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Published:
— *with international search report*
— *with amended claims*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/49636 A1

(54) Title: AN ANTIDIABETIC COMPOSITION OF AMINO ACIDS

(57) Abstract: Present invention provides a selected composition of L-Lysine Hydrochloride, L-Leucine, L-Glycine, L-Cysteine hydrochloride and L-Glutamic acid, with very effective and safe antidiabetic properties and which in addition to reducing blood glucose levels in diabetes, has the potential to alleviate the secondary complications of the diabetes. The invention describes the composition of the above amino acids, process for the preparation of different formulations, including the preferred ranges and their use as anti diabetic agents.

AN ANTIDIABETIC COMPOSITION OF AMINO ACIDS

Technical Field

This invention relates to a novel composition consisting of bio-active and bio-acceptable amino acids for treatment and control of various forms of diabetes.

Diabetes Mellitus is an insidious disease, which has no cure at present and is considered to be a major global health problem. About 160 million people all over the world are currently estimated to be suffering from diabetes which according to WHO predictions is likely to cross the 300 million mark by the end of 2025. Incidence of diabetes has increased by 40% among the people in the age group of 40 and by 70% for people in the age group of 30 during 1990-1998. In India alone over 20 million diabetes patients need treatment. Diabetes is estimated to affect a significantly higher percentage of the population in India when compared with other countries. It is noticed that the Indian racial groups settled in other countries also show a higher rate of incidence when compared to other nationalities. These facts clearly indicated that Indians are more prone to diabetes.

There are two major types of diabetes (1) Insulin Dependant Diabetes Mellitus (IDDM) and (2) Non Insulin Dependant Diabetes Mellitus (NIDDM). It is shown that incidence of insulin dependent diabetes is particularly high in Indian children below the age of 15 when compared to

statistics from other countries. Insulin dependent diabetes is shown to have a strong genetic aetiology. Conventional treatment for this type of diabetes is administration of insulin extraneously in a rigorous and disciplined manner.

Non insulin dependent diabetes can be controlled by proper and regulated diet and exercise. Blood sugar levels may thus be controlled and medication is avoided at least in the early stages of detection and in borderline cases. However, chronic cases require treatment both insulin and other anti-diabetic drugs. Incidence of this type of diabetes is also found to be on the increase and various anti-diabetic drugs and formulations are available in the market. These drugs act by stimulating insulin secretion, improving absorption of glucose and by increasing insulin sensitivity. In non-insulin dependent diabetes, insulin production may be normal but impaired glucose absorption and consequent increase in blood sugar level may be due to insulin resistance. Major problems facing chronic diabetic patients are diabetic retinopathy, nephropathy, diabetic neuropathy, non-healing diabetic ulcers, neuritis, cataract retinopathy and heart ailments.

Proteins are known to stimulate insulin secretion and they do play an important role in the absorption and utilization of dietary carbohydrates. While there have been suggestions on the utility of amino acids for the control of blood sugar in hyperglycemic patients based on animal experiments there has been no systematic evaluation of their utility. For example, it has been reported that cataractogenis could be prevented by

preventing glycation of lens proteins mediated by chronic diabetes. This, in turn, has lead to the suggestion lysine or a mixture of amino acids could be useful in the treatment of diabetes. However, it is reported that lowering of blood sugar is not immediate and sustained which lead to the hypothesis that the mechanism may be mostly through the scavenging of glucose by the amino acids administered (Sulochana et. al. Exp. Eye Research (1998) 67-, 597). Similarly while it is reported that amino acids can stimulate insulin release (Fajans et. al.), the pancreatic beta cells do not respond equally well to all amino acids. It was not clear as to what extent, the different capacities of individual amino acids to stimulate insulin release are related to differences in their metabolism (Hellmann et al., Biochem J (1971) 123, 513). For example, studies on rabbit and rat pancreas have indicated that leucine is able to elicit insulin release in a glucose free medium. Lysine has also been reported to ameliorate cataractogenesis in rats (Sulochana et al. Insight Vol. XV(3) 1997.

In addition to the free individual amino acids, such as leucine and lysine, glutathione, the tripeptide containing glycine, glutamic acid and cysteine residues has been studied with respect to its effect on diabetic animals and patients. It has been surmised that diabetes condition lowers the glutathione content in the liver and reduces the activity of Super oxide dismutase, an enzyme responsible for the elimination of active oxygen radicals, administration of glutathione to diabetic rats led to the recovery of liver glutathione concentration due to increased Super oxide dismutase activity. The impairment of glutathione metabolism weakens the defence

mechanism against oxidative stress that the cells experience during the absorption phase of glucose. It has been reported that presence of diabetic complications correlated negatively with the concentration of reduce glutathione. Experimental work with animals has shown the protective effect of glutathione on beta cell toxicity in trials aimed at reducing the beta cell damage in insulin dependent diabetes.

