Trigonelline: A Plant Alkaloid with Therapeutic Potential for Diabetes and Central Nervous System Disease

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Abstract: There is evidence that *Trigonella foenum-graecum* L. (fenugreek), a traditional Chinese herb, and its components are beneficial in the prevention and treatment of diabetes and central nervous system disease. The pharmacological activities of trigonelline, a major alkaloid component of fenugreek, have been more thoroughly evaluated than fenugreek's other components, especially with regard to diabetes and central nervous system disease. Trigonelline has hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities, and it has been shown to reduce diabetic auditory neuropathy and platelet aggregation. It acts by affecting β cell regeneration, insulin secretion, activities of enzymes related to glucose metabolism, reactive oxygen species, axonal extension, and neuron excitability. However, further study of trigonelline's pharmacological activities and exact mechanism is warranted, along with application of this knowledge to its clinical usage. This review aims to give readers a survey of the pharmacological effects of trigonelline, especially in diabetes, diabetic complications and central nervous system disease. In addition, because of its pharmacological value and low toxicity, the reported adverse effects of trigonelline in experimental animal models and humans are briefly reviewed, and the pharmacokinetics of trigonelline are also discussed.

Keywords: Alkaloid, antibacterial, anti-tumor, central nervous system disease, diabetes, diabetic complications, hypoglycemia, anti-hyperlipidemia, neuroprotective, pharmacokinetics, toxicology, trigonelline, *Trigonella foenum-graecum* L.

INTRODUCTION

The purification of trigonelline (hulubajian in Chinese, Fig. (**1**)), chemically known as *N*-methylnicotinic acid ($C_7H_7NO_2$), from *Trigonella foenum-graecum* L. (fenugreek) (Fig. (**2**)) has facilitated research of the therapeutic effects of trigonelline-containing plants. Trigonelline, a plant alkaloid, was first isolated from the seeds of fenugreek, which is a legume crop used as a spice [1]. Fenugreek is one of the oldest medicinal plants and originates in East Asia and Northern Africa [2, 3]. There are as many as 260 species of fenugreek. Fenugreek is the only widely cultivated species of the genus *Trigonella*. Various portions of fenugreek, including seeds, leaves, and extracts, have been extensively used as antidiabetics in various model systems [4, 5]. Initial animal and human experiments showed possible hypoglycemic and hypolipidemic properties of fenugreek seed powder taken orally. Fenugreek has been utilized for centuries as a folk medicine to treat a wide range of diseases, including diabetes, fever, and abdominal colic, and as a poultice for abscesses, boils, and carbuncles [4, 6, 7].

The content of the active pharmacological constituent of fenugreek, trigonelline, content is approximately 0.1-0.15% of the seed weight [8]. The other components include steroids, alkaloid, polyphenolic substances, volatile constituents, and amino acids [9, 10]. Trigonelline, a plant hormone that is widely distributed in plants within the subclass Dicotyledonae [11], also exists in several animal species, such as arthropods, bryozoans, cnidarians, coelenterates, crustaceans, echinoderms, marine poriferans, mollusks, marine fishes, and mammals. Accumulation of trigonelline occurs in the seeds of various legume species and coffee. It also appears in mammalian urine following administration of nicotinic acid. Trigonelline has been reported to have hypoglycemic, hypolipidemic, sedative, antimigraine, antibacterial, antiviral, and anti-tumor effects, and to improve memory retention and inhibit platelet aggregation [12-18].

Trigonelline is a vitamin B6 derivative with a bitter taste. Its content in green coffee beans is between 0.6 and 1%. When coffee is roasted at 230°C, approximately 85% of the trigonelline is broken down to nicotinic acid, with few trigonelline molecules remaining in the roasted beans. As a secondary metabolite formed from nicotinate, trigonelline is produced in green coffee beans by nicotinic acid (pyridinium-3-carboxylic acid) methylation using methionine, a sulfur-containing amino acid [19]. Trigonelline, most likely the most substantial element that contributes to undue bitterness in coffee, is 100% water soluble. Structure-activity studies of trigonelline are still lacking. Trigonelline has a zwitterionic structure similar to that of a substrate D-amino acid and is a useful active site probe for D-amino acid oxidase. The affinity of trigonelline for D-amino acid oxidase at the enzyme-bound flavin adenine dinucleotide 3-imino group is higher than in the deprotonated state in the neutral state, unlike benzoate, which is a monoanionic competitive inhibitor [20].

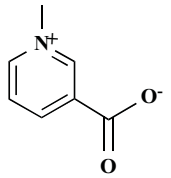

Fig. (1). Chemical structure of trigonelline.

We attempted to identify any relevant published experiments concerning the pharmacological effects of trigonelline *in vivo* and/or *in vitro*. We scanned several electronic bibliographic databases including PubMed, Google Scholar, Scirus, SciFinder Scholar, Web of Science, China Biological Medicine Database, and China National Knowledge Infrastructure Database using combined search terms such as "trigonelline", "*N*-methylnicotinic acid", "hulubajian" (in Chinese), "fenugreek", "*Trigonella foenum-graecum* L.", "pharmacological effects", and "toxicology". We also searched by hand through the reference lists of identified articles.

