Study of lipid profile and parieto-temporal lipid peroxidation in AlCl₃ mediated neurotoxicity. modulatory effect of fenugreek seeds

Yosra Belaïd-Nouira¹*, Hayfa Bakhta¹, Mohamed Bouaziz², Imen Flehi-Slim¹, Zohra Haouas¹ and Hassen Ben Cheikh¹

Abstract

Background: Peroxidation of lipid (LPO) membrane and cholesterol metabolism have been involved in the physiopathology of many diseases of aging brain. Therefore, this prospective animal study was carried firstly to find out the correlation between LPO in posterior brain and plasmatic cholesterol along with lipoprotein levels after chronic intoxication by aluminium chloride (AlCl₃). Chronic aluminum-induced neurotoxicity has been in fact related to enhanced brain lipid peroxidation together with hypercholesterolemia and hypertriglyceridemia, despite its controversial etiological role in neurodegenerative diseases. Secondly an evaluation of the effectiveness of fenugreek seeds in alleviating the engendered toxicity through these biochemical parameters was made.

Results: Oral administration of AlCl₃ to rats during 5 months (500 mg/kg bw i.g for one month then 1600 ppm via the drinking water) enhanced the levels of LPO in posterior brain, liver and plasma together with lactate dehydrogenase (LDH) activities, total cholesterol (TC), triglycerides (TG) and LDL-C (Low Density Lipoproteins) levels. All these parameters were decreased following fenugreek seeds supplementation either as fenugreek seed powder (FSP) or fenugreek seed extract (FSE). A notable significant correlation was observed between LPObrain and LDL-C on one hand and LDHliver on the other hand. This latter was found to correlate positively with TC, TG and LDL-C. Furthermore, high significant correlations were observed between LDHbrain and TC, TG, LDL-C, LPObrain as well as LDHliver.

Conclusion: Aluminium-induced LPO in brain could arise from alteration of lipid metabolism particularly altered lipoprotein metabolism rather than a direct effect of cholesterol oxidation. Fenugreek seeds could play an anti-peroxidative role in brain which may be attributed in part to its modulatory effect on plasmatic lipid metabolism.

Background

A close relationship between lipid peroxidation and hypercholesterolemia and/or hyperlipidemia has been evidenced in plasma, liver and aorta through many studies in animals and humans [1]. These factors contribute to the process of several pathologies namely atherosclerosis, but also seem implicated in the physiopathology of neurodegenerative diseases particularly Alzheimer’s disease (AD) [2]. Indeed, lipid peroxidation is considered as the most prominent form of oxidative damage in neurodegenerative lesions due to the brain’s relative enrichment in polyunsaturated fatty acids [3]. On the other hand, an increasing number of reviews implicating cholesterol metabolism in the development of AD have been published [4] which suggest cholesterol as a target for treatment. However, although it is admitted that an animal model able to reproduce all the cognitive, behavioural, biochemical, and histopathological abnormalities observed in AD patients does not exist [5], a partial reproduction of some AD hallmarks, using Al salts, have been achieved [6].

In this study, we aimed firstly to evaluate the relationship between plasmatic cholesterol metabolism and lipid peroxidation in posterior brain (parieto-temporal and occipital regions), previously found to be preferentially affected by AD [7], using Al, a potential etiological factor...
in AD [8], as neurotoxicant. Chronic aluminum-induced neurotoxicity has been in fact related to enhanced brain lipid peroxidation [9] together with hypercholesterolemia and hypertriglyceridemia [10].

Moreover, in the search for a new drug which may offer neuroprotection by controlling both hypercholesterolemia and lipid peroxidation, we have tested fenugreek seeds. Fenugreek (Trigonella foenum-graecum), a legume cultivated predominantly in Asia, the Mediterranean and North African regions for the edible and medicinal values of its seeds, has been reported to have antioxidative and hypocholesterolemic properties [11]. Trigonella is also known for its multiple pharmacological effects including its antidiabetic, antineoplastic, anti-inflammatory, antiulcerogenic, antipteric, antitumor and immuno-modulatory effects [12]. The active components of fenugreek seeds behind their most common properties (i.e. hypoglycemic, hypocholesterolemic, hypotriglyceridemic and antiperoxidative) have been described as polyphenolic flavonoids [13], steroid sapoains [14], polysaccharides mainly galactomannans [15] and 4-hydroxyisoleucine [16]. Nevertheless, the neuroprotective effect of fenugreek seeds was mainly restricted to studies on diabetes [17-19] except for a single in vitro study which have demonstrated the acetylcholinesterase enzyme inhibitory potential of standardized extract of fenugreek seeds [12]. Thereby, the role of fenugreek seeds in neurodegenerative diseases and especially against aluminum-induced changes has not so far been considered.

**Results**

**Lipid profile and glucose levels in blood**

Plasmatic levels of total cholesterol (+42.3%), LDL-C (+63.52%), triglycerides (+81.81%), and glucose (+97.85%) were found to be significantly increased in Al-treated rats (Table 1), as compared to controls. No significant changes were observed in rats consuming fenugreek seeds and treated (AlCl3+FSP, AlCl3+FSE) or not (FSP, FSE) with AlCl3.

