Research Article

Pharmacological Studies of Pitrashish 8 in 1 Juice (Animal study)

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Herbs used in Pitrashis 8 in 1 Juice

Kerela: ***Momordica charantia***

Momordica charantia often called bitter melon, bitter gourd or bitter squash. Its Bengali name is Kerela. Scientific Classification of this plant is, **Kingdom**: Plantae, **Order:** Cucurbitales, **Family**: Cucurbitaceae, **Genus**: *Momordica*.

Chemical Composition

Momordica charantia has a non-nitrogenous neutral principle charantin, and on hydrolysis gives glucose and a sterol. The fruit pulp of M. charantia has soluble pectin but no free pectic acid. Galactouronic acid is also obtained from the pulp. M.charantia fruits glycosides, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids. The presence of an unidentified alkaloid and 5-hydroxytryotamine is also reported. The ether extract residue of the alcoholic concentrate from the leaves of M. charantia is reported to reveal hypoglycemic activity comparable to that of tolbutamide. The pure protein termed as P-insulin extracted from M. charantia fruits in crystalline form is also tested.

Uses

Popularity of Momordica charantia in various systems of traditional medicine for several ailments (antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies) focused the investigator's attention on this plant.

A lot has been written lately about the very beneficial aspects of bitter melon in the treatment of diabetes. Bitter melon has a host of bitter chemicals, which are hypoglycemic in action. It also has at least one substance that is like the insulin secreted by the human pancreatic glands. Hence, bitter melon is extremely effective in the treatment of diabetes mellitus. Doctors all over the world prescribe having either bitter melon juice early in the morning or to include it in some other fashion in the daily diet. Regular use of bitter melon over a period of time helps to bring the blood sugar level down.

Amla: *Phyllanthus emblica*

Phyllanthus emblica, the Indian gooseberry or amla , is a deciduous tree of the **Family:** Phyllanthaceae, **Order:** Malpighiales, **Genus:** *Phyllanthus*. It is known for its edible fruit of the same name. Phyllanthus has a remarkable diversity of growth forms including annual and perennial herbs, shrubs, climbers, floating aquatics, and pachycaulous succulents.

Chemical Composition

The specific contents are disputed, and the overall antioxidant strength of amla may derive instead from its high density of ellagitannins such as emblicanin A , emblicanin B, punigluconin and pedunculagin. It also contains punicafolin and phyllanemblinin A, phyllanemblin other polyphenols: flavonoids, kaempferol, ellagic acid and gallic acid.

Phyllanthus emblica juice contains high levels of ascorbic acid (vitamin C). Ayurvedic preparations that contain Phyllanthus emblica may increase the concentration of ascorbic acid by up to three times. Various parts of this plant show antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective, gastroprotective, and chemopreventive properties.

Medical Uses

In medicine, dried and fresh Phyllanthus emblica is used, including the fruit, seed, leaves, root, bark, and flowers. Traditionally, Phyllanthus emblica is used alone and in combination with various Ayurvedic herbs for various medical conditions, including pancreatitis, hepatitis, inflammation, cancer, diabetes, obesity, kidney disease, and stomach problems. It is also considered a natural adaptogen.

Harad: *Terminalia bellirica*

Terminalia bellirica, known as "Bahera" or Beleric or bastard myrobalan, is a large deciduous tree common on plains and lower hills in Southeast Asia, where it is also grown as an avenue tree .Terminalia belerica is growing widely throughout the Indian subcontinent, Sri Lanka and SE Asia. This plant is come under the **Kingdom:** Plantae, **Order:** Myrtales, **Family:** Combretaceae, **Genus:** Terminalia. In the Traditional system of medicine like Ayurveda, Siddha and Unani, medicinal uses have been described as it is works in disease of every system. Glucoside, Tannins, Galliacid, Ellagicacid, Ethylgalate, Gallylglucose, Chebulanic acid are mainly believed to be responsible for its wide therapeutic actions.

Chemical constitutes

Glucoside (bellericanin) , Gallo-tannic acid, Coloring matter, resins and a greenish yellow oil.Ellargic acid, gallic acid, lignans(termilignan and thanni lignan), 7-hydroxy 3’4’ (methylene dioxy) flavone and anolignan B10. Tannins, ellargic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyllemblin, β-sitosterol, mannitol, glucose, fructose and rhamnose.

Medicinal uses
antimicrobial, anticancer, antioxidant, antidiarrhoeal, analgesic, immumomodulatory, antihypertensive, antisolmonella, hepatoprotective, antispasmodic and bronchodilatory activities. Further the plant is used in the treatment of gastric ulcer, constipation, general debility, piles. antidiabetic activity- e plant extracts significantly decreased the serum levels of total cholesterol, triglycerides, low density lipoprotein cholesterol, urea, uric acid and creatinine in diabetic rats.