Earlier experimental data has suggested that diabetes bring about considerable oxidative stress which leads to organic damage in the long run. There is also evidence that glutathione, the master physiological anti-oxidant confers protection against the degenerative changes resulting from oxidative stress.

Administration of glutathione orally, however, may not be the right answer for solving the problems associated with control of diabetes, mediated through the use of use of glutathione, since there are doubts that administration of oral glutathione may not provide the blood levels required for pharmacological activity.

In summary, therefore, it is obvious that present day knowledge on the potential utility of amino acids for the control of hyperglycemia suffer from lack of information and evidence on the appropriate composition of amino acids required to meet the challenges of developing a product which has the ability to control blood sugar levels, reduce to the extent possible the long-term complications of chronic diabetes, tackle the problems of Insulin resistance and provide a physiologically acceptable preparation with little or no side effects.

Disclosure of the invention

The object of the present invention is to address the basic drawbacks associated with the current management of diabetes, particularly, Non-insulin Dependent Diabetes Mellitus by developing an anti-diabetic preparation which combines many of the desirable features required for an ideal Anti-Diabetic Drug. The essence of the present invention is related to the development of a novel concept for a novel and innovative method of treatment of acute and chronic diabetes and their attendant long-term complications, which affect the various tissues and organs in the human body. The new approach is to utilize the most desirable combination of amino acids, based on their effect on insulin secretion, glucose absorption, insulin resistance, oxidative stress, diabetic cataractogenesis and retinopathy, and vascular complications.

Surprisingly, it has now been found that a composition of amino acids essentially containing lysine, leucine, cysteine, glycine and glutamic acid, the last three being the building blocks for the anti-oxidant glutathione exhibit excellent antidiabetes properties. The influence of glutathione as an effective anti-oxidant for reducing the oxidative stress in tissues and cells is well documented. There is also evidence that diabetes induces increased oxidative stress in cells and tissues, which may further aggravate problems of glucose absorption. Decreased levels of glutathione and increase in the levels of glutathione oxidation products are noticed in diabetic patients. It is observed that glutathione is minimally absorbed and has low bioavailability.

We have noticed that administration of component amino acids for the biosynthesis of glutathone namely glycine, glutamic acid and cysteine lead to increase in bioavailability of glutathione in vivo.

The present invention resides in the remarkable finding that a composition of amino acids essentially containing lysine, glycine, leucine, glutamic acid and cysteine which may contain other biologically active and biologically acceptable amino acids has powerful anti diabetic properties. Such compositions are found to exhibit blood sugar reducing properties and also inhibit insulin resistance. Several long-term secondary complications of diabetes such as cataractogenesis, retinopathy, diabetic neuropathy and heart diseases are minimized on treatment with compositions of this invention.

A unit dosage of the composition of this invention may have the following composition:

L- Lysine hydrochloride	-125.00 to 1000 mg
L- Leucine	- 25.85 to 206.80 mg
L- Cysteine hydrochloride	- 16.58 to 132.64 mg
L- Glycine	- 10.275 to 82.20 mg
L- Glutamic acid	- 20.01 to 160.08 mg

In addition to the above referenced amino acids, any known biologically acceptable amino acids may also be added to the composition.

The composition of amino acids formulated, according to this

invention, were tested in Streptozotocin induced diabetic rats for its antihyperglycemic activity, in accordance with the defined protocol, with the positive (Phenformin) control and solvent control comparisons.

Albino rats of wistar strain of either sex weighing between the narrow range of weight of 150 to 200 gms were selected and were provided with water and standard commercial rat feed ad libitum. Animals were housed at temperature of 18 to 25 degrees C. Diabetes were induced in the rats by the administration of Streptozotocin at a single dose 50mg/kg, intraperitonially to overnight fasted animals. Blood glucose levels were estimated by obtaining drop of blood from the tip of the rats tail and placing on a strip of lifescan Inc., USA and the values were measured using the glucometer. Blood glucose is estimated at intervals of 48 and 72 hours after Streptozotocin administration, to the overnight fasted animals which was considered as zero blood glucose value. Animals with blood glucose levels 180 to 300 mg/dl were selected for study. In all, there were 3 groups one for amino acid composition, one for Phenformin and one for solvent control. The effect of the preparation of amino acids on blood glucose levels, was measured and evaluated in comparison to solvent and positive control groups. Amino acid composition and Phenformin were administered orally as suspension in 0.3% CMC twice a day for 6 days and blood glucose levels were measured on the 6^{th} day. The statistical significance of the antihyperglycemic activity vis-à-vis solvent and positive control was evaluated using ANOVA.