HDL-C level was unchanged in Al-treated groups (AlCl3, AlCl3+FSP and AlCl3+FSE), but significantly increased when fenugreek seeds were administrated alone either as FSP (+24.4%) or FSE (+10.2%).

**Estimation of lipid peroxidation levels (TBARS)**

After 5 months of exposure to AlCl3, a significant raise of TBARS levels occurred increasingly in liver (+44.65%), plasma (+47.56%) and brain (+78.42%) whereas no significant changes were noted in (AlCl3+FSP) and (AlCl3+FSE) groups (Table 2). In (FSP) and (FSE) treated rats, TBARS levels were not significantly different from controls except in the liver where we noticed a slight but significant increase for both groups.

**Lactate dehydrogenase (LDH) activity**

As shown in Table 2 AlCl3 treatment induced an important increase in plasmatic LDH release (+120.7%) and relatively similar increase in liver and brain (+47.43% and +36.39% respectively).

**Correlation between measured parameters**

Correlation analysis of TBARS and LDH in plasma, liver and brain with plasmatic lipid and glucose profile are illustrated in Table 3.

**TBARS versus lipid profile**

TBARS in brain, liver and plasma correlated positively with TC, TG and LDL-C and negatively with HDL-C. Distinctions existed yet between compartments. In fact, Table 1 Lipid profile and blood glucose level in different experimental groups.

| Parameter | Control | AlCl3 | AlCl3+FSP | AlCl3+FSE | FSP | FSE |
|-----------|---------|-------|-----------|-----------|-----|-----|
| TC        | 1.56 ± 0.009 | 2.22a* ± 0.36 | 1.56b* ± 0.01 | 1.61b* ± 0.06 | 1.56 ± 0.08 | 1.55 ± 0.10 |
| HDL-C     | 0.49 ± 0.02 | 0.44 ± 0.01 | 0.57b*c ± 0.01 | 0.50h* ± 0.004 | 0.61b* ± 0.03 | 0.54a* ± 0.01 |
| LDL-C     | 0.85 ± 0.02 | 1.39b* ± 0.03 | 0.89c* ± 0.01 | 0.84h* ± 0.04 | 0.83 ± 0.05 | 0.80 ± 0.05 |
| TG        | 0.66 ± 0.02 | 1.20b* ± 0.06 | 0.58h* ± 0.02 | 0.74h* ± 0.04 | 0.58 ± 0.14 | 0.68 ± 0.03 |
| Glc       | 8.39 ± 0.03 | 166b* ± 1.30 | 8.18b* ± 0.08 | 7.95c* ± 0.29 | 8.31 ± 0.02 | 8.18 ± 0.24 |

Values are expressed as means ± SD; n = 10 for each treatment group.

* Significant difference from the control group at p < 0.05.

b Significant difference from the AlCl3-intoxicated group at p < 0.05.

c Significant difference between AlCl3+FSP and AlCl3+FSE groups at p < 0.05.

p < 0.001.

All parameters are expressed as mmol/l.
in brain as in liver, LDH correlated strongly with TC, TG and mainly LDL-C \( (r = 0.821 \text{ and } r = 0.864 \text{ respectively; } p < 0.01). \) In plasma, similar mild correlations were observed for all parameters \( (0.462 < r < 0.583). \)

**TBARS versus LDH**

In brain like in liver, LDH leakage was correlated positively to TBARS in brain, liver and plasma. The most prominent correlations were recorded between plasmatic TBARS and brain LDH \( (r = 0.768; p < 0.01). \) No significant correlation was found between plasmatic LDH and TBARS in brain, liver and plasma.

**Brain TBARS versus liver TBARS**

A significant but moderate positive correlation was reported between TBARS from different compartments \( (r \leq 0.528). \)

**Brain LDH versus liver LDH**

LDH leakage in the brain correlated positively with that observed in the liver \( (r = 0.780; p < 0.01). \) The correlation was weaker, yet statistically significant, between its levels in plasma and those in brain and liver \( (r = 0.588 \text{ and } r = 0.559 \text{ respectively; } p < 0.01). \)

**Levels of glycemia versus measured parameters**

Variations of all measured parameters were correlated to blood glucose level. The most prominent correlation was observed for LDL-C \( (r = 0.909; p < 0.01), \) correlations to total cholesterol and triglycerides were also high \( (r = 0.798 \text{ and } r = 0.769 \text{ respectively; } p < 0.01). \) Lipid peroxidation in plasma seemed more affected by glycemia than that in brain and liver \( (r = 0.764; r = 0.708 \text{ and } r = 0.418 \text{ respectively; } p < 0.01) \) whereas LDL release in these two organs was strongly correlated to

---

**Table 2 Thiobarbituric acid-reactive substances (TBARS) and lactate dehydrogenase (LDH) activity in brain, liver and plasma of different experimental groups.**

| Parameter | Control | AlCl₃ | AlCl₃+FSP | AlCl₃+FSE | FSP | FSE |
|-----------|---------|-------|-----------|-----------|-----|-----|
| **Brain** |         |       |           |           |     |     |
| TBARS     | 3.43 ± 0.35 | 6.12 ± 0.12 | 3.76 ± 0.21 | 2.46 ± 0.28 | 4.07 ± 0.02 | 4.15 ± 0.37 |
| LDH       | 84.9 ± 1.27 | 1158 ± 7.21 | 86.4 ± 3.65 | 93.3 ± 0.20 | 83.05 ± 1.58 | 83.13 ± 2.64 |
| **Liver** |         |       |           |           |     |     |
| TBARS     | 2.15 ± 0.18 | 3.11 ± 0.24 | 2.75 ± 0.20 | 2.32 ± 0.15 | 3.02 ± 0.07 | 2.88 ± 0.25 |
| LDH       | 191.4 ± 6.45 | 282.2 ± 11.2 | 199.9 ± 0.80 | 185.3 ± 2.62 | 195.7 ± 3.49 | 184.9 ± 0.30 |
| **Plasma** |         |       |           |           |     |     |
| TBARS     | 0.82 ± 0.03 | 1.21 ± 0.09 | 0.79 ± 0.02 | 0.91 ± 0.02 | 0.88 ± 0.01 | 1.05 ± 0.02 |
| LDH       | 485.5 ± 14.0 | 1071.5 ± 5.59 | 891.5 ± 20.3 | 914.0 ± 0.01 | 575.5 ± 120.0 | 463.0 ± 30.8 |

Values are expressed as means ± SD; \( n = 10 \) for each treatment group.

* Significant difference from the control group at \( p < 0.05. \)

** Significant difference from the AlCl₃-intoxicated group at \( p < 0.05. \)

* Significant difference between AlCl₃+FSP and AlCl₃+FSE groups at \( p < 0.05. \)

\( * p < 0.001. \)

In brain and liver TBARS and LDH are expressed respectively as: nmol MDA/mg protein and UI/g of tissue; in plasma TBARS and LDH are expressed respectively as: nmol MDA/l and UI/l.

---

**Table 3 Pearson correlation coefficients assessed between parameters measured in all experimental groups.**

|          | n = 60 | TBARSBrain | TBARSLiver | TBARSPlasma | LDHBrain | LDHLiver | LDHPlasma | Glc | TC | TG | HDL | LDL |
|----------|--------|------------|------------|-------------|-----------|----------|-----------|-----|----|----|-----|-----|
| TBARSBrain | 0.414* | 0.528** | 0.509** | 0.736** | 0.256 | 0.708** | 0.556** | 0.490** | -0.225 | 0.767** |
| TBARSLiver | 0.492** | 0.480** | 0.409* | 0.130 | 0.418* | 0.394* | 0.280 | -0.024 | 0.350* |
| TBARSPlasma | 0.768** | 0.613** | 0.261 | 0.764** | 0.594** | 0.725** | -0.247 | 0.640** |
| LDHBrain | 0.780** | 0.588** | 0.823** | 0.783** | 0.795** | -0.424** | 0.821** |
| LDHLiver | 0.559** | 0.840** | 0.782** | 0.685** | -0.518** | 0.864** |
| LDHPlasma | 0.511** | 0.530** | 0.462** | -0.330** | 0.583** | 0.909** |
| Glc | 0.798** | 0.769** | -0.441** | 0.909** | 0.675** | 0.669** |
| TC | 0.657** | -0.548** | 0.669** | -0.296 | 0.800** |
| TG | 0.657** | -0.548** | 0.669** | -0.296 | 0.800** |
| HDL | 0.798** | 0.769** | -0.441** | 0.909** | 0.675** | 0.669** |
| LDL | -0.378* |

Data are presented as \( r \) values.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed)

---

Belaïd-Nouira et al. Lipids in Health and Disease 2012, 11:16
http://www.lipidworld.com/content/11/1/16
blood glucose level ($r = 0.823$ and $r = 0.840$ respectively; $p < 0.01$).

Flavonoids and 4-Hydroxyisoleucine identification

To elucidate the structures of phenolic compounds mainly flavonoids in fenugreek seeds, the aqueous methanol extract was analyzed with diode array detection. HPLC analysis of the fractions showed the presence of peaks with flavonoid type UV spectra (two bands, $\lambda_{\text{max}}$ of band 1 between 320 and 350 and $\lambda_{\text{max}}$ of band 2 between 250 and 270 nm). Table 4 lists the identified compounds with their retention time. The structure assignment of flavonoids for which no standards were available was based on a systematic search for molecular ions using extracted ion mass chromatograms and comparing those with data in the literature. Thus, the ESI mass spectrum in positive ionization mode. The spectrum exhibited a molecular ion $[M + H]^+$ at $m/z$ 449 and an aglycone ion at $m/z$ 287. The loss of 162 amu from the pseudo-molecular ion indicated the presence of a hexosyl moiety. The $\lambda_{\text{max}}$ of UV spectrum at 345 and 265 nm suggested that flavonoid 2 was a kaempferol 3-O-glucoside. Flavonoid 3 exhibited a base peak $[M + H]^+$ at $m/z$ 579 and also an intermediate ion at 433 and an aglycone ion at $m/z$ 271. The loss of 146 amu from pseudo-molecular ion indicated the loss of sugar rhamnose and the loss of 162 amu from the intermediate ion was due to the loss of glucose. The obtained MS spectra combined to the UV spectra ($\lambda_{\text{max}}$ at 338-266) suggested that compound 3 was apigenin 7-O-rutinoside. On the other hand, by comparing their HPLC retention time, UV spectra, and mass spectra with the data obtained from standard in-house libraries, compounds 4, 5 and 6 were identified as naringenin, quercetin and vitexin respectively. Another amino acid compound, 4-hydroxyisoleucine (1) was identified using an LC-MS apparatus in the positive mode. The spectrum exhibited a molecular ion at $m/z$ 148 $[M + H]^+$ with fragments at $m/z$ 138, 130, and 118. The obtained mass fragments were consistent with those described earlier [20].

Discussion

The major effects of aluminium-induced neurotoxicity has been related to lipid peroxidation via free radical production [21]. In the present experiment, there was a significant increase in LPO after aluminium exposure in terms of MDA levels in liver, blood and especially in brain which confirms the susceptibility of brain to oxidative insult. In brain, LPO correlated significantly with lipid profile components in plasma principally LDL-C. Thereby, it seems that the assayed endproducts of LPO in posterior brain result in a great part from LDL oxidation. Low-density lipoproteins are in fact highly vulnerable to oxidative modifications especially when it is triggered by metal ions like aluminium [22]. The strong correlation found between LDL-C and glycemia during this experiment suggest that hyperglycemia induced by aluminium ingestion is the primary cause of LDL oxidation. Glycated LDL is in fact a preferred target for oxidative modifications [23]. A similar but stronger correlation was found between LDL-C and LDH release in brain. LDH leakage has been used as a marker of Al toxicity [24]. It was found to occur simultaneously with the elevation of LPO [25] as a result of cell membrane deterioration. However, neuronal LPO is not the only cause of cerebral damage that links LDH to LDL-C. Indeed, oxidized LDL have been found to induce apoptosis of mouse cerebrovascular endothelial cells [26] which supports the hypothesis that LPO plays a role in AD through linking agents contributing to blood-brain barrier disruption [27]. On the other hand, unlike LPO, LDH leakage in posterior brain appears to be highly associated to plasmatic TC and TG levels. This might be explained in part by the link between LDH and LDL-C given that hypercholesterolemia and even hypertriglyceridemia lead to LDL-C increase and oxidation. The LDL-C/TG ratio has yet been suggested to be an important predictor of LDL oxidation [28]. Nevertheless, increasing circulating cholesterol could have a direct effect on LDH release as hypercholesterolemia enhances intra-neuronal accumulation and deposition in brain of β-amyloid protein [29], which is considered to induce oxidation [30] and plays a pivotal role in Alzheimer’s disease. Changes in the extra-cerebral cholesterol levels could also induce modifications in brain cholesterol and low-density lipoprotein receptors present in the blood-brain barrier [31].

Table 4 Compounds detected in fenugreek seed extract with their retention times, UV spectra and mass spectral data.

| N° | Flavonoid                  | Retention time (min) | UV $\lambda_{\text{max}}$ (nm) | $[M+H]^+ (m/z)$ | $[I+H]^+ (m/z)$ | $[A+H]^+ (m/z)$ |
|----|---------------------------|----------------------|---------------------------------|----------------|----------------|----------------|
| 1  | 4-hydroxy-isoleucine      | 1.6                  | 260                             | 148            | -              | -              |
| 2  | Kaempferol 3-O-glucoside  | 8.7                  | 265, 345                        | 449            | -              | 287            |
| 3  | Apigenin 7-O-rutinoside   | 12.3                 | 338, 266                        | 579            | 433            | 271            |
| 4  | Naringenin                | 13                   | 288, 330                        | 273            | -              | -              |
| 5  | Quercetin                 | 2                    | 371, 255                        | 303            | -              | -              |
| 6  | Vitexin                   | 9.1                  | 268, 336                        | 433            | 341            | -              |

a APCI-MS (positive mode) data for the protonated molecular ion  
b APCI-MS (positive mode) data for protonated intermediate molecular ions  
c APCI-MS (positive mode) data for the protonated aglycone ion
Otherwise, the increase in circulating cholesterol due to AI administration indicates a loss of membrane integrity [32] as it was confirmed by LDH release in brain, liver and blood. Similarly, AI exposure can result in AI accumulation in the liver leading to a disturbance of lipid metabolism and an elevation of serum cholesterol [33]. This explains the high correlation between LDH in liver and both LDH and TBARS in brain. This correlation is in fact indirect because mediated by cholesterol.

Regarding the cerebral protective effect of fenugreek seeds, we found that co-administration of fenugreek seeds either as FSP or FSE with Al reduced significantly levels of TBARS and LDH in brain. These results indicate that fenugreek seeds are endowed with anti-peroxidative properties in brain which may be mediated either by direct or indirect effects on brain. It is likely that LPO and consequently LDH inhibition is owing to the antiradical and antioxidant potential of polyphenolic flavonoids of *Trigonella* seeds emphasized through *in vitro* and *in vivo* experiments [18,34-37]. Five flavonoids (kaempferol 3-O-glucoside, apigenin 7-O-rutinoside, naringenin, quercetin and vitexin) were in fact detected in this extract using LC-MS/MS. The well known hypoglycemic (quercetin and vitexin) were in fact detected in this study. However, correlations established in this study showed that FSP and FSE were effective in lowering plasma cholesterol, triglyceride and LDL-cholesterol in AlCl₃-treated rats which is in line with the previous studies [17,38]. However, correlations established in this study between LPO and LDH in brain and lipid profile suggest that fenugreek seeds exert their neuroprotective effect via controlling lipid and lipoprotein metabolism. Indeed, our study showed that FSP and FSE were effective in lowering plasma cholesterol, triglyceride and LDL-cholesterol in AlCl₃-treated rats which is in line with the previous studies [20,39,40]. Several mechanisms, in addition to various components have been suggested to explain the lipid-lowering effect of fenugreek seeds. These include a direct effect on cholesterol metabolism by inhibiting the key enzymes involved in cholesterol and fatty acid synthesis. Number of studies has shown that steroid saponin extracted from fenugreek seeds has the ability to modify cholesterol status by its capacity to bind both cholesterol and bile acids [41]. Diosgenin, a steroidal sapogenin extracted from fenugreek seeds, has in fact been shown to reduce TC as well as LDL-C in high-cholesterol fed quails [42]. On the other hand, trigonelline, an alkaloid isolated from fenugreek seeds, was found able to normalize the rate of lipogenesis in streptozotocin induced hyperglycemic rats by stimulating hepatic lipogenic enzymes [43]. A recent study carried by Vijayakumar et al. [44] has proven that precipitable protein/peptide or associated factors could be responsible for improvement in serum lipid profile through hypolipidemic effect on adipocytes and liver cells leading to decreased TG and cholesterol synthesis in addition to enhanced LDL receptor-mediated LDL uptake. Nevertheless, the lipid-lowering compounds in fenugreek seeds could be of a polyphenolic nature. Indeed, Wilox et al. [45] provided evidence that naringenin not only decrease cholesterol biosynthesis but also inhibit acyl transferase (ACAT), a key enzyme involved in the esterification and absorption of cholesterol, secretion of hepatic LDL cholesterol, and cholesterol accumulation in the arterial wall. Naringenin is in fact a well known flavonoid which was detected in our extract. This effect could explain the significant increase in plasmatic HDL-C following TC decrease when fenugreek seeds are administrated to AlCl₃-treated rats. Increasingly, hypolipidemic effect of fenugreek seeds has been attributed to the presence of 4-hydroxyisoleucine an atypical branched-chain amino acid derived from fenugreek [46] also detected in the used FSE. However, the action of 4-hydroxyisoleucine or galactomannan on lipid profile, like other components of fenugreek seeds, could be due to achievement of normoglycemia where there was no further degradation of already accumulated lipids for otherwise glucose starved cells [43]. Hypercholesterolaemia and consequently the increase of TG and LDL-C are in fact highly correlated to hyperglycemia. 4-Hydroxyisoleucine was shown to display an insulinotrophic property in vitro, stimulate insulin secretion in vivo, and improve glucose tolerance in normal rats and dogs and in rat model of type 2 DM [47]. Other components of *Trigonella* seeds having hypoglycemic effects include arginine, tryptophan, ascorbic acid, niacin, nicotinic acid, chromium, copper, magnesium, manganese, zinc, gentianine, choline and quercetin, a flavonoid also detected in our extract [48]. On the other hand, the significant hepato-protective effect of fenugreek seeds as evidenced by decreased levels of TBARS and LDH may be a secondary indirect mechanism for its neuroprotective effect, taking into account the high observed correlation between hepatic injury induced by AlCl₃ and LPO in brain. Eventually, the decrease of LPO and LDH in brain after fenugreek administration might also be attributed to its oestrogenic constituents (saponines, trigoniosides, flavonoids) [49]. Their action could be direct since phytostrogens have shown potential neuroprotective properties [50] or indirect thanks to their hypocholesterolemic effect [51].

Finally, it is worthwhile to mention that in this study, fenugreek seeds were given either as FSP 5% or FSE (100 mg/kg) in order to make a useful comparison as for the best form of administration. The dose of powdered fenugreek seeds was equated to the therapeutic dose suggested for humans and has been subjected to nutritional and safety evaluation [52] and the dose of fenugreek seed extract was established based on a previous study which has proved its safety and therapeutic effect [36]. Although many compounds present in the whole fenugreek seed could play a role in the described actions, similarities between the effects of the whole seed (FSP) in
one hand and the effects of the seed extract (FSE) on the other hand, suggest that a mixture of flavonoids and 4-hydroxyiseuleucine was enough to generate neuroprotection against Al toxicity.

**Conclusion**

Even if preliminary, the present study demonstrates that aluminium-induced LPO could arise from alteration of lipid metabolism and that is probably related to altered lipoprotein metabolism rather than a direct effect of cholesterol oxidation. Using fenugreek seeds either as FSP or FSE could be neuroprotective thanks to their antioxidant activity and also their ability to control hypercholesterolemia, hypertriglyceridemia and hyperglycemia. Synergism among well defined components endowed with pleiotropic actions is one of the characteristics of *Trigonella foenum-graecum* seeds. The whole fenugreek seed could be suggested as a regular nutrient to protect brain from chronic aluminium toxicity. However, further investigations are imperative to adjust quantitatively the active compounds in the fenugreek seed extract and provide a base for the development of natural drugs.

**Materials and methods**

**Reagents**

Aluminum chloride (AlCl₃, 6H₂O), analytical grade) and all used chemicals were of analytical grade and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, France).

**Animals**

Sixty female Wistar rats (weighing 208-220 g) were obtained from the Central Pharmacy (SIPHAT, Tunis, Tunisia). They were fed pellet diet, purchased from the Industrial Society of Rodents’ Diet (SICO, Sfax, Tunisia) and tap water *ad libitum*. Animals were kept in an air-conditioned room (temperature 22 ± 3°C and relative humidity of 40%) with a 12 h light/dark cycle. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the local Ethics Committee.

**Plant material**

**Preparation of fenugreek seeds powder (FSP)**

*Trigonella* seeds purchased from the local market were finely powdered and mixed at 5% in ground standard rat feed (i.e. 5 g of dry ground *Trigonella* seeds in 95 g of ground rat food).

**Preparation of fenugreek seeds extract (FSE)**

An aqueous methanol fenugreek seeds extract was prepared according to the protocol described by Kaviarasan et al. [34]. In brief, fenugreek seeds were finely powdered, mixed with 80% methanol and kept at room temperature for 5 days. The mixture was then filtered and the solvent was evaporated to get the residue. This latter was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 mL/L). Extraction was then carried out for 36 h at room temperature and the combined ethyl acetate layer was concentrated. The residue was lyophilised and stored at -20°C. The extract was dissolved in double-distilled water for oral administration.

**Characterization of fenugreek seed extract by LC-MS/MS analysis**

The LC-MS/MS experiments were performed as described previously by Belghith-Hadrich et al. [20] using an Agilent 1100 LC system consisting of a degasser, a binary pump, an autosampler and a column heater. The column outlet was coupled with an Agilent MSD Ion Trap XCT mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out in a personal computer with Data Analysis software (Chemstations). For the chromatographic separation, a Zorbax 300 Å Extend-C-18 Column (2.1 × 150 mm) was used. The flavonoids and other compounds were identified using a combination of HPLC with diode array detection and liquid chromatography coupled with an electrospray ionization mass spectrometry (ESI-LC-MS/MS) on the basis of their UV spectra and mass spectra and by comparison of the spectra with those of available authentic standards.

**Study design**

Rats were treated according to the protocol established by Gong et al. [53] in which a brain dysfunction model was established. In brief, rats, randomly distributed into six groups of ten animals each, were given a daily AlCl₃ solution (500 mg/kg, i.g) for control) for the first month, and then fed with an AlCl₃ solution (1600 ppm in drinking water) for up to 5 months. Three months after Al administration, rats were given either FSP 5% in powdered rat feed or FSE (100 mg/kg, i.g) for two months together with AlCl₃.

**Blood and tissue collection**

Blood samples were collected under anesthesia by cardiac puncture in heparinized tubes. Plasma was separated from the blood cells by centrifuging the blood at 3,000xg for 15 min at 4°C and stored in aliquots at -20°C until analysis. Livers and brains were removed quickly from animals, washed in ice-cold physiological saline. Then, multiple lobes of the liver from each rat were cut out, minced and homogenized (10% w/v) separately in ice-cold 1.15% KCl-0.01 mol/L sodium, potassium phosphate...
buffer (pH 7.4) in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 10,000g for 20 min at 4°C, and the resultant supernatant was stored at -80°C to be used for different enzyme assays.

Regarding the brain, temporal and parietal lobes were isolated from one hemisphere of each brain by free hand dissection on ice according to the method of Głowinski and Iversen [54]. Brain tissue was minced and homogenized (10% w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4) then centrifuged at 10,000g for 30 min at 4°C, the resultant supernatant was used for different enzyme assays.

Biochemical assays

**Determination of blood lipids and glucose levels**

Plasmatic cholesterol, triglycerides, glucose, total protein content and HDL-C levels were quantified by enzymatic methods using commercial kits (Randox-Antrim, UK). Plasmatic LDL-C level was calculated according to the Friedewald equation [55].

**Lipid Peroxidation Estimation**

The extent of lipid peroxidation was assessed by measuring the content of thiobarbituric acid reactive substances (TBARS) following the method of Yoshioka et al. [56] in plasma and the method of Buege and Aust [57] in liver and brain. TBARS were expressed as malondialdehyde (MDA) amount using freshly diluted malondialdehyde bis dimethylacetel as standard.

**Lactate dehydrogenase activity**

The activity of LDH in plasma, liver and brain was measured using commercial reagent kits (Randox-Antrim, UK).

**Statistical Analysis**

Data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by the post hoc Tukey's test, used for comparison. Bivariate correlation between variables using Pearson's correlation coefficient r value was used. Values were considered statistically significant when p < 0.05. Statistics were done using IBM SPSS Statistics 19.

**Acknowledgements**

This work was supported by the « Ministère Tunisien de l’Enseignement Supérieur, de la Recherche Scientifique et de la Technologie » We acknowledge the significant contribution of all experts who participated in this study especially Dr. Oussama Ayara for English proofreading.

**Author details**

1Research unit of Genetic (02/UR/08-03), Laboratory of Histology and Cytogenetic, Faculty of Medicine, Monastir, Tunisia. 2Laboratory of Bioprocesses, Center of Biotechnology, University of Sfax, Tunisia.

**Authors’ contributions**

YB-N conceived this study, designed it, analyzed and interpreted the data and wrote the manuscript. HB participated in the study design and data acquisition. MB carried out the plant extract characterization. IF-S carried out plasmatic lipids assays. ZH helped to draft and revise the manuscript. HBC drafted and revised the manuscript. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Received:** 28 December 2011 **Accepted:** 26 January 2012 **Published:** 26 January 2012

**References**

1. Bular H: High cholesterol diet supplemented with sunflower seed oil but not olive oil stimulates lipid peroxidation in plasma, liver, and aorta of rats. The Journal of Nutritional Biochemistry 1995, 6:547-550.
2. LaFepla FM: An array of genes implicated in Alzheimer’s disease. Neurobiology of aging 2006, 27:1078-80.
3. Bassett CN, Montine TJ: Lipoproteins and lipid peroxidation in Alzheimer’s disease. The Journal of nutrition, health & aging 2003, 7:24-29.
4. Cossec J-C, Marquier C, Panchal M, Lazar AN, Duyckaerts C, Potier M-C: Cholesterol changes in Alzheimer’s disease: methods of analysis and impact on the formation of enlarged endosomes. Biochimica et biophysica acta 2010, 1801:839-45.
5. Pan R, Qiu S, Lu D-xiang, DongJ: Curcumin improves learning and memory ability and its neuroprotective mechanism in mice. Chinese medical journal 2008, 121:822-9.
6. Kakkar V, Kaur IP: Evaluating potential of curcumin loaded solid lipid nanoparticles in aluminium induced behavioural, biochemical and histopathological alterations in mice brain. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 2011, 49:2906-13.
7. Novotny R, Louis S: Alzheimer Disease. Life Sciences 2001, 1-6.
8. Flaten T: Aluminium as a risk factor in Alzheimer’s disease, with emphasis on drinking water. Brain Research Bulletin 2001, 55:187-196.
9. Fraga CG, Oteiza PI, Golub MS, Gershwin ME, Keen CL: Effects of aluminum on brain lipid peroxidation. Toxicology Letters 1990, 51:213-219.
10. Newany A-Sa, Salama AP, Hussen HM, Yousef MI: Propolis alleviates aluminium induced lipid peroxidation and biochemical parameters in male rats. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 2009.
11. Diet PP, Misar A, Mujumdar AM, Ghashidke S: Pre-treatment of Syndrex protects mice from becoming diabetic after streptozotocin injection. Phytoterapia 2010, 81:403-412.
12. Sarweshkumar N, Mukherjee PK, Bharda S, Saha RP: Acetylicholineresterase enzyme inhibitory potential of standardized extract of Trigonella foenum graecum L, and its constituents. Phytomedical: International journal of phytopharmacology and phytopharmacy 2010, 17:292-5.
13. Gupta R, Nair S: Antioxidant flavonoids in common Indian diet. South Asian J Prev Cardiol 1999, 3:83-94.
14. Petit PR, Sauvare YD, Hillane-Buys DM, Leconte OM, Baisac YG, Ponsin GR, Ribes GR: Steroid saponins from fenugreek seeds: extraction, purification, and pharmacological investigation on feeding behavior and plasma cholesterol. Steroids 1995, 60:674-80.
15. Madar Z, Shomer I: Polyaccharidic composition of a gel fraction derived from fenugreek and its effect on starch digestion and bile acid absorption in rats. Journal of Agricultural and Food Chemistry 1990, 38:1535-1539.
16. Broca C, Briel V, Cruciani-Guglielmacci C, Manteghetti M, Rouault C, Derouet P, Riskaal M, Pau B, Petit P, Ribes G, Ktorza A, Geiss R, Reach G, Taiouis M: Insulinotropic agent ID-1101 (4-hydroxyisoleucine) activates insulin signaling in rat, American journal of physiology. Endocrinology and metabolism 2004, 287:E463-71.
17. Siddiqui MR, Taha A, Moorthy K, Hussain ME, Basir SF, Baquer N: Amelioration of altered antioxidant status and membrane linked functions by vanadate and Troglitazone in alloxan diabetic rat brains. Journal of biosciences 2005, 30:483-90.
18. Genet S, Kiel RK, Baquer NZ: Alterations in antioxidant enzymes and oxidative damage in experimental diabetic rat tissues: Effect of vanadate and fenugreek (Trigonella foenum graecum). Molecular and cellular biochemistry 2002, 236:7-12.
19. Mohammad S, Mohamad S, Taha A, Rameez R, Basir SF, Baquer NZ: Lower doses of vanadate in combination with trigonella restore altered
carbohydrate metabolism and antioxidant status in alloxaan-diabetic rats. *Clinica chimica acta; international journal of clinical chemistry* 2004, *342*:105-14.

20. Belguith-Hadriche O, Bouaziz M, Jarroussi K, El Feki A, Sayadi S, Makni-Ayedi F. Lipid-lowering and antioxidant effects of an ethyl acetate extract of fenugreek seeds in high-cholesterol-fed rats. *Journal of agricultural and food chemistry* 2010, *58*:2116-22.

21. Gupta V, Anitha S, Heggde ML, Zecca L, Garuto RM, Ravid R, Shankar SK, Stein R, Shannahgavuvi P, Jagannatha Rao KS. Aluminium in Alzheimer’s disease: are we still at a crossroad? *Cellular and Molecular Life Sciences* 2005, *62*:143-158.

22. Ferretti G, Baccetti T, Marchionni C, Dousset N. Effect of non-enzymatic glycation on aluminium-induced lipid peroxidation of human high density lipoproteins (HDL), *Nutrition,* metabolism, and cardiovascular diseases: *NMC&D* 2004, *14*:538-65.

23. Sobal G, Menzel J, Sinzinger H. Why is glycated LDL more sensitive to oxidation than native LDL? *A comparative study.* *Prostaglandins, leukotrienes, and essential fatty acids* 2000, *63*:177-86.

24. Anane R, Creppy EE. Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase-catalase and vitamins E and C. *Human & experimental toxicology* 2001, *20*:477-81.

25. Perez S, Sergent O, Morel P, Chevanne M, Dubos MR, Cillard P, Cillard J. [Kinetics of lipid peroxidation induced by UV beta rays in human keratinocyte and fibroblast cultures], Comptes rendus des séances de la Société de biologie et de ses filiales 1995, *199*:453-65.

26. Lin Y, Chang H, Tseng C, Chiu W, Lin J, Chen R. Resveratrol protects against Oxidized LDL-induced Breakdown of the Blood-Brain Barrier by Lessening Disruption of Tight Junctions and Apoptotic Insults to Mouse Cerebrovascular Endothelial Cells. *The Journal of Nutrition* 2010, *140*:2187-2192.

27. Cai Z, Yan Y, Lan L, Wang F, Huang H, Wang Y, Zhao Y. Serum level of MPP-2, MPP-9 and Ox-LDL in Alzheimer’s disease with hyperlipidemia. *Journal of Medical Colleges of PLA* 2007, *22*:352-56.

28. Briza P, Tonola G, Carusillo F, Malaquarera M, Maolli M, Muremeci S. Plasma Lipid Composition and LDL Oxidation, *Clinical Chemistry and Laboratory Medicine* 2003, *41*:56-60.

29. Zatta P. The role of metals in neurodegenerative processes: aluminum, manganese, and zinc. *Brain Research Bulletin* 2003, *62*:15-28.

30. Schippings S, Kountush A, Ahtz S, Buhmann C, Strenzburger H-J, Mann U, Müller-Thomsen T, Biesigel U. Increased lipoprotein oxidation in Alzheimer’s disease. *Free Radical Biology and Medicine* 2000, *28*:351-360.

31. Silva VS, Cordeiro JM, Matos MJ, Oliveira CR, Gonçalves PP. Aluminum accumulation and membrane fluidity alteration in synaptosomes isolated from rat brain cortex following aluminum ingestion: effect of cholesterol. *Neuroscience research* 2002, *44*:181-93.

32. Sarin S, Gupta V, Gill KD. Alterations in lipid composition and neuronal injury in primates following chronic aluminum exposure. Biological trace element research 1997, *59*:133-43.

33. Wilhelm M, Jaeger DE, Schüll-Cablitiz H, Häfner D, Igel H. Hepatic clearance and retention of aluminum: studies in the isolated perfused rat liver. *Toxicology letters* 1996, *86*:257-63.

34. Kaviarasan S, Viswanathan P, Anuradha CV. Fenugreek seed (Trigonella foenum graecum) polyphenols inhibit ethanol-induced collagen and lipid accumulation in rat liver. *Cell biology and toxicology* 2007, *23*:373-83.

35. Srinivasan K. Fenugreek (Trigonella foenum-graecum): A Review of Health Beneficial Physiological Effects. *Food Reviews International* 2006, *22*:203-224.

36. Basu TK, Sircamroon A. Health Benefits of Fenugreek (Trigonella foenum-graecum leguminosse), In *Bioactive Foods in Promoting Health.* Edited by: Watson RR, Preedy VR. San Diego: Academic Press; 2010:435-45.

37. Al-Mutairi HY, Nasrat NA, Ounass GA, Abu-Samak M, Al-Mizan KA, Salmi M. The hypocholesterolemic and antioxidative effect of dietary diosgenin and chromium chloride supplementation on high-cholesterol fed Japanese quails, *Pakistan journal of biological sciences: PJBS* 2011, *14*:425-32.

38. El-Soud NHA, Khalil M, Hussein J, Oraby F, Farraye ARH. Antidiabetic Effects of Fenugreek Alkaloid Extract in Streptozotocin Induced Hyperglycemic Rats. *Journal of Applied Sciences Research* 2007, *3*:1073-1083.

39. Mijayakumar MV, Pandey V, Mishra GC, Bhat MK. Hypolipidemic effect of fenugreek seeds is mediated through inhibition of fat accumulation and upregulation of LDL receptor. *Obesity* (Silver Spring, Md.) 2010, *18*:667-74.

40. Wilcox LJ, Borradaile de L, Dreu E, Huff MW. Secretion of hepatocyte apolipoprotein B is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *Journal of Lipid Research* 2001, *42*:725-34.

41. Jetté L, Hanley L, Eugeni K, Levens N. 4-Hydroxyisoleucine: a plant-derived treatment for metabolic syndrome. *Current opinion in investigational drugs* (London, England: 2000) 2009, *10*:353-8.

42. Sauvage Y, Petit P, Broca C, