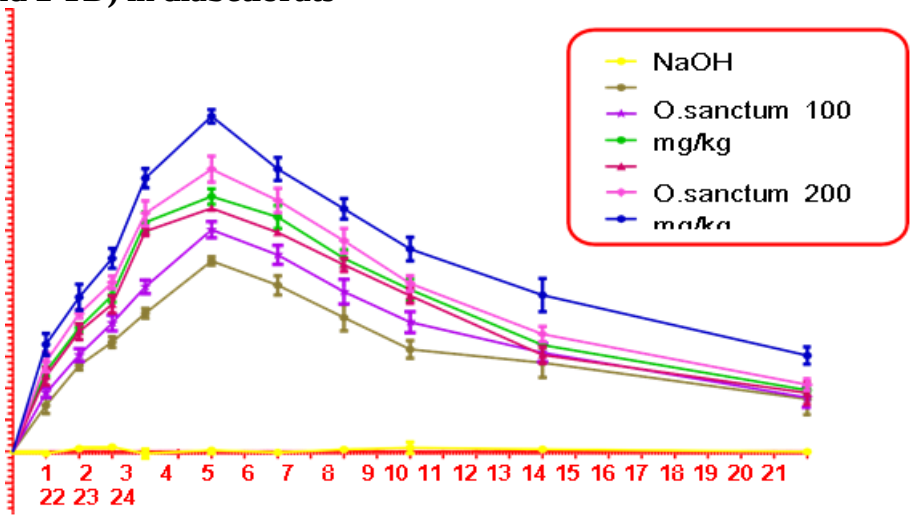
AQELOS (100 mg/kg, p.o.) has shown no significant reduction in serum glucose levels. The group treated with repeated doses of glimepiride TD (0. 072 mg/ 200g, p.o) followed by single dose of AQELOS (200 and 400 mg/kg, p.o) has shown a significant reduction in serum glucose levels at 4-8 h and 4-12 h respectively.
When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride 2 TD (0. 144 mg/ 200g, p.o) followed by single dose of AQELOS (100 and 200 mg/kg, p.o.) has shown a significant reduction in serum glucose levels at 6-8 h and 16-24 h. The group treated with repeated dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) followed by single dose of AQELOS (400 mg/kg, p.o.) has shown a significant reduction throughout the experimental study.
All the groups have shown maximum reduction in serum glucose levels at 6th h only.

When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride TD (0. 072 mg/ 200g, p.o) followed by single dose of AQELOS (100 mg/kg, p.o.) has shown no significant reduction in serum glucose levels. The group treated with repeated doses of glimepiride TD (0. 072 mg/ 200g, p.o) followed by single dose of AQELOS (200 and 400 mg/kg, p.o) has shown a significant reduction in serum glucose levels at 4-8 h and 4-12 h respectively.
When compared to glimepiride 2 TD (0. 144 mg/ 200g, p.o) treated group, the group treated with repeated doses of glimepiride 2 TD (0. 144 mg/ 200g, p.o) followed by single dose of AQELOS (100 and 200 mg/kg, p.o.) has shown a significant reduction in serum glucose levels at 6-8 h and 16-24 h. The group treated with repeated dose of glimepiride 2 TD (0. 144 mg/ 200g, p.o) followed by single dose of AQELOS (400 mg/kg, p.o.) has shown a significant reduction throughout the experimental study.
All the groups have shown maximum reduction in serum glucose levels at 6th h only.

Fig.no. 1 Percentage reduction in serum glucose with repeated dose treatment of *O. sanctum* (100, 200 and 400 mg/Kg) and repeated dose treatment of glimepiride (½ TD, TD and 2 TD) in diabetic rats



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Conclusion:-

Herb–drug interaction is an important issue affecting the efficacy and safety of therapeutic treatments. The present study has shown a dose dependent increase in anti-diabetic action of AQELOS and glimeperide when administered as single doses. Further there is an increase in the hypoglycaemic action of glimepiride when administered in combination with various doses of the AQELOS which confirms a positive herb-drug interaction between *O. sanctum* and glimepiride.

In order to avoid hypoglycaemia care must be taken i.e. blood glucose levels are to be checked regularly, during concurrent administration of both drugs *O. sanctum* and glimepiride in the same diabetic individual.

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