Baheda: *Belliric Myrobalan*

Kingdom: Plantae, **Class:** Magnoliopsida, **Order:** Myrtales, **Family:** Combretaceae, **Genus:** Terminalia, **Species:** T. bellirica, **Popular Name(s):** Beleric Myrobalan, Bibhitaki, Bahera, Bahira, Bilhitak, Baheda, Vibhidhaka, Bastard Myrobalan,BeddaNut. Beleric is a large deciduous tree found throughout India, in areas up to an altitude of 1,000 meters. The tree takes a height of 30 meters, while the bark is brownish grey in color. They blossom in the month of May. The fruits are ovoid grey drupes and the kernels are sweet, but narcotic. The tree is found in abundance in Madhya Pradesh, Uttar Pradesh, Punjab and Maharashtra. It is known as vibhitaki, karshaphala and kalidruma in Sanskrit and bahera in Hindi.

Chemical Constitutes
Sitosterol, gallic acid, ellagic acid, chebulagic acid, galloyl glucose, fatty acid, protein, oxalic acid, tannin, palmitic acid, oleic acid, linoleic acid, galactose, glucose, ethyl gallate.

Medical Uses
The herb is an excellent remedy for stomach disorders like diarrhea, indigestion, antidiabetic activity constipation and stomach worms. Belliric Myrobalan is helpful in alleviating cough. It is an important herbal ingredient in Ayurvedic medication for treating eye disorders. Beleric is a rejuvenative and laxative. It proves beneficial for hair, throat and eyes. Beleric seed oil or fruit paste is applied on swollen and painful parts. The seed oil gives excellent results in skin diseases and premature graying of hair. Fruit pieces are baked and chewed for cough, cold, hoarseness of voice and asthma. Beleric fruit is powdered and used to dress wounds to arrest the bleeding. Beleric fruits and kernels are used in making medicated hair oil, used to alleviate pain and burning sensation, boost hair growth and impart black color to the hair. The paste of the fruit is applied on eyelids, in case of conjunctivitis. The herb is used in various eye ailments, such as myopia, corneal opacity, pterigium, immature cataract, chronic and acute infective conditions. Beleric helps in loss of appetite, flatulence, thirst, piles and worms. The ripened fruit acts as an astringent and anti-diarrheal. The decoction of the kernels is used in case of excessive thirst and vomiting. Beleric plant alleviates cough, relieves blocked phlegm, controls bleeding in the sputum and eases bronchospasms. It prevents ageing, imparts longevity, boosts immunity, improves mental faculties and enhances the body resistance against diseases. It helps in lowering cholesterol and blood pressure.

Jamun: *Syzygium cumini*

Syzygium cumini is slow growing species; it can reach heights of up to 30 m and can live more than 100 years. Its dense foliage provides shade and is grown just for its ornamental value. At the base of the tree, the bark is rough and dark grey, becoming lighter grey and smoother higher up. The wood is water resistant. **Order:** Myrtales, **Family:** Myrtaceae, and **Genus:** *Syzygium*.

Chemicals Constitutes

Jambolan is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar and seed extract has lowered blood pressure by 34.6% and this action is attributed to the ellagic acid content. The seeds have been reported to be rich in flavonoids, a well-known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes and also found to have high total phenolics with significant antioxidant activity.

Medicinal Uses

This plant is acrid, sweet, digestive, astringent to the bowels, anthelmintic and used for the treatment of sore throat, bronchitis, asthma, thirst, biliousness, dysentery and ulcers. It is also a good blood purifier. The fruit is acrid, sweet, cooling and astringent to the bowels and removes bad smell form mouth, biliousness, stomachic, astringent, diuretic and antidiabetic. The fruit has a very long history of use for various medicinal purposes and currently has a large market for the treatment of chronic diarrhea and other enteric disorders. The seed is sweet, astringent to the bowels and good for diabetes.

In the early 1960s to 1970s, some preliminary reports on the antidiabetic activity of different parts of jambolan in diabetic animals were reported. Most of these studies have been conducted using crude preparation of the plant without pointing out their chemical profile and antidiabetic action in animals is not fully understood. A number of herbal formulations were also prepared in combination with this plant available in market which showed potential antidiabetic activity and are used regularly by diabetic patients on the advice of the physicians. It exhibits hypolipidemic effect in diabetic rats. Reduces tissue damage in diabetic rat brain. Prevents the cataract development in diabetic rats. Attenuates the progression of renal damage in diabetic mice.

Neem: *Azadirachta indica*

Azadirachta indica, also known as Neem, Nimtree, and Indian Lilac is a tree in the mahogany family Meliaceae. It is one of two species in the genus ***Azadirachta***, and is native to India, Pakistan, and Bangladesh growing in tropical and semi-tropical regions. Neem tree is the official tree of the Sindh Province and is very common in all cities of Sindh, there are projects underway for planting this tree in all over Sindh Province. This plant is come under the **order** of Sapindales, **Family:** Meliaceae, **Genus:** *Azadirachta*.

Chemicals Constitutes

Nimbin (sulphur-free crystalline product with melting point at 205 °C, empirical composition $C_7H_{10}O_2$), nimbinin (with similar principle, melting at 192 °C), and nimbidin (cream-coloured containing amorphous sulphur, melting at 90–100 °C). nimbidin as the main active antibacterial ingredient, and the highest yielding bitter component in the neem . These compounds are stable and found in substantial quantities in the Neem. They also serve as natural insecticides.

