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Model Test for Oral Hypoglycemic Activity Of Parthenium Weed In Albino Mice

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Abstract: Experiments were carried out to assess the impact of Parthenium hysterophorus L. on hypoglycemic effect of albino mice. Swiss albino mice in laboratory condition was given alloxan dose 50 mg/kg body wt. through i.p. route. After 24 hours glucose level of mice were observed. Then different dilution of Parthenium as given orally and after 2 hour again blood-glucose level was checked. The alloxan induced glucose level was significantly lowered without damaging other physiological parameters. So Parthenium hysterophorus may reduce alloxan induced diabetes mellitus.

Keywords : Parthenium hysterophorus, Alloxan, Hypoglycemia, Diabetes mellitus.

1. Introduction

Parthenium is an annual herb with a deep taproot and an erect stem that becomes woody with age. Parthenium is native of West Indies.

Scientific name: Parthenium hysterophorus L.

Common names: Carrot weed, white top, Congress grass, star weed

Family: Asteraceae

Taxonomic position: Division: Magnoliophyta

Class: Magnoliopsida

Order: Asterales

2. Distribution:

Argentina, Australia, Bangladesh, China, Cuba, Dominican Republic, Ethiopia, Haiti, Honduras, India, Jamaica, Madagascar, Mauritius, Mexico, Mozambique, Nepal, New Caledonia, Pakistan, Papua New Guinea, Puerto Rico, South Africa, Sri Lanka, Swaziland, Trinidad, the United States of

America, Venezuela, Vietnam and West Indies. Parthenium probably entered India before 1910 (through contaminated cereal grain), but went unrecorded until 1956.[1] Since 1956, the weed has spread like wildfire throughout India.[2]

3. Description:

An annual herb, erect, up to 2 m in height; the stem is branched and covered with trichomes. Leaves are pale green, lobed, hairy, initially forming a basal rosette of strongly dissected leaves that are up to 30 cm in length, close to the soil, alternate, sessile, irregularly dissected and bipinnate, having small hairs on both the sides, resembling the leaves of carrot.Each flower contains five seeds, which are wedge-shaped, black, 2 mm long with thin white scales.[3] A large single plant produces up to 100,000 seeds in its lifecycle.[4]

4. Habitat:

Parthenium grows luxuriantly in wastelands and vacant lands, orchards, forestlands, flood plains, agricultural areas, scrub / shrublands, urban areas, overgrazed pastures and along roadsides and railway tracks. Drought, and subsequent reduced pasturecover, creates the ideal situation for the parthenium weed to establish itself.[5] It prefers alkaline, clay loam to heavy black clay soils, but tolerates a wide variety of soil types. The weed grows well in areas where the annual rainfall is greater than 500 mm and falls dominantly in summer.[6] It can grow up to an elevation of 2200 m above sea level.

5. Similar species:

Parthenium hysterophorus can be confused with Ambrosia artemisiifolia (annual ragweed), Ambrosia psilostachya (perennial ragweed), Ambrosia confertiflora (burr ragweed) and Ambrosia tenuifolia (lacy ragweed) when in the vegetative stage of growth. However, P. hysterophorus can be distinguished from all these species by its ribbed stems, and also by white flower-heads (capitula) when it is in flower.

6. Chemical Constituents:

Chemical analysis of P. hysterophorus has indicated that all its parts including trichomesand pollen contain toxins called sesquiterpene lactones (SQL).P. hysterophorus contains a bitter glycoside parthenin, a major sesquiterpene lactone. Other phytotoxic compounds or allelochemicals are hysterin, ambrosin, flavonoids such as quercelagetin 3,7-dimethylether, 6-hydroxyl kaempferol 3-0 arabinoglucoside, fumaric acid. P- hydroxy benzoin and vanillic acid, caffeic acid, p courmaric, anisic acid, p-anisic acid, chlorogenic acid, ferulic acid, sitosterol and some unidentified alcohols.[7]

7. Toxicity:

7.1. Impact On Crops: Due to the presence of chemicals like parthenin, hysterin, hymenin and ambrosin the weed exerts strong allelopathic effects on different crops grown in association.