This review considers the relevant pharmacological research concerning trigonelline folk usage that has been published since the late 1950s and provides a comprehensive review of the characteristics of trigonelline. The pharmacological activity of trigonelline has been more thoroughly examined than those of other components of fenugreek, especially with regard to diabetes and central nervous system disease. Progress in the study of the pharmacological properties and mechanisms of trigonelline has been reported, with particular emphasis on activities relevant to diabetes and its complications and central nervous system disease.

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Fig. (2). *Trigonella foenum-graecum* L. (fenugreek).

There are several recent reviews concerning the pharmacological effects of fenugreek, especially its antidiabetic and hypolipidemic properties [21-24]. An excellent review of the distribution, biosynthetic and degradation pathways, and biological roles of trigonelline was also recently published by Ashihara [25]. However, no comprehensive review of the pharmacology of trigonelline has yet been published. Therefore, we here review the pharmacology (*in vivo* and *in vitro*), pharmacokinetics, and toxicology of trigonelline.

EFFECTS ON DIABETES AND ITS COMPLICATIONS

Fenugreek is often included as an essential ingredient in traditional medicine prescriptions intended to treat diabetes and its complications. As the main alkaloid constituent of fenugreek, trigonelline is a potential treatment for certain disorders related to diabetes, such as hyperglycemia, hyperlipidemia, insulin resistance, and diabetic auditory neuropathy. Accumulating evidence indicates that trigonelline provides protection against diabetes and its complications in various animal models (Table **1**).

Hypoglycemic Effect

In a genetic model of diabetes (KK-A^y obese mice), administration of trigonelline and nicotinic acid lowered blood glucose levels in oral glucose tolerance tests (OGTT) carried out on days 22-23 after feeding, indicating that both trigonelline and nicotinic acid improve glucose tolerance in diabetes with obesity. The fasting serum insulin levels were significantly lower in mice fed trigonelline and showed a lowering tendency in mice fed nicotinic acid, than in the control mice. The liver glucokinase/glucose-6-phosphatase ratios were higher and serum levels of tumor necrosis factor (TNF)-α lower in the trigonelline and nicotinic acid-fed mice compared to the control mice, suggesting that the regulation of glucokinase/glucose-6-phosphatase and TNF-α by trigonelline and nicotinic acid were closely related to the suppression of diabetes in KK-A^y mice [12]. Our recent study also showed that in low-dose streptozocin- and high-carbohydrate/high-fat diet-induced type 2 diabetes in rats, 4-week treatment with trigonelline decreased the blood glucose levels of the diabetic to near those of the control rats [26]. Trigonelline counteracted the hyperglycemic effect of cortisone when administered concomitantly, but not when administered 2 h after cortisone administration, in non-diabetic rabbits. In Sabra albino rats with alloxan-induced diabetes, trigonelline exhibited a mild and transient hypoglycemic effect [27, 28].

Inhibition of Intestinal Glucose Uptake

Trigonelline inhibits intestinal sodium-dependent glucose-uptake, as shown *in vitro* using rabbit intestinal brush border membrane vesicles. However, trigonelline (10 mM) did not inhibit glucagon-induced glycogen phosphorylase activity [29].

Improvement in Glucose Tolerance

Coffee consumption has been associated with a decreased risk of type 2 diabetes. Ingestion of the major coffee components chlorogenic acid (1 g) and trigonelline (500 mg) significantly reduced glucose and insulin concentrations 15 min after a 2-h OGTT compared with placebo (1 g mannitol) in a group of 15 overweight men. Decaffeinated coffee, chlorogenic acid, and trigonelline all failed to affect area under the curve values for glucose or insulin during the OGTT. Therefore, chlorogenic acid and trigonelline reduce early glucose and insulin responses during the OGTT [30]. In OGTTs in non-obese type 2 diabetic Goto-Kakizaki (GK) rats, a pumpkin paste concentrate-fed group maintained a lower glucose level than the control group between 15 min and 60 min after glucose ingestion. The active compounds in the pumpkin extract were isolated and identified as trigonelline and nicotinic acid. Diets containing trigonelline and nicotinic acid improved and tended to improve glucose tolerance, respectively [13]. However, in this and another study published by Yoshinari *et al.* [12], no positive control was used.

Improvements in Insulin Resistance and β Cell Regeneration

In OGTTs administered to non-obese type 2 diabetic GK rats, insulin levels increased after 15 min in trigonelline-fed GK rats and then gradually decreased over the next 120 min. In contrast, the insulin level gradually increased over 120 min in control GK rats, suggesting that trigonelline improves insulin sensitivity [13]. In our studies, trigonelline decreased insulin levels and increased pancreas weights, pancreas to body weight ratios, insulin sensitivity indexes, and pancreatic insulin content in type 2 diabetic rats [26].