Medical Uses

Anti-inflammatory, Antidiabetic , Anti-thelmintic, Antiarthritic , Antipyretic , Hypoglycaemic , Antigastric ulcer, Spermicidal ,Antifungal, Antibacterial, Anti-inflammatory, Antibacterial, Antiviral, Antiulcer effect, Antimalerial, Antifungal Anticancer, Antioxident, Anti-inflammatory ,Immunmodulatory. Effect on central nervous system, pathogenic fungi, bacteria, viral, protozoan and Helminthes are sensitive to neem preparations, with antiseptic properties. NSO and leaves extract significantly inhibited fertility in males, but not anti-ovulatory, hence “sensal” a contraceptive.

Gurmar: *Gymnema sylvestre*

Gymnema sylvestre is an herb native to the tropical forests of southern and central India and Sri Lanka. Chewing the leaves suppresses the sensation of sweet. This effect is attributed to the presence of the eponymously named gymnemic acids. G. sylvestre has been used in herbal medicine as a treatment for diabetes for nearly two millennia. This plant comes under the **Order** of Gentianales, **Family:** Asclepiadaceae, **Genus:** *Gymnema*.

Chemical constitutes

G. sylvestre leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemasides. Besides this, other plant constituents are flavones, anthraquinones, hentri-acontane, pentatriacontane, α and β-chlorophylls, phytin, resins, dquercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives. Gymnemic acids have antidiabetic, antisweetener and anti-inflammatory activities. The antidiabetic array of molecules has been identified as a group of closely related gymnemic acids after it was successfully isolated and purified from the leaves of G. sylvestre. Later, the phytoconstituents of G. sylvestre were isolated, and their chemistry and structures were studied and elucidated.

Medicinal Uses

Gymnema reduces the taste of sugar when it is placed in the mouth. From extract of the leaves were isolated glycosides known as gymnemic acids, which exhibit anti-sweet activity. This effect lasts up to about 2 hours. Some postulate that the herb may block sugar receptors on the tongue. This effect was observed in isolated rat neurons.

The active ingredients are thought to be the family of compounds related to gymnemic acid: purified gymnemic acids are widely used as experimental reagents in taste physiology and have also an anti-

diabetic effect in animal models, reduce intestinal transport of maltose in rats when combined with acarbose, and reduce absorption of free oleic acid in rats.
Historically, the leaves were used for stomach ailments, constipation, water retention, and liver disease; however, these claims are not supported by scientific studies
This *in vitro* data suggests that extracts derived from Gymnema sylvestre may be useful as therapeutic agents for the stimulation of insulin secretion in individuals with diabetes. The rise in insulin levels may be due to regeneration of the cells in the pancreas. G. sylvestre can also help prevent adrenal hormones from stimulating the liver to produce glucose in mice, thereby reducing blood sugar levels. Clinical trials with type 2 diabetics in India have used 400 mg per day of water-soluble acidic fraction of the Gymnema leaves administered for 18–20 months as a supplement to the conventional oral drugs.

Methi: *Trigonella foenum-graecum*

Fenugreek (**Trigonella foenum-graecum**) is an annual plant in the family Fabaceae with leaves consisting of three small obovate to oblong leaflets. It is cultivated world wide as a semi-arid crop, and especially its seeds are a common ingredient in dishes from the Indian Subcontinent. This plant come under the **Order** of Fabales, **Family:** Fabaceae, **Species:** *Trigonella*

Chemical Composition
Fenugreek is a natural source of iron, silicon, sodium and thiamine. Fenugreek contains mucilagins which are known for soothing and relaxing inflamed tisuues. Fenugreek seeds contain alkaloids, including trigonelline, gentianine and carpine compounds. The seeds also contain fibre, 4-hydroyisoleucine and fenugreekine, a component that may have hypoglycemic activity. The mechanism is thought to delay gastric emptying, slow carbohydrate absorption and inhibit glucose transport. Fenugreek may also increase the number of insulin receptors in red blood cells and improve glucose utilization in peripheral tissues, thus demonstrating potential anti-diabetic effects both on the pancreas and other sites. The amino acid 4-hydroxyisoleucine, contained in the seeds, may also directly stimulate insulin secretion.

Effects Fenugreek on Sugar Decreasing and Diabetes
Fenugreek seed powder in the diet reduces blood sugar and urine sugar with concomitant improvement in glucose tolerance and diabetic symptoms in type 2 diabetic patients. The hypoglycemic effects of fenugreek have been attributed to several mechanisms. In vitro the amino acid 4-hydroxyisolcucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells; it was observed that **4- hydroxyisoleucine** extracted from fenugreek seeds has insulin tropic activity. This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. In human studies, fenugreek reduced the area under the plasma glucose curve and increased the number of insulin receptors, although the mechanism for this effect is unclear. In humans, fenugreek seeds exert hypoglycemic effects by stimulating glucose dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of alpha-amylase and 203ignali, two intestinal enzymes involved in carbohydrate metabolism.