7.1.1. Poor germination and crop growth.

7.1.2. Affects nodulation in legumes due to inhibition of activity of nitrogen fixing and nitrifying bacteria viz., Rhizobium, Actynomycetes, Azotobactor and Azospirillum.

7.1.3. Parthenium produces enormous quantity of pollen (on an average 624 million/plant), which is carried away at least to short distance in clusters of 600-800 grains, and settles on the vegetative and floral parts, including stigmatic surface inhibiting fruit setting in crops like tomato, brinjal, beans, capsicum and maize.

7.1.4. Reduction in yield up to 40% in agricultural crops and 90% in forage crops.[8]

7.1.5. The weed acts as an alternate host for many diseases caused by viruses in crop plants.

7.2. Impact On Humans:

7.2.1. Direct contact with plant causes contact dermatitis. Adult males are more sensitive than females while children below twelve years age are unlikely to be affected.

7.2.2. Even the presence of pollen in the air is allergic to some and may result in diseases like fever and asthma.[9]

7.2.3. It is a major cause of Allergic, Trinities Sinusitis, affecting about ten percent of the people who live near it (Tower & Subarao, 1992).

7.2.4. It reduces yield of milk and weight of animals.

7.2.5. The major components of toxic being parthenin and other phenolic acids such as caffeic acid, vanillic acid, ansic acid, ρ-anisic acid, chlorogenic acid and parahydroxy benzoic acid are lethal to human beings and animals.[10]

8. Use Of Perthenium:

Parthenium is reported to have insecticidal, nematicidal and herbicidal properties. It is also used for composting. The odour of the plant is apparently disagreeable to bees and they can be easily kept away by carrying a handful of Parthenium flower heads. A root decoction of the plant is used in treating amoebic dysentery. Sub-lethal doses of parthenin, a toxin recovered from Parthenium, exhibited antitumor activity in mice and the drug can either cure mice completely or increase their survival time after they had been injected with cancer cells. Parthenin is also found to be pharmacologically active against neuralgia and certain types of rheumatism.[11]

9. Diabetes Mellitus :

It is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipaemia, negative nitrogen balance and sometimes ketonaemia. A widespread pathological change is thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency. Normal glucose level is 80-120 mg/dl.

Two major types of diabetes mellitus are-

Type I Insulin-dependent diabetes mellitus (IDDM), juvenile onset diabetes mellitus: There is β cell destruction in pancreatic islets; majority of cases are auto immune (type1A) antibodies that destroy β cells are detectable in blood, but some are idiopathic (type1B)-no β cell antibody is found.

Type II Noninsulin-dependent diabetes mellitus (NIDDM), maturity onset diabetes mellitus: There is no loss or moderate reduction in β cell mass; insulin in circulation is low, normal or even high, no anti-β-cell antibody is demonstrable; has a high degree of genetic predisposition; generally has a late onset (past middle age). Over 90% cases are type 2 DM.

10. Oral Hypoglycemic Agent :

There agents lowers blood glucose levels & are effective orally. The chief drawback of insulin is—it must be given by injection. Orally active drugs have always been searched.

11. Drug Used In The Model Test For Hyperglycemic Activity In Albino Mice :

11.1 Alloxan :

About Alloxan Drug : Alloxan (2,4,5,6-tetraoxypyrimidine;2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution. Brugnatelli originally isolated alloxan in 1818 and the name was given by Wohler and Liebig in 1838.[12] Moreover, the compound was discovered by von Liebig and Wohler in 1828 and has been regarded as one of the oldest named organic compounds that exist. The name Alloxan emerged from the merging of two words, i.e., Allantoin and Oxaluric acid. Allantoin is a product of uric acid excreted by the foetus in the allantois and oxaluric acid has been derived from oxalic acid and urea that is found in urine. Additionally, the alloxan model of diabetes induction was first described in rabbits by Dunn, Sheehan and McLetchie in 1943.[13] Alloxan was originally prepared by the oxidation of uric acid by nitric acid. The monohydrate is simultaneously prepared by oxidation of barbituric acid by chromium trioxide. Moreover , alloxan has been regarded as a strong oxidizing agent that forms a hemiacetal with its reduced reaction product; dialuric acid, in which a carbonyl group is reduced to a hydroxyl group, that is called alloxantin.[14] The drug has been noted to exert its diabetogenic action when administered parenterally, i.e., intravenously, intraperitoneally or subcutaneously. Furthermore, the dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Moreover, alloxan has been demonstrated to be non-toxic to the human beta-cells, even in very high doses, the reason of which may be attributed to the differing glucose uptake mechanisms in humans and rodents.[15-16]