Effects on Incretin

In a group of 15 overweight men, decaffeinated coffee, chlorogenic acid, and trigonelline all failed to affect the overall levels of glucagon-like peptide-1 or the gastric inhibitory peptide secretion pattern following an OGTT. Decaffeinated coffee slightly increased the total glucagon-like peptide-1 concentration 30 min

Table 1. Effects of Trigonelline on Diabetes and its Complications *In Vivo* and *In Vitro*

Property	Model	Species/Exposure	Dose/Concentration	Effects	References
1. Hypoglycemic Effect	Type 2 Diabetic Mice	Male KK-A^y/Ta Jcl and C57BL/6J mice (6 weeks old)	0.056% of the Diet for 28 Days	(1) Improved glucose tolerance in diabetes with obesity on day 22-23; (2) increased liver glucokinase/glucose-6-phosphatase ratio and decreased serum TNF-α	[12]
	Streptozocin- and high-carbohydrate/high-fat diet-induced Type 2 diabetes	Sprague-Dawley Rats (180-220 g)	50 mg/kg (*i.g.*) for 28 days	Progressive lowering of blood glucose	[26]
	Alloxan-induced Diabetes	Sabra Albino Rats	250 or 1000 mg/kg via a stomach tube or in the drinking water	Mild and transient hypoglycemic effect	[27, 28]
2. Inhibition of intestinal glucose uptake	*In vitro* using rabbit intestinal brush border membrane vesicles	New Zealand White Rabbit	IC_{50}=19 mM	Inhibition of intestinal sodium-dependent glucose uptake	[29]
3. Increased glucose tolerance	Overweight Men	15 overweight Men (Mean age 39.9±16.5 years and body mass index 27.6±2.2 kg/m^2)	500 mg/kg (*i.g.*) 15 min following a 2 h, 75 g OGTT	Reduction in blood glucose; no effect on the glucose area under the curve values during the OGTT	[30]
	Non-obese type 2 diabetic rats	Male Wistar (Normal Control) and GK rats (8 weeks old)	0.056% of the diet for 43 Days	Lowering of the glucose level between 15 and 60 min of the OGTT compared to controls, i.e., improved glucose tolerance	[13]
4. Improved insulin resistance and β cell regeneration	Non-obese type 2 diabetic rats	Male Wistar (Normal Control) and GK rats (8 weeks old)	0.056% of the diet for 43 Days	Increase in the insulin level after 15 min of the OGTT; gradual decrease over the next 120 min (unlike the gradual increase observed in the control rats)	[13]
	Type 2 Diabetic Mice	Male KK-A^y/Ta Jcl and C57BL/6J mice (6 weeks old)	0.056% of the diet for 28 Days	Reduction in fasting serum insulin	[12]
	Overweight Men	15 overweight men (Mean age 39.9±16.5 years)	500 mg/kg (*i.g.*) 15 min following a 2 h, 75 g OGTT	Reduction in insulin levels; no affect on insulin area under the curve values during the OGTT	[30]
	Streptozocin- and high-carbohydrate/high-fat diet-induced Type 2 diabetes	Sprague-Dawley Rats (180-220 g)	50 mg/kg (*i.g.*) for 28 days	(1) Decreased serum insulin level and increased insulin sensitivity index; (2) increased pancreas weight, pancreas to body weight ratio, and insulin content in pancreas	[26]
5. Incretin	Overweight Men	15 Overweight Men (Mean age 39.9±16.5 years)	500 mg/kg (*i.g.*) 15 min following a 2 h, 75 g OGTT	No effect on overall glucagon-like peptide-1 or gastric inhibitory peptide secretion pattern following an OGTT	[31]
6. Hypolipidemic Effect	Non-obese type 2 diabetic rats	Male Wistar (Normal Controls) and Goto-Kakizaki (GK) rats (8 weeks old)	0.056% of the diet for 43 Days	(1) Decreased serum and liver triglyceride levels; (2) decreased liver fatty acid synthase activity and increased liver carnitine palmitoyl transferase and glucokinase activities	[13]
	Type 2 Diabetic Mice	Male KK-A^y/Ta Jcl and C57BL/6J mice (6 weeks old)	0.056% of the diet for 28 Days	Decreased triglyceride levels in the liver and adipose tissue	[12]
	Streptozocin- and high-carbohydrate/high-fat diet-induced Type 2 diabetes	Sprague-Dawley rats (180-220 g)	50 mg/kg (*i.g.*) for 28 days	Decreased serum total cholesterol and triglyceride levels	[26]
	Rats (Model not provided)	Rats (Strain and weight not provided)	Dose and route of administration not provided	Reduction in total and free plasma cholesterol levels	[32]
7. Antioxidant Effect	HT-29 cells	HT-29 cells	≥30 μM (24 h incubation)	Diminished cellular reactive oxygen species level	[33]
	Streptozocin- and high-carbohydrate/high-fat diet-induced Type 2 diabetes	Sprague-Dawley rats (180-220 g)	50 mg/kg (*i.g.*) for 28 days	Decreased malonaldehyde and nitric oxide contents and increased superoxide dismutase, catalase, glutathione, and inducible nitric oxide synthase activities in pancreas	[26]
8. Treatment of Diabetic auditory neuropathy	Diabetic auditory neuropathy induced by streptozocin (100 mg/kg *i.p.*, one dose)	Male Institute for Cancer Research mice (7 weeks old)	10 mg/kg (*i.g.*) for 9 weeks	Rescue of the hearing threshold shift and delayed latency of the auditory evoked potential induced by streptozocin	[16]
	Auditory Neuropathy induced by increasing doses of pyridoxine	Male Institute for Cancer Research Mice (7 weeks old)	10 mg/kg (*i.g.*) for 5 weeks	Rescue of the hearing threshold shift, Delayed latency of the auditory evoked potential, and sensory fiber loss induced by pyridoxine intoxication	[17]