Medicinal Uses
Helps treat diabetes and reduce Cholesterol. It has been proven to be an excellent remedy for reducing level of bad Cholesterol levels from our body. It is also used to Aids Digestio,prevent hair

loss, moisturisation of hair, Helps In Losing Weight, antidote for skin problems, prevents dandruff and stren

Toxicological Studies of Pitrashis 8 In 1 Juice

Toxicity Study

In the evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is an initial step. It provides information about a number of health hazards likely to arise from short-term exposure by the oral route. Data from an acute toxicity study may serve as a basis for classification, labeling and also in establishing a dosage regimen in sub-chronic and other studies. They also may show good therapeutic activity. Thus it helps in the determination of minimum dose, which can produce desired results in 50 % population.

In any toxicity experiment animals are treated with drugs and observed for toxic manifestations. To increase the chances of toxic manifestations the dose is chosen much higher than the therapeutic dose and for a longer duration. Drug is administered to a group of animals to get statistically reliable results. For acute toxicity study a group of animals are taken comprising of 5-10 animals in each group. Generally mice or rats are employed for the study. Sometimes dogs or monkeys are also used for this purpose. However, determination of therapeutic dose, emetic dose, and minimum symptomatic or toxic dose is greatly encouraged as these give ideas about the extent of toxic manifestations when given at a particular dose.

The animals used for the acute toxicity study are kept in identical laboratory condition at least two weeks for acclimatization to the working environment. They are fasted for 18 hours prior to drug administration. Drug is given once a day either orally or parenteraly. The dose should be such that at least three doses should cause less than 100% mortality. Now the number of deaths in each group is recorded after 24 hours and 72 hours. But here we observed that up to **5 ml/kg** body weight of the juice there was no death in treated animals. So it can be said that PITRASHIS 8 in 1 MAGIC JUICE is **safe** in animals.

Here we observed some toxicological parameter to establish how much safe it is. These parameters are following.

RESULT OF TOXICITY STUDY

1. Liver Function Test

1) Determination of Alkaline Phosphates after treatment with PITRASHIS 8 IN 1 MAGIC JUICE

Test	Control	Treated with 8 IN 1 MAGIC JUICE
Alkaline Phosphates	320.2±2.50	317.6± 3.35*

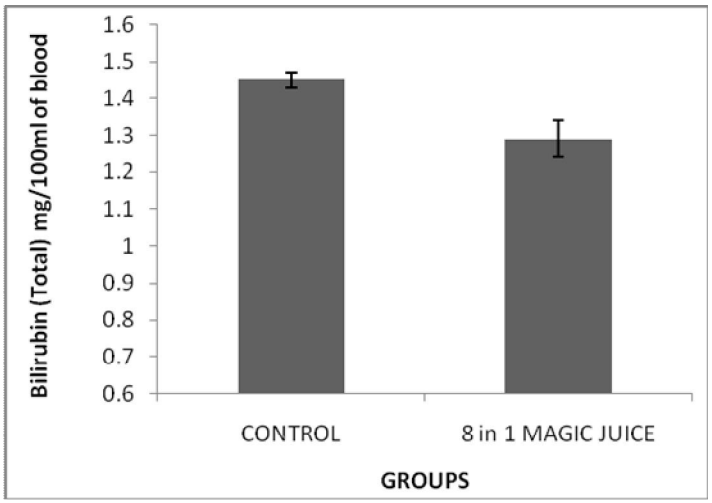
No. of Animals: 20*Statistically there is no change.

2) Determination of total Bilirubin after treatment with PITRASHIS 8 IN 1 MAGIC JUICE

Test	Control	Treated with 8 IN 1 MAGIC JUICE
Bilirubin (Total) mg/100ml of blood	1.45±0.02	1.29±0.05*

No. of Animal: 20

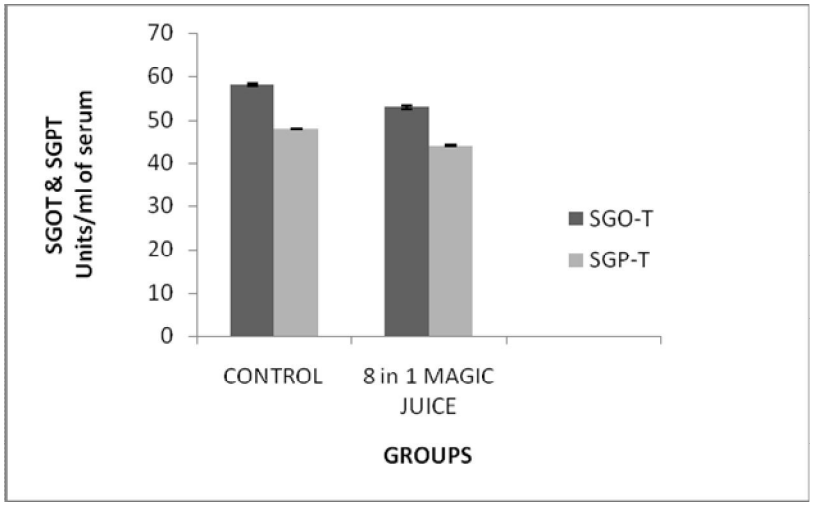
*Statistically there is no change.



3) Determination of SGO-T & SGP-T levels after treatment with PITRASHIS 8 IN 1 MAGIC JUICE

Test (Units/ml of serum)	Control	Treated with 8 IN 1 MAGIC JUICE
SGO-T	58±0.30	53±0.35*
SGP-T	48±0.21	44± 0.09*

No. of Animal: 20
*Statistically there is no change.

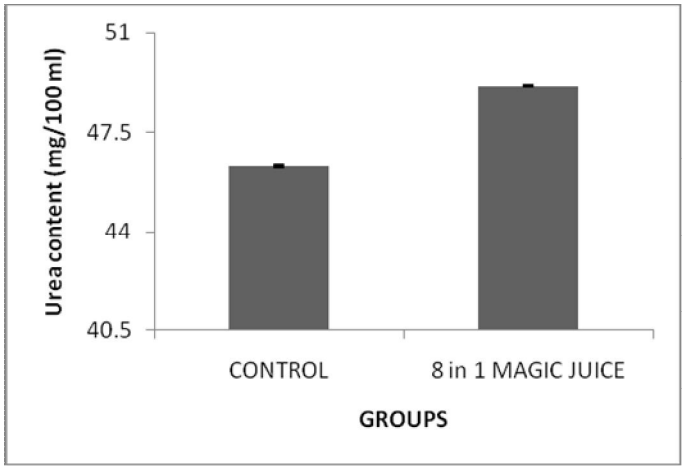


1. Kidney Function Test

Determination of Urea levels after treatment with PITRASHIS 8 IN 1 MAGIC JUICE

Test	Control	Treated with 8 IN 1 MAGIC JUICE
Urea mg/100 ml of blood	46.6±0.03	49.1±0.02*

No. of Animals: 20
*Statistically there is no change



CONCLUSION

Above toxicological data revealed that **Pitrashish** 8 in 1 Magic Juice and control data are more or less same. It is clear from the toxicological studies in animal that Pitrashish 8 in 1 Magic Juice is safe.

1. The mechanism of alloxan action

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyura-cil) was first described by Brugnatelli in 1818. Wöhler and Liebig used the name “alloxan” and described its synthesis by uric acid oxidation (for review see Lenzen and Panten 1988). The diabetogenic properties of this drug were reported many years later by Dunn, Sheehan and McLethie (1943), who studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic islets. Since then, alloxan diabetes has been commonly utilized as an animal model of insulin-dependent diabetes mellitus (IDDM).

Alloxan exerts its diabetogenic action when it is administered parenterally: intravenously, intraperito-neally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse (Eizirik *et al*. 1994). The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg b.w. (Gruppuso *et al*. 1990, Boylan *et al*. 1992). When alloxan is given intraperito-nealy or subcutaneously its effective dose must be 2-3 times higher. The intraperitoneal dose below 150 mg/kg b.w. may be insufficient for inducing diabetes in the rat (Katsumata *et al*. 1992, 1993). Fasted animals are more susceptible to alloxan (Katsumata *et al.* 1992, Szkudelski *et al.* 1998), whereas increased blood glucose provides partial protection (Bansal *et al*. 1980, Szkudelski *et al*. 1998).

The mechanism of alloxan action has been intensively studied, predominantly *in vitro*, and is now characterized quite well. Using isolated islets (Weaver *et al*. 1978b) and perfused rat pancreas (Kliber *et al*. 1996) it was demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose. This phenomenon appeared just after alloxan treatment and was not observed after repetitive exposure of islets to this diabetogenic agent (Weaver *et al*. 1978b). The sudden rise in blood insulin concentration was also observed *in vivo* just after alloxan injection to rats (Szkudelski *et al*. 1998). Alloxan-induced insulin release is, however, of short duration and is followed by complete suppression of the islet response to glucose,even when high concentrations (16.6 mM) of this sugar were used (Kliber *et al*. 1996).

Alloxan is a hydrophilic and unstable substance. Its half-life at neutral pH and 37 □ C is about 1.5 min and is longer at lower temperatures (Lenzen and Munday 1991). On the other hand, when a diabetogenic dose is used, the time of alloxan decomposition is sufficient to allow it to reach the pancreas in amounts that are deleterious.

The action of alloxan in the pancreas is preceded by its rapid uptake by the B cells (Weaver *et al*. 1978a, Boquist *et al*. 1983). Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining alloxan diabetogenicity. Another aspect concerns the formation of reactive oxygen species (Heikkila *et al*. 1976). A similar uptake of alloxan also takes place in the liver. However, the liver and other tissues are more resistant to reactive oxygen species in comparison to pancreatic B cells and this resistance protects them against alloxan toxicity (Malaisse *et al*. 1982, Tiedge *et al*. 1997). The formation of reactive oxygen species is preceded by alloxan reduction. In B cells of the pancreas its reduction occurs in the presence of different reducing agents. Since alloxan

exhibits a high affinity to the SH-containing cellular compounds, reduced glutathione (GSH), cysteine and protein-bound sulfhydryl groups (including SH-containing enzymes) are very susceptible to its action (Lenzen and Munday 1991). However, other reducing agents such as ascorbate may also participate in this reduction (Zhang *et al*. 1992). Lenzen *et al.* (1987) proposed that one of the SH-containing compounds essential for proper glucose-induced insulin secretion is glucokinase (EC 2.7.1.2), being very vulnerable to alloxan. Alloxan reacts with two -SH groups in the sugar-binding side of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. Glucose can protect glucokinase against the inactivation hindering the access of alloxan to the -SH groups of the enzyme (Lenzen *et al*. 1987, 1988, Lenzen and Mirzaie-Petri 1991).

Dialuric acid is formed as a result of alloxan reduction. It is then re-oxidized back to alloxan establishing a redox cycle for the generation of superoxide radicals (Munday 1988). The reaction between alloxan and dialuric acid is a process in which intermediate alloxan radicals (HA) and an unidentified "compound 305" (maximum absorption at 305 nm) is formed. The latter appears when alloxan is reduced by GSH (Sakurai and Ogiso 1991). Superoxide radicals areable to liberate ferric ions from ferritin and reduce them to ferrous ions. Fe^{3+} can also be reduced by alloxan radicals (Sakurai and Ogiso 1995). Moreover, superoxide radicals undergo dismutation to hydrogen peroxide:

$O_2^- + O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$

This reaction may occur spontaneously or may be catalyzed by superoxide dismutase (EC 1.15.1.1)

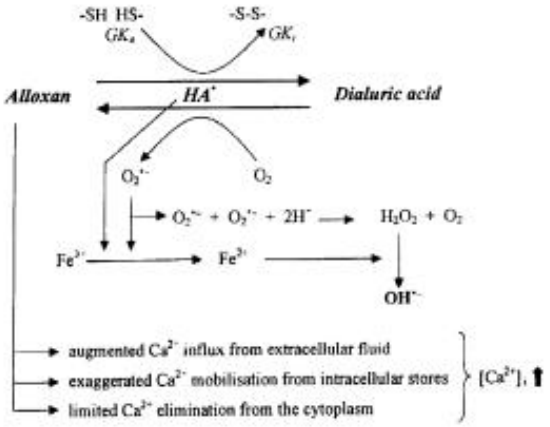


Fig. 1: The mechanism of alloxan-induced reactive oxygen species generation in B cells of rat pancreas. GKa, GKi – glucokinase active and inactive, respectively; HA – alloxan radicals; $[Ca^{2+}]_i$ – intracellular calcium concentration

(Malaisse 1982). In the presence of Fe^{2+} and hydrogen peroxide, highly reactive hydroxyl radicals are then formed according to the Fenton reaction (Fig. 1): $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-$

The action of hydroxyl radicals following alloxan treatment was demonstrated *in vitro* (Grankvist 1981, Munday 1988) and *in vivo* (Kurahashi *et al*. 1993).

One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in B cells exposed to alloxan (Takasu *et al*. 1991a, Sakurai and Ogiso 1995). DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair. Some inhibitors of poly ADP-ribosylation can partially restrict alloxan toxicity. This effect is, however, suggested to be due to their ability to scavenge free radicals rather than to a restriction of poly ADP-ribosylation initiated by alloxan (Sandler and Swenne 1983, LeDoux *et al*. 1988). Superoxide dismutase, catalase (EC 1.11.1.6) (Grankvist *et al*. 1979, Grankvist 1981, Jörns *et al*. 1999) and non-enzymatic scavengers of hydroxyl radicals (Ebelt *et al*. 2000) were also found to protect against alloxan toxicity. Therefore, chemicals rendering anti-oxidative properties and inhibiting poly ADP-ribosylation can attenuate alloxan toxicity.

It has been argued that glucose counteracts alloxan cytotoxicity *in vitro* and *in vivo*. This ability, however, is not only the result of the protection of glucokinase. The protective effect of glucose against necrotic death of B cells may be due to interaction of the sugar with the glucose transporter GLUT2 resulting in limited alloxan uptake (Jörns *et al*. 1997).

It has been previously proposed that the action of glucose is also related to its metabolism and to the increased generation of reducing equivalents (NADH and NADPH) accelerating the recirculation of glutathione. GSH is known to provide protection against free radicals (Donnini *et al*. 1996). It may thus divert hydrogen peroxide from the pathway leading to the formation of hydroxyl radicals (Malaisse 1982, Malaisse-Lagae *et al*. 1983, Pipeleers and van de Winkel 1986):

$$GSSG + 2\ NADPH \rightarrow 2\ GSH + 2\ NADP^{+}$$
$$H_2O_2 + 2\ GSH \rightarrow GSSG + 2\ H_2O$$

Moreover, Sakurai and Ogiso (1991) observed that the *in vitro* generation of hydroxyl radicals in the presence of alloxan strongly depends on GSH concentration. GSH in low concentrations potentiated the formation of these radicals, whereas the oxygen consumption, autoxidation of dialuric acid and formation of hydroxyl radicals were significantly inhibited in higher concentrations. GSH at high concentrations can also inhibit HA$^{\bullet}$ generation and directly neutralize hydroxyl radicals. Thiyl radicals (GS) formed in this reaction are then converted to GSSG:

$$GSH + OH^{\bullet -} \rightarrow GS^{\bullet} + H_2O$$
$$GS^{\bullet} + GS^{\bullet} \rightarrow GSSG$$

Indeed, in rat islets incubated with alloxan the GSH content and GSH/GSSG ratio were decreased (Malaisse *et al*. 1982), whereas glucose evoked the opposite effect.

In the *in vivo* experiment, glucose given to rats 20 min prior to alloxan partially restricted alloxan-induced increase in the activity of glutathione peroxidase (EC 1.11.1.9) and mitigated the drop of liver nonprotein -SH groups (especially reduced glutathione) (Szkudelski *et al*. 1998). The protective action of this sugar is, however, strongly glucose and alloxan dose-dependent (Harman and Fischer 1982, Gorray *et al*. 1983).

It has been proposed that disturbances in intracellular calcium homeostasis constitute an important step in the diabetogenic action of alloxan. This concept was confirmed by *in vitro* and *in vivo* experiments demonstrating that alloxan elevates cytosolic free Ca^{2+} concentration in pancreatic B cells (Kim *et al*. 1994, Park *et al*. 1995). This effect arises from several events: alloxan-induced calcium influx from extracellular fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination from the cytoplasm. The calcium influx may result from the ability of alloxan to depolarize pancreatic B cells (Dean and Matthews 1972). Depolarization of the cell membrane opens voltage-dependent calcium channels and enhances calcium entry into cells. Alloxan was also found to exert a stimulatory effect on mitochondrial Ca^{2+} efflux with simultaneous inhibitory action on Ca^{2+} uptake by mitochondria (Nelson and Boquist 1982, Lenzen *et al*. 1992). The restriction of calcium removal from the cells due to alloxan-induced inhibition of liver plasma membrane Ca^{2+}-ATPase was also reported (Seckin *et al*. 1993). The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by H_2O_2 since hydrogen peroxide itself exerts a similar effect on calcium concentration in B cells (Park *et al*. 1995).

Thus, the previously mentioned sudden rise in insulin release from B cells treated with alloxan (Weaver *et al*. 1978b, Kliber *et al*. 1996) may be one of the effects of alloxan-induced augmentation in cytosolic Ca^{2+} concentration (Weaver *et al*. 1978b, Kim *et al*. 1994). The exaggerated concentration of this ion contributes to supraphysiological insulin release and, together with reactive oxygen species, causes damage of pancreatic B cells.

The results of experiments with calcium channel antagonists have confirmed the important role of cytosolic calcium in the cytotoxic action of alloxan.Pretreatment of rats with verapamil prevented the alloxan-induced increase in B cell Ca^{2+} concentration and abolished the stimulatory effect of alloxan on insulin release (Kim *et al*. 1994). The calcium channel antagonists (verapamil and diltiazem) also suppressed hyperglycemia and the onset of alloxan diabetes in rats (Katsumata *et al*. 1992, Kim *et al*. 1994).

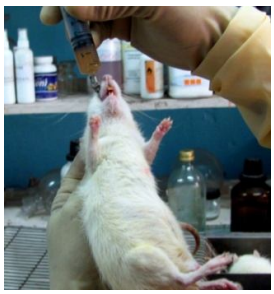
Summing up, the toxic action of alloxan on pancreatic B cells, described many years ago by Dunn *et al.* (1943), are the sum of several processes such as oxidation of essential -SH groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis.

Many investigators suggested that the selectivity of alloxan action is not quite satisfactory. Recent experiments confirmed this objection. The diabetogenic dose of alloxan was found to decrease -SH groups accompanied by a simultaneous rise in glutathione peroxidase activity in the rat liver two minutes after its administration (Szkudelski *et al*. 1998). At the same time, the blood insulin concentration rose dramatically. This exaggerated insulinemia did not evoke, however, any significant reduction of blood glucose suggesting impaired peripheral insulin sensitivity in the short time after alloxan treatment (Szkudelski *et al*. 1998). It was also observed that alloxan intensified basal and epinephrine-induced lipolysis in isolated rat adipocytes and insulin failed to restrict this effect (Kandulska *et al*. 1999).

Thus, using alloxan to evoke diabetes, animals should be examined after proper period of time to minimize side effects of alloxan action. It should also be emphasized that the range of the

diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic causing the loss of many animals. This loss is most likely due to kidney tubular cell necrotic toxicity, in particular when too high doses of alloxan are administered (Lenzen *et al.* 1996).

MATERIALS AND METHODS



Product details

The **PITRASHIS** 8 in 1 JUICE was collected from Pitrashish Global,Herbertpur,Dehradun(Uttarakhand),India .

Chemicals and Reagents used

Alloxan was purchased from Lobachemical laboratory Regent and Fine chemical. Glibenclamide used was purchased from medical store of Aventis Pharma Ltd., Goa, India product's. The other chemicals and reagents used were of analytical grade.

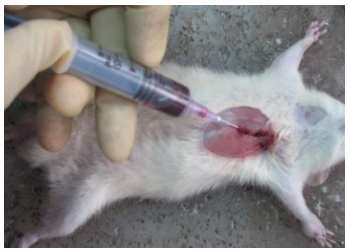
Animals

Healthy adult male Sprague-dawley rats (150-200gms) having age between 2-4months were obtained from Indian Institute of Chemical Biology (IICB, Kolkata, India) were grouped and housed in polypropylene cages under 12hrs light dark cycle at 24±1ºC with free access to standard pellet diet and water ad libitum. The animals were allowed to acclimatize to the lab condition for 7days prior to the experiment.

Oral Glucose Tolerance Test

Rats were divided into four groups with 10 in each group (n=10) and were administered orally with Normal saline, 8 in 1 MAGIC JUICE at doses 1ml/kg B.W , standard drug Glibenclamide (10mg/kg B.W)and one is only diabetic induced respectively. Glucose load of 2gm/kg B.W was administered orally 30mins after administration of the extract. The blood glucose level was estimated at 0, 30, 60, 90, 120 mins after withdrawing blood via retro-orbital plexus of the eye under light ether anesthesia. The glucose level was estimated using the standard GOD-POD kit method (Span Diagnostics Ltd, Surat,India).

Induction of Diabetes



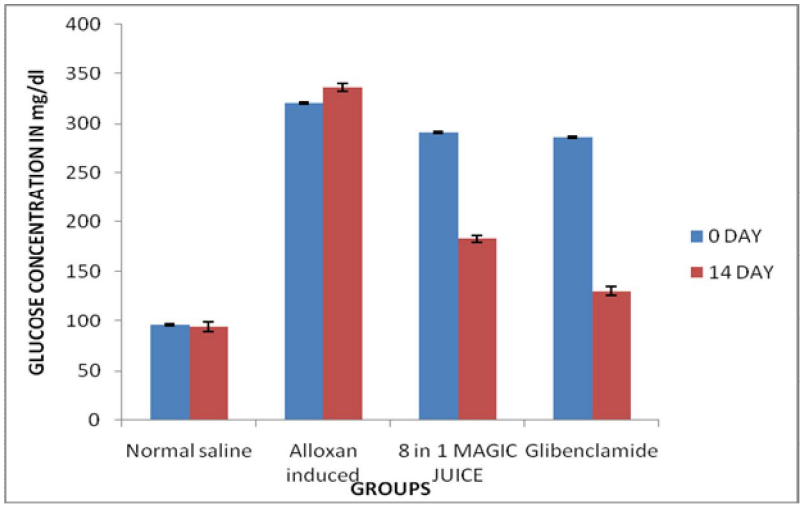
The animals were fasted overnight and were administered with a single intraperitoneal injection of Alloxan (160mg/kg,B.W) in 0.1M freshly prepared citrate buffer pH 4.5. Normal control group received citrate buffer. As alloxan causes severe destruction of pancreatic cells leading to the death of the animal due to hypoglycaemia, so glucose at 10gm/kg B.W dose was provided 6hrs after administration. After 72hrs, the animals were screened for fasting blood glucose (FBG) and the animals with FBG≥250mg/dl of blood were included in the study.

Experimental design and antidiabetic activity

In the experiment 40 rats were used which were divided in four groups with 10 animals in each group (n=10). Group I served as normal control group receiving 0.1ml/kg B.W normal saline solution. Group II, III were alloxan induced diabetic rats treated orally with Glibenclamide (10mg/kg, B.W), 8 in 1 MAGIC JUICE(1ml/kg, B.W) respectively for 14 days and group IV only diabetic induced. Rats were sacrificed by cardiac puncture at 14th day and blood glucose were estimated by GOD-POD kit (Span diagnostics, Surat, India).

Effect of PITRASHIS 8 in 1 MAGIC JUICE on blood glucose level in alloxan induced rats

Group	0 day	14 day
Normal saline	96.35±5.12	94.18±4.90
Alloxan induced	320.65±3.20	335.85±3.95
8 in 1 MAGIC JUICE	290.15±3.95	183.25±3.26
Glibenclamide	285.30±4.16	130.45±4.65



Effect on PITRASHIS 8 in 1 MAGIC JOUICE on body weight of rats

DAY	BODY WT. OF GLIBENCLAMIDE	BODY WT. OF 8 in 1 MAGIC JUICE	BODY WT. OF CONTROL
1.	142.75±0.06	152.80±0.31	123.65±0.27
2	138.40±0.35	153.05±0.56	124.23±0.34
3	138.15±1.50	153.65±0.28	124.47±0.63
4	140.75±0.65	152.95±0.61	124.98±0.28
5	138.55±0.83	152.40±0.23	125.15±0.18
6	137.40±0.36	151.25±0.57	125.35±0.93
7	137.50±0.23	151.50±0.63	125.85±0.26
8	136.45±0.82	150.75±0.81	126.20±0.18
9	136.15±0.34	150.05±0.39	126.65±0.56
10	135.95±0.93	149.65±0.91	126.83±0.83
11	135.10±0.12	149.03±0.58	127.69±0.21
12	134.75±0.67	148.25±0.61	128.05±0.31
13	134.50±1.23	147.85±0.73	129.14±0.57
14	134.10±0.95	147.60±0.28	130.12±0.87

CONCLUSION

It is revealed from the pharmacological studies in animals that **PITRASHIS** 8 in 1 JUICE possess blood glucose lowering capacity.

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