11.1.1. Phases of Diabetes Induction:

Alloxan has been used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic beta-islets. Alloxan induces a multiphasic blood glucose response when injected into to an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultrastructural beta cell changes ultimately leading to necrotic cell death. The first phase that comes into view within the first minutes after alloxan injection is transient hypoglycemic phase that lasts maximally for 30 minutes.[17] This little hypoglycemic response has been noted to be the result of a transient stimulation of insulin secretion that was confirmed by an increase of the plasma insulin

concentration. The underlying mechanism of this transient hyperinsulinemia may be attributed to a temporary increase in ATP availability due to inhibition of glucose phosphorylation through glucokinase inhibition. The 2nd phase appearing one hour after administration of alloxan leads to rise in blood glucose concentration. Moreover, the plasma insulin concentration has been noted to decrease at the same time. This is the first hyperglycemic phase after the first contact of the pancreatic beta cells with the toxin.[18-20] This hyperglycemic phase lasts for 2-4 hours which is accompanied by decreased plasma insulin concentrations. These changes are a result of inhibition of insulin secretion from the pancreatic beta cells that is attributed to the induction due to their beta cell toxicity. The 3rd phase is again a hypoglycemic phase that is noted 4-8 hours after the alloxan injection, which lasts for several hours.[21] The flooding of circulation with insulin occurs as a result of the alloxan-induced secretory granule and cell membrane rupture resulting in severe transitional hypoglycemia. In addition, other subcellular organelles are also ruptured that include cisternae of rough endoplasmic reticulum and the golgi complex. Moreover, the outer and inner membranes of the mitochondria loose structural integrity in this particular phase. These changes are irreversible and highly characteristic for a necrotic cell death of pancreatic islets. The last and the 4th phase of the blood glucose response is the final permanent diabetic hyperglycemic phase during which complete degranulation and loss of the integrity of the beta cells within 24-48 hr after administration of the alloxan takes place.[22] Surprisingly, the non-beta cells and other endocrine and non-endocrine islet cell types along with extra pancreatic parenchyma remain intact, providing the evidence of selective toxic action of alloxan. Thus, alloxan injection has been noted to induce an insulin-dependent type I like diabetes syndrome and all the morphological features of beta cell destruction are characteristic for a necrotic cell death.

11.1.2. Mechanism of Action:

Alloxan-induced diabetes has been commonly employed as an experimental model of insulin dependent diabetes mellitus. The mechanism of alloxan action has been thoroughly studied which currently can be characterized quite well. Several experimental studies have demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment.[23] This particular alloxan-induced insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used. Further, the alloxan action in the pancreas is preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features determining alloxan diabetogenicity. Moreover, in pancreatic beta cells, the reduction process occurs in the presence of different reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups.[24] Alloxan reacts with two -SH groups in the sugar binding site of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. As a result of alloxan reduction, dialuric acid is formed which is then re-oxidized back to alloxan establishing a redox cycle for the generation of reactive oxygen species (ROS) and superoxide radicals. The superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous and ferric ions. In addition, superoxide radicals undergo dismutation to yield hydrogen peroxide (H_2O_2) in the presence of superoxide dismutase. As a result, highly reactive hydroxyl radicals are formed according to the Fenton reaction in the presence of ferrous and H_2O_2. Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. The fragmentation of DNA takes place in the beta cells exposed to alloxan that causes DNA damage,

which stimulates poly ADP-ribosylation , a process participating in DNA repair. Antioxidants like superoxide dismutase, catalase and the non- enzymatic scavengers of hydroxyl radicals have been found to protect against alloxantoxicity. In addition, the disturbance in intracellular calcium homeostasis has also been reported to constitute an important step in the diabetogenic action of alloxan. It has been noted that alloxan elevates cytosolic free Ca^{2+} concentration in the beta cells of pancreatic islets.[25] The calcium influx is resulted from the ability of alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca^{2+} ion further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause damage of beta cells of pancreatic islets.

12. Method For Oral Hypoglycemic Activity As Model Test :

12.1 : Procedure :

12.1.1. Extraction Procedure :

12.1.1.1. Collection of parthenium weed from field and cut the roots from each plant.

12.1.1.2. Shade drying the herb for 5 days.

12.1.1.3. After shade drying transfer the plant for tray drying for 1 day at temperature 40 degree centigrade.

12.1.1.4. Grind the herb in hand mill and sieved the grinded material to obtain fine particles and residue particles (coarse particles) were separated.

12.1.1.5. Weigh the fine & coarse particles separately.

12.1.1.6. Extract the coarse particles with chloroform by percolation method and collect the extract in a beaker and keep it in cool temperature.

12.1.1.7. Extract the fine particles with methanol by soxhlet apparatus and collect the extract in a beaker and keep it in cool temperature.

12.1.1.8. Evaporate both the extract in water bath after 2 days.

12.1.1.9. Take the both residue and dilute it with 100ml distilled water in 100ml volumetric flask & keep it for overnight at cool temperature.

12.1.1.10. Take the dilution of coarse particles extract for chemical test.

12.1.1. 11. Take the dilution of fine particles extract for model test. Take the mother dilution and further dilute it by taking 1ml from it to 50ml volumetric flask with 50 ml distilled water. Further dilution from 1st dilution by taking 1ml from it to a 50ml volumetric flask by adding 50 ml distilled water.

12.2. Induced Diabetes In Mice With Allxoan :

12.2.1. Dose of alloxan in mice is 50 mg/kg.

12.2.2. Make the dilution of alloxan in 100ml water for injection in 100ml volumetric flask.

12.2.3. Inject the dilution of the drug in mice (i.p. route).

12.2.4. Observe the glucose level in mice after 24 hours.

12.3. Model Test Procedure :

12.3.1. Take 9 swiss albino mice and divided into 3 groups. Each groups contains 3 mice.

12.3.2. Inject the dilution of the Alloxan in mice (i.p route).

12.3.3. Observe the glucose level in each mice after 24 hours.

12.3.4. The glucose level is rise up in each mice.

12.3.5. Then treat each 3 groups of mice with three different dilution of perthenium extract (fine particles extract) given by orally.

12.3.6. Then keep the 3 groups of mice for 2 hours.

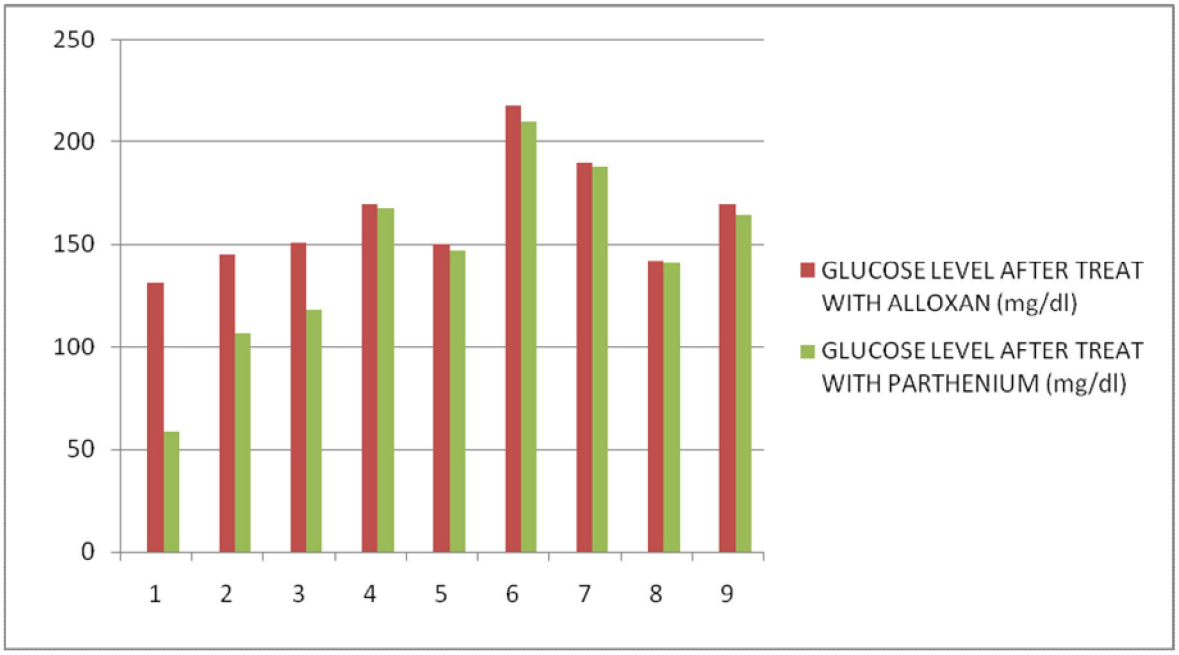
12.3.7. Then check the glucose level of each mice.

13. Result Of Model Test :

Table 1 : Observation of hypoglycemic activity or Parthenium weed :

Group	Marking of mice	Weight of mice (gm.)	Dose of Alloxan (ml.) (i.p.)	Dose of parthenium(oral)	Observation After Alloxan	After parthe -nium
Group A (mother dilution)	A^1	23.3	2.3	1 ml	131mg/dl	59mg/dl
	A^2	25.00	2.5	1 ml	145mg/dl	107mg/dl
	A^3	25.25	2.5	1 ml	151mg/dl	118mg/dl
Group B (1^{st} dilution)	B^1	21.21	2.1	1 ml	170mg/dl	168 mg/dl
	B^2	29.1	2.9	1 ml	150mg/dl	147mg/dl
	B^3	33.7	3.3	1 ml	218mg/dl	210mg/dl
Group C (2^{nd} dilution)	C^1	38.1	3.8	1 ml	190mg/dl	188mg/dl
	C^2	24.1	2.4	1 ml	142mg/dl	141mg/dl
	C^3	33.1	3.3	1 ml	170mg/dl	165mg/dl

Chart 1 : Graphical representation of hypoglycaemic activity of Parthenium weed :



14. Discussion:

Following alloxan administration, alloxan is concentrated in the islets of Langerhans and in the liver where it is reduced to dialuric acid. This acid is unstable in aqueous solutions and undergoes oxidation back to alloxan, accompanied by generation of O_2, H_2O_2 and OH radicals by fenton type reaction (Uchigata, 1982). Latest research has found alloxan to be an effective pro-oxidant selectively cytotoxic to ß cells of the pancreatic islets of langerhans (Shanti, 1994). Hydroxy radicals generated cause single stranded breaks in the islets cell DNA. There is a two-fold increase in lipid conjugated dienes, the primary products of lipid peroxidation (Shanti and Ramakrishnan 1994). The glutathione and catalase activity which can scavange these free radicals are present in large amounts in the liver. On the contrary, the beta islet cells of the pancreas have low quantities of these enzymes and are extremely vulnerable to free radical injury (Halliwell 1989). Alloxan induced experimental diabetes is also associated with marked reduction of anti oxidant enzyme superoxide dismutase activity in islets cells. In antioxidant enzyme superoxide dismutase activity (Halliwell,1989).

After statistical analysis of glucose level in mice, after creating diabetes in mice (by alloxan) the glucose level is reduced to normal value while treating with parthenium dilution. The glucose level is reduced in group a mice very significantly (i.e. glucose level before treating with parthenium is 145 mg/dl & after treating with parthenium the glucose level is reduced to 107 mg/dl).

15. Conclusion :

The chemical induction of diabetes appears to be the most popularly used procedure in inducing diabetes mellitus in experimental animals. The foremost drug-induced diabetic model is the alloxan diabetes that is capable of inducing type I diabetes mellitus in experimental animals. Hence, alloxan induced diabetes model appears to be the most reliable and easily reproducible method of inducing

diabetes mellitus in experimental animals. After performing the model test with parthenium the glucose level is reduced in group A mice while treated with mother dilution of parthenium (59mg/ml).But 1st & 2nd dilution of mother dilution cannot reduced the sugar level in mice.

So,It is concluded that parthenium dilution may be reduced glucose level in diabetes mellitus.

So, Parthenium may have oral hypoglycemic activity.

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