after ingestion (before the OGTT) relative to placebo (2.7 pM), but this change was not accompanied changes in glucose or insulin secretion. These findings do not support the hypothesis that coffee acutely improves glucose tolerance through effects on the secretion of incretin hormones. However, the chronic effects of ingestion of coffee and its major components still need to be investigated [31].

Hypolipidemic Effect

Trigonelline reduced the total and free plasma cholesterol levels in rats [32]. The serum and liver triglyceride levels in the trigonelline- and nicotinic acid-fed GK rats were lower than those in the control GK rats. Trigonelline and nicotinic acid decreased liver fatty acid synthase activity, and increased liver carnitine palmitoyl transferase and glucokinase activities in GK rats. These results suggest that the regulation of these enzymes by trigonelline and nicotinic acid is closely related to the suppression of both triglyceride accumulation and the progression of diabetes [13]. The triglyceride levels in the liver and adipose tissue of mice fed trigonelline and nicotinic acid were lower than those of control mice, indicating that trigonelline and nicotinic acid reduce the changes in lipid levels accompanied with diabetes [12]. Our study showed that 4-week treatment with trigonelline decreases total serum cholesterol and triglyceride levels in type 2 diabetic rats [26].

Antioxidant Effect

Epidemiological studies suggest that coffee can reduce the risk of degenerative diseases such as type 2 diabetes, cardiovascular disease, and cancer. These beneficial effects have been partially attributed to the antioxidant activity of coffee. Cellular reactive oxygen species (ROS) levels were distinctly diminished by trigonelline treatment of HT-29/Caco-2 cells, underscoring trigonelline's contribution to the antioxidant effects of coffee [33]. In our study, diabetic rats treated with trigonelline for 4 weeks showed decreased malonaldehyde and nitric oxide contents, and increased superoxide dismutase, catalase, glutathione, and inducible nitric oxide synthase activities in the pancreas. These findings suggested that trigonelline had beneficial effects in the diabetic pancreas and acts by up-regulating antioxidant enzyme activities and decreasing lipid peroxidation [26]. Another study showed that trigonelline had an antioxidant effect on liposome peroxidation [34]. However, trigonelline has almost no hydroxyl radical-scavenging activity, as shown using electron spin resonance methods [35].

Treatment of Diabetic Auditory Neuropathy

Auditory neuropathy is a hearing disorder characterized by an abnormal auditory brainstem response. In one study, auditory brainstem responses, auditory middle latency responses, and otoacoustic emissions were evaluated to assess auditory neuropathy induced by diabetes. Coffee and trigonelline ameliorated the hearing threshold shift and the delayed latency of the auditory evoked potential observed in diabetic neuropathy in mice. These findings show that certain diabetic mouse models are accompanied by auditory neuropathy and that coffee consumption could facilitate recovery from diabetes-induced auditory neuropathy. Furthermore, trigonelline might be the active constituent in coffee (in this regard) [16].

In another study, experimental auditory neuropathy was induced in mice by treatment with increasing dosages of pyridoxine, and the mice were examined for ≤ 10 weeks following the last pyridoxine treatment. The pyridoxine-treated mice exhibited an increase in the hearing threshold shift and delayed latency of both the auditory brainstem response and the auditory middle latency response in a dose-dependent manner. The extent of auditory nerve fiber loss also increased in a dose-dependent manner following pyridoxine intoxication. Trigonelline ameliorated the hearing threshold shift, delayed the latency of the auditory evoked potential, and improved sensory fiber loss induced by pyridoxine intoxication. Thus, high-dose pyridoxine administration can be used to produce a mouse model for auditory neuropathy, and trigonelline may facilitate recovery of pyridoxine-induced auditory neuropathy [17].

EFFECTS ON THE CENTRAL NERVOUS SYSTEM DISEASE

Traditional medicine prescriptions have been used for various central nervous system diseases, such as memory loss, epilepsy, and migraine. As the main alkaloid component of fenugreek, trigonelline could be a useful treatment for certain disorders related to the central nervous system. Accumulating evidence indicating that trigonelline has neuroprotective effects in several animal and cellular models of brain disorders (Table **2**).

Improvement in Memory

Starting 14 days after *i.c.v.* injection of amyloid β(25-35) in male ddY mice, trigonelline, donepezil hydrochloride (0.5 mg/kg), or vehicle (tap water) was administered orally once daily for 15 days. Mice were trained in a Morris water maze for 5 days starting 21 days after the i.c.v. administration of amyloid β(25-35). Six days after the last acquisition test, a retention test was performed in the water maze. The number of crossings over a previous platform position was significantly decreased in the amyloid β(25-35)-injected group compared with the saline-injected group. The change in the number of crossings was rescued by treatment with trigonelline, suggesting that trigonelline treatment improves memory retention [14].

Sedative and Antimigraine Effects

When administered to rats, trigonelline elevates the seizure threshold, indicating that it may act as a sedative [36]. Trigonelline has also been suggested to have antimigraine properties [37].

Anti-histamine and Anti-Cholinergic Effects

Using an isolated organ bath preparation method, researchers have shown that trigonelline has significant anti-histamine effects on guinea pig ileum, anti-cholinergic effects on rat colon, and uterine stimulant effects on rat uterus [38].

Increase in Functional Neurite Outgrowth

Extension of dendrites and axons in neurons may compensate for neural loss and rescue damaged neuronal networks in the brains of patients with dementia. Among the extracts of raw and roasted coffee beans, a methanol fraction of the ethanol extract from raw beans (at a dose of 1 μg/ml) significantly increased the percentage of human neuroblastoma SK-N-SH cells with neurites. Among the subfractions of the methanol fraction was a basic fraction that, at 5 μg/ml, significantly increased neurite outgrowth. Trigonelline was identified as the active ingredient in this regard. Trigonelline increased the percentage of cells with neurites after 3 and 6 days of treatment. In addition, 6-day treatment with trigonelline increased the number of neurites that were positive for phosphorylated neurofilament-H, which is correlated with axonal extension [39]. Trigonelline also induces dendritic and axonal regeneration in rat cortical neurons. In these experiments, amyloid β(25-35) was added to the culture medium (with or without trigonelline) after three days of culture, and trigonelline was shown to prevent both dendritic and axonal atrophy induced by amyloid β(25-35) in a dose-dependent manner [14].

Increase in Neuronal Excitability

Whole cell patch clamp recording and intracellular Ca^{2+} imaging were carried out on rat cultured dorsal root ganglion neurons. At high concentrations, trigonelline decreased spike frequency adaptation, causing dorsal root ganglion neurons to switch from firing single action potentials to multiple firing. This increased excitability was associated with an increase in

Table 2. Effects of Trigonelline on Central Nervous System Disease *In Vivo* and *In Vitro*

Property	Model	Species/Exposure	Dose/Concentration	Effects	References
1. Memory improvement	14 days after *i.c.v.* injection of amyloid β(25-35)	Male ddY mice (6 weeks old)	500 mg/kg orally once daily for 15 days	Increased number of crossings over a previous platform position in the water maze test	[14]
2. Sedative and antimigraine effects	Normal Rats	Rats (Strain not given)	(Not Given)	Elevated seizure threshold	[36, 37]
3. Anti-histaminic and anti-cholinergic effects	Isolated organ bath preparation	Guinea pig ileum and rat colon and uterine	1 g/L	(1) Anti-histamine effect on guinea pig ileum; (2) anti-cholinergic effect on rat colon; (3) stimulant effect on rat uterus	[38]
4. Increased functional neurite outgrowth	Human Neuroblastoma SK-N-SH cells in culture	Human Neuroblastoma SK-N-SH Cells	30 μM for 3 or 6 Days	(1) Increased percentage of cells with neurites; (2) increased number of neurites positive for phosphorylated neurofilament-H	[39]
	Amyloid β(25-35) addition to medium 3 days after initiation of culture	Cultured Rat Cortical Neurons	30 and 100 μM added at the time of amyloid β(25-35) addition	(1) Increased dendritic and axonal regeneration; (2) dose-dependent reduction in both dendritic and axonal atrophy induced by amyloid β(25-35)	[14]
5. Protection against cerebral ischemia	Primarily cultured dorsal root ganglion neurons	Rats (2 Days Old)	0.1 mM	(1) Decreased spike frequency adaptation/neuronal switch from single action potentials to multiple firing; (2) increase in excitability coupled to enhanced KCl-evoked Ca^{2+} influx	[40]
6. Inhibition of $GABA_A$ receptors	Xenopus oocytes expressing α_1 and β_1 subunits of bovine $GABA_A$ receptors	Bovine $GABA_A$ receptors	0.5 mM	Competitive inhibition of GABA-elicited responses	[41]
7. Weak inhibition of acetylcholinesterase	Ellman's method and thin layer chromatography	Acetylcholinesterase enzyme from bovine erythrocytes	IC_{50}=233±0.12 μM	Weak inhibition of acetylcholinesterase	[42]
8. Stimulation of dopamine release	Pheochromocytoma Cells	After loaded with fluo-4 direct dye and incubated for 30 min, fluorescence was measured immediately after addition of the compound, every 2 s for a total of 1 min	4.977 nM to 0.4977 nM	(1) Increased neurotransmitter release by 136 ± 31.0% (at 4.977 μM); (2) increased Ca^{2+} mobilization (EC_{200}= 439 ± 475 μM)	[44]
9. Weak inhibition of amyloid β peptide aggregation	Surface plasmon resonance-based biosensors	Values of surface plasmon resonance shift angle were recorded 20 min after the end wash	1.0 mM in PBS containing 0.002% ammonia	(1) No effect on amyloid β deposition and surface plasmon resonance angle shift; (2) log D value of -3.31; (3) affinity for amyloid β of 0.28×10^8 M^{-1}	[45]

KCl-evoked Ca^{2+} influx. The mechanism of action of trigonelline was shown to involve inhibition of voltage-activated K^+ currents. Trigonelline increases the excitability of dorsal root ganglion neurons and thus may affect development, osmoregulation, and chemical defenses [40].

Inhibition of $GABA_A$ Receptors

In Xenopus oocytes expressing exogenous bovine $GABA_A$ receptors, trigonelline was reported to inhibit GABA-elicited responses in a competitive manner (K_i=13 mM) [41]. This inhibition of $GABA_A$ receptors, the main inhibitory neurotransmitter receptors, by trigonelline may contribute to central nervous system stimulation.

Weak Inhibition of Acetylcholinesterase

Acetylcholinesterase inhibitors provide symptomatic relief of some of the clinical manifestations of Alzheimer's disease. The *in vitro* acetylcholinesterase inhibitory activity of trigonelline was measured using Ellman's method in a 96-well microplate assay and a thin layer chromatography bioassay. Trigonelline weakly inhibited acetylcholinesterase. Galanthamine, which was used as a positive control, inhibited acetylcholinesterase with an IC_{50} of 0.21 μM [42]. In contrast, Orhan *et al.* [43] found that trigonelline (1 g/L) did not inhibit acetylcholinesterase or butyrylcholinesterase *in vitro* using the Ellman method in an enzyme-linked immunosorbent assay.

Stimulation of Dopamine Release *In Vitro*

Because coffee and caffeine are known to affect the limbic system, dopamine release and Ca^{2+} mobilization in pheochromocytoma cells after stimulation with trigonelline was investigated. Trigonelline significantly induced neurotransmitter release by 136 ± 31.0% at a concentration of 4.977 μM (1:100 dilution) and stimulated Ca^{2+} mobilization with an EC_{200} value of 439 ± 475 μM [44].

Weak Inhibition of Amyloid-β Peptide Aggregation

Trigonelline did not affect amyloid-β peptide deposition and surface plasmon resonance angle shift decreased from 125.7 ± 15.6 m° for control incubation (amyloid-β peptide injected alone) to

121.9 ± 25.6 m° for trigonelline (-3.31) demonstrate the predominantly hydrophilic character of the compound at physiological pH. The affinity of trigonelline for the amyloid-β peptide is 0.28×10^8 M^{-1}, which demonstrates relatively weak binding. The results indicate that the hydrophilicity of trigonelline governs its very weak interaction with amyloid-β [45].

OTHER PHARMACOLOGICAL EFFECTS

Antibacterial Effects

A potential antibacterial effect of trigonelline was assayed using the following bacteria and their isolated strains: Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis. Trigonelline was also tested against two fungi, Candida albicans and Candida parapsilosis, using the microdilution method. Trigonelline displayed a very high antibacterial activity towards the following organisms and their isolated strains: Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Enterococcus faecalis, Bacillus subtilis, Candida albicans, and Candida parapsilosis. It showed especially high anti-Candida activity at 4 μg/ml. Trigonelline is ineffective against methicillin-resistant Staphylococcus aureus and extended-spectrum β-lactamase strains [15]. However, Peddie *et al.* [46] found that trigonelline had no antibacterial activity towards Escherichia coli under hyperosmotic conditions, showing no evidence of osmoprotection or urea protection. Trigonelline failed to inhibit the growth of Escherichia coli and Listeria innocua [47]. Staphylococcus aureus did not accumulate trigonelline, so trigonelline is not a potential antistaphylococcal agent for use in urinary tract infections [48].

Trigonelline also has antiadhesive properties and interferes with *Streptococcus mutans'* sucrose-independent adsorption to saliva-coated hydroxyapatite beads. This effect was observed both when trigonelline was present in the adsorption mixture and when it was used to pretreat the beads, suggesting that trigonelline may adsorb to a host surface, preventing tooth receptors from interacting with bacteria [49]. Trigonelline was tested in nine strains of enterobacteria using the disc diffusion method and was shown to inhibit their growth. Using the microtiter plate method, researchers found that trigonelline acted as a natural antimicrobial agent (with an IC_{50} of 2.2±0.5 mg/ml and an IC_{90} of 3.8±0.8 mg/ml) against *Salmonella enterica* [50].

Bacteria adapt to high osmolarity by activating osmoregulated betaine porters and accumulating organic osmolytes intracellularly. Several inhibitors of uropathogens, including urea and organic acids, have been identified in the urine. Trigonelline promotes rapid growth by balancing osmotic forces and stabilizing macromolecular structures against attacks from urea and low pH. However, trigonelline can also enhance urea toxicity [51]; High concentrations (250 mmol, 10 times that of glycine betaine) of trigonelline, added under hypertonic conditions, inhibit glycine betaine accumulation in osmotically stressed Madin Darby canine kidney cells *in vitro*. Glycine betaine, which accumulates in the inner medulla of the kidney, balances the hyperosmotic environment and counters urea denaturation in mammals. Because α-substituted betaines were accumulated by bacteria and not by Madin Darby canine kidney cells, these betaines may be a good basis for the design of antimicrobial agents [52]. Trigonelline also has antiseptic activity [37].

Antiviral Effects

Trigonelline was tested on the DNA virus herpes simplex (type 1) and the RNA virus parainfluenza (type 3). Trigonelline significantly inhibits the survival of herpes simplex virus (type 1), with the greatest non-toxic cytopathogenic effect observed at a concentration of 1.6 μg/ml [15].

Weak Antiparasitic Effects

Trigonelline has weak growth inhibitors of *Plasmodium falciparum* with inhibitory concentration 50% values >100 μg/mL and of *Trypanosoma brucei brucei* and *Leishmania donovani* with efficacy concentration 50% values both >125 μg/mL [53].

Anti-Tumor Effects

Nicotinic acid and trigonelline inhibit invasion of AH109A cells (a rat ascites hepatoma cell line) at concentrations of 2.5-40 μM, without affecting proliferation. The invasive activity of AH109A cells is greater when cells have previously been cultured in a ROS-generating system. Nicotinic acid and trigonelline suppressed the ROS-induced increase in invasive capacity [18]. Trigonelline showed anticarcinogenic activity in P-388 lymphocytic leukemia in mice [54]. Trigonelline has also been reported to have effects in cervical and liver cancer [37].

Oxidative cellular stress initiates Nrf2 translocation into the nucleus, thus inducing antioxidant response element-mediated expression of Phase II enzymes involved in detoxification and antioxidant defense. In addition to the known Nrf2 activator 5-O-caffeoylquinic acid, pyridinium derivatives such as the *N*-methylpyridinium ion have been identified as potent activators of Nrf2 nuclear translocation and expression of selected antioxidative Phase II enzymes in human colon carcinoma cells (HT29). In fact, the substitution pattern at the pyridinium core structure determines the impact of a given compound on Nrf2-signaling. In contrast, trigonelline interferes with Nrf2 activation, effectively suppressing *N*-methylpyridinium ion-mediated induction of Nrf2/antioxidant response element-dependent gene expression. Certain types of coffee, including the raw material and that produced during the roasting process, have increased effects on Nrf2 translocation. The formation of deactivating constituents of the Nrf2/antioxidant response element pathway has a critical role in the chemopreventive properties of trigonelline [55]. Trigonelline was the first chemically identified hormone found to cause cell cycle arrest in complex animal tissues [56].

Reduced Platelet Aggregation

Trigonelline has been reported to weakly inhibit platelet aggregation, but it had no effect on human tissue plasminogen activator-producing cells. However, oral administration of trigonelline-containing fractions of coffee to human subjects resulted in a shortening of plasma euglobulin lysis time [57].

PHARMACOKINETICS

To examine the pharmacokinetic properties of trigonelline, a recent study treated rabbits with fenugreek extract (*i.g.*) or trigonelline (*i.v.*), obtained biological samples, and purified proteins via precipitation with methanol and acetonitrile. An Asahipak NH_2P-50 column was used, with a mobile phase consisting of acetonitrile-water (90:10) at a flow-rate of 1.2 ml/min, a detection wavelength of Ultra Violet 265 nm, and a column temperature of 30°C. The calibration curve was linear in the range from 0.98 mg/L to 31.28 mg/L, with r=0.9986; the detection limit of this method was 50 μg/L. The concentration-time curves of trigonelline in rabbits after administration fit one-compartment (*i.g.*) and two-compartment (*i.v.*) open models, respectively. The main parameters for the fenugreek extract (*i.g.*) were as follows: $T_{1/2(Ka)}$=0.9 h, $T_{1/2(Ke)}$=2.2 h, V=0.64 L/kg, area under the curve=1.93 mg/min/L. The main parameters for trigonelline (*i.v.*) were as follows: $T_{1/2}\alpha$=10.8 min, $T_{1/2}\beta$=44.0 min, K_{21}=0.044 min^{-1}, K_{10}=0.026 min^{-1},

K_{12}=0.017 min^{-1}, area under the curve=931.0 mg/min/L. Trigonelline had a moderate rate of absorption and high rate of elimination in the rabbit. The method used was simple and accurate, with good reproducibility [58].

From 0 to 48 h after trigonelline (*i.v.*, 8 mg/kg) administration in rats, urine samples were collected and purified through C_{18} solid-phase extraction cartridges for analysis by liquid chromatography multi-stage tandem mass spectrometry. The structures of trigonelline metabolites were elucidated according to the changes in the molecular weights of the metabolites (ΔM) and their cleavage patterns in electrospray ionization ion trap multi-stage tandem mass spectrometry. Two phase I metabolites, two phase II metabolites, and the parent drug were identified in rat urine. The liquid chromatography multi-stage tandem mass spectrometry method is rapid, high sensitive, specific, and suitable for the identification of trigonelline and its metabolites in rat urine [59].

After direct injection of trigonelline into the upper end of the duodenum in specific pathogen-free and germ-free rats, the remaining trigonelline in the contents of small intestine was determined after fractionation by ion-exchange and Nucher column chromatography and using high performance thin-layer chromatography. In both specific pathogen-free and germ-free rats, the remaining trigonelline in the sac of small intestine markedly decreased with time. Trigonelline was not destroyed by the small intestinal microflora, and the majority of the trigonelline was absorbed from the small intestine [60]. Trigonelline slightly quenched the fluorescence of human serum albumin, and when the reaction temperature was increased (to 37 or 47°C), the binding reaction was negatively affected. (At 37 and 47°C, respectively, the binding constants were 749.03 L/mol and 106.29 L/mol, and the binding sites value were 1.0077 and 0.7625). Therefore, electrostatic forces mediate the majority of trigonelline's binding to human serum albumin [61].

TOXICOLOGY

Human exposure to trigonelline occurs when trigonelline-containing plants are consumed in the diet. Common foods containing trigonelline include barley, cantaloupe, corn, onions, peas, soybeans, and tomatoes. Exposure can also occur upon the consumption of herbal remedies, coffee, or fish, mussels, or crustaceans containing trigonelline. Approximately 5% of the niacin consumed is converted into trigonelline. Upon oral administration of trigonelline to female volunteers, approximately 20% of the dose was excreted in the urine as trigonelline, and approximately 9% was excreted as N'-methyl-2-pyridone-5-carboxylic acid [62, 63]. All of the orally administered trigonelline was recovered unchanged in rat urine [64]. The oral and subcutaneous median lethal dose (LD_{50}) doses of trigonelline were found to be approximately 5000 mg/kg in rats [27]. When female Sabra albino mice were fed 50 mg/kg trigonelline daily for 21 days, no changes in the weight of the liver, kidney, thymus, thyroid, adrenals, uterus, or ovaries were identified, and no mice died during the experiment [27]. Cats fed 3500 mg trigonelline daily for 62 to 70 days had no visible adverse reactions [65]. At doses of less than 20 mg/kg, trigonelline had no cardiovascular effects [66]. In Madin-Darby bovine kidney cells, 1.6 μg/ml trigonelline had comparable cytotoxicity to the reference (acyclovir, 1.6 μg/ml) [15].

Trigonelline was not mutagenic at concentrations of up to 10,000 μg/plate in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 in either the presence or absence of metabolic activation [38]. A thermal decomposition product prepared by heating trigonelline at 250°C was mutagenic in *Salmonella typhimurium* strain TA98 in the presence of metabolic activation [67]. Trigonelline heated in combination with individual amino acids or glucose was also mutagenic. When trigonelline was heated in combination with multiple amino acids and glucose, the reaction products were mutagenic in *Salmonella typhimurium* strains TA98 and YG1024 but not strain YG1029. In the absence of S9, the heated trigonelline, amino acids, and glucose reaction products were mutagenic in strains TA98 and YG1029, but were toxic to YG0124. Trigonelline was not mutagenic at concentrations of up to 7400 μg/plate in a L5178Y $TK^{+/-}$ mouse lymphoma mutation assay, either with and without metabolic activation.

CONCLUSIONS

In summary, trigonelline has beneficial effects in several human diseases, notably diabetes and central nervous system disease. Its properties include hypoglycemic, hypolipidemic, improving diabetic auditory neuropathy, neuroprotective, antimigraine, sedative, antibacterial, antiviral, and anti-tumor activities; it also improves memory retention and inhibits platelet aggregation. The mechanisms underlying these effects are related to modulation of β cell regeneration, insulin secretion, activity of glucose metabolism-related enzymes, reactive oxygen species generation and scavenging, axonal extension, and neuron excitability. More investigation is required to further elucidate possible mechanisms of trigonelline action, such as its effects on transcriptional pathways and antioxidant actions. Before trigonelline can be used clinically in the treatment of diabetes and its complications or central nervous system disease, further *in vivo* and *in vitro* study is required, especially with regard to its mechanisms of action.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

GK	=	Goto-Kakizaki
OGTT	=	Oral Glucose Tolerance Test
ROS	=	Reactive Oxygen Species
TNF-α	=	Tumor Necrosis Factor α

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