Results

The blood glucose levels after administration of amino acids were 45% lower than that of the control group and 29% lower than that of the Phenformin group (Positive control) indicating significant anti-diabetic activity for the amino acid composition.

It is to be understood that the dosage levels required for anti-diabetic activity are to be evaluated through pharmacokinetic studies and human trials. Since the composition of the present invention contains nutritional principles, safety is guaranteed and toxic effects are bound to be minimum.

The composition can be administered in a solid dosage form such as plain and coated tablets/hard gelatine capsules/soft gelatine capsules/powder in sachet/liquid dosage form like solution/suspension and parental preparations.

Different dosage forms are prepared using the conventional excipients and the composition of the excipients may change depending upon the dosage form formulated.

Tablets or capsules are formulated by using suitable excipients like microcrystalline cellulose, potato starch, methyl cellulose, hydroxy propyl cellulose, dicalcium phosphate, poly ethylene glycol, hydroxy propyl methyl cellulose, talc, magnesium stearate etc. are used and the process for tabletting normally, not necessarily the same steps.

1. Dispensing

2. Sieving of raw material
3. Dry mixing
4. Wet granulation
5. Drying
6. Dry granulation
7. Blending
8. Compression

The capsules are formulated in two ways. They are

1. Direct filling of the amino acids with or without excipients in capsules.
2. Filling of extruded and spheronized granules into hard gelatine capsules. These granules are filled in 1/2/0/00 size capsules.

The same composition with or without the excipients can be used as a powder in a suitable packing like sachet etc..

This composition can be formulated into a liquid using pharmaceutical vehicles like sorbitol, water, sugar syrup, propylene glycol, glycerine in addition to flavours, buffers, antioxidants and preservatives.

EXAMPLE 1

The following non-limiting example shall serve to describe the invention.

The composition and process for extrusion and spheronisation

technique is as follows.

Granules I	**mg/cap**
L-Lysine hydrochloride	250.00
L-Leucine	51.70
L-Cysteine hydrochloride	33.16
L-Glycine	20.55
Microcrystalline cellulose	45.00
Potato starch	5.99
Methyl cellulose	4.00
Hydroxy propyl cellulose	2.00
Tartrazine	0.60
P.E.G. 6000	2.00

Granules II	**mg/cap**
L-Glutamic acid	40.02
Microcrystalline cellulose	14.60
Potato starch	5.98
Methyl cellulose	2.00
Hydroxy propyl cellulose	2.00
Indigo carmine	0.20

The above ingredients are sieved through 40# and mixed in a suitable mixer for 30 mnts. And water is added slowly to obtain a soft mass. This is passed through an extruder and then spheronised. The spheronised granules are dried at 45 deg C for about 3 hours or till moisture content is less than 2.0% w/w.

The above 2 granules are mixed in a suitable mixer for 1 hour. These granules are filled in 'O' size capsules.

The description given as above with respect to the material (excipients etc.) and process of the extrusion and spheronosation is an example. The same granules may be produced with different excipients and by modifying the process steps in the same technique.

These two granules can also be taken as single granules, by taking all the materials together.

EXAMPLE 2

The following non-limiting example shall serve to described the invention.

All the 5 amino acids namely, L-lysine hydrochloride, L-leucine, L-glycine, L-cysteine hydrochloride, L-glutamic acid were blended geometrically and the blended mix was used to test the hypoglycaemic effect in rats. Thirty six diabetic rats of either sex of Albino strain were used in the study. Animals were given streptozotocin (50mg/kg) to induce diabetes. To one group, the amino acid mix in 0.3% sodium carboxy methyl cellulose dispersion was administered twice a day, at a dose of 131.796 mg per dose for six days and blood glucose levels were checked at initial and at sixth day. However, the drug mix is administered for six days, twice a day. The other group was administered only 0.3% sodium carboxy methyl cellulose dispersion (solvent control group) at the same time intervals as that of the

test group. The results are as follows :

Group	Blood Glucose levels	
	Initial	6th day
Control	260	510
Test	284	281
Positive control (Phenformin)	275	398

As can be seen from the above data, the blood glucose levels of the test group were 45% lower than that of the control group and 29% lower than that of positive control group. The amino acid mixture could contain the raise in blood glucose level, in test group from the initial level, where as solvent treated control group continued to show significantly increasing blood glucose levels.

CLAIMS :

1. An anti-diabetic amino acid composition which comprises a combination essentially of L-Lysine, L-Leucine, L-Glutamic acid, L-Glycine and L-Cysteine and/or pharmaceutically acceptable salts thereof.

2. The composition as claimed in claim 1, a unit dose of which comprises of:

L- Lysine hydrochloride	- 125.00	to	1000	mg
L- Leucine	- 25.85	to	206.80	mg
L- Cysteine hydrochloride	- 16.58	to	132.64	mg
L- Glycine	- 10.275	to	82.20	mg
L- Glutamic acid	- 20.01	to	160.08	mg,

the balance being other amino acids and excipients.

3. The composition as claimed in claims 1 and 2, wherein L-Lysine is one part by weight, L-Leucine is 0.2068 part by weight, L-cysteine hydrochloride is 0.1326 part by weight, L-glycine is 0.0822 part by weight and L-glutamic acid is 0.1600 part by weight, the balance being diluents, excipients and other amino acids.

4. The composition as claimed in claim 1, which consists of 250 mg of L-Lysine hydrochloride, 51.7 mg of L-Leucine, 33.16 mg of L-cysteine hydrochloride, 20.55 mg of L-glycine and 40.02 mg of L-

5. glutamic acid in addition to excipients, colourants and other amino acids.

6. The composition as claimed in claims 1 to 4 in the form of powder, tablets, soft and hard gelatin capsules, suspensions, syrups, parenteral preparations.

7. The composition as claimed in claim 5, wherein the composition is in the form of pellets or spheronised granules.

8. Use of a composition essentially consisting of lysine, leucine, glycine, glutamic acid, and cysteine in treating Diabetes Mellitus.

9. Use of the composition as claimed in claim 7, either alone or in a mixture with known hypoglycemic agents and/or insulin for treating diabetes.

AMENDED CLAIMS

[received by the International Bureau on 20 March 2002 (20.03.02);
original claims 1-9 replaced by new claims 1-8 (2 pages)]

1. An anti-diabetic amino acid composition which comprises a combination of L-Lysine, L-Leucine, L-Glutamic acid, L-Glycine and L-Cysteine and/or pharmaceutically acceptable salts thereof.

2. The composition as claimed in claim 1, a unit dose of which comprises of:

L- Lysine hydrochloride	- 125.00	to	1000	mg
L- Leucine	- 25.85	to	206.80	mg
L- Cysteine hydrochloride	- 16.58	to	132.64	mg
L- Glycine	- 10.275	to	82.20	mg
L- Glutamic acid	- 20.01	to	160.08	mg,

the balance being excipients.

3. The composition as claimed in claims 1 and 2, wherein L-Lysine is one part by weight, L-Leucine is 0.2068 part by weight, L-Cysteine hydrochloride is 0.1326 part by weight, L-Glycine is 0.0822 part by weight and L-Glutamic acid is 0.1600 part by weight, the balance being diluents and excipients .

4. The composition as claimed in claim 1, which consists 250mg of L-Lysine hydrochloride, 51.7mg of L-Leucine, 33.16mg of L-Cysteine hydrochloride, 20.55mg of L-Glycine and 40.02mg of L-Glutamic acid in addition to excipients and colourants.

5. The composition as claimed in claims 1 to 4 in the form of powder, tablets, hard gelatin and soft gelatin capsules, suspensions and syrups.

6. The composition as claimed in claim 5, wherein the composition is in the form of pellets or spheronised granules.

7. Use of a composition consisting of Lysine, Leucine, Glycine, Glutamic acid and Cysteine in treating diabetes mellitus.

8. Use of the composition a consisting of Lysine, Leucine, Glycine, Glutamic acid and Cysteine either alone or in a mixture with known hypoglycemic agents and/or insulin for treating diabetes.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 00/00128

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: A61K 31/198, 9/00, A61P 3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, PAJ, HCAplus, medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 60 255722 A (OTSUKA PHARM. CO. LTD.) 17 December 1985 (17.12.85) (abstract) World Patent Index (online) London, U.K.: Derwent Pub. Ltd. Retrieved from EPOQUE, DW198605, Acc.No. 1986-033308 *abstract.*	1-9
X	WO 82/03773 A1 (BAXTER TRAVENOL LABORATORIES INC.) 11 November 1982 (11.11.82) *abstract; claims.*	1-6,8,9
X	US 4279917 A (TAKAMI et al.) 21 July 1981 (21.07.81) *claims.*	1-6
X	EP 0917826 A1 (SCHWARTZ R. S.) 26 May 1999 (26.05.99) *abstract.*	1-7

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed

„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2001 (21.08.2001)	30 August 2001 (30.08.2001)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer KRENN Telephone No. 1/53424/435

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 00/00128

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8,9
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8 and 9 are directed to a therapeutic method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition (see PCT-Article 17, rule 39.1.iv.).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 00/00128

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
EP	A1	917826	26-05-1999	ES	UA	1037109	01-01-1998
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JP	A2	60255722	17-12-1985	JP	B4	3080126	24-12-1991
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				ZA	A	8202608	30-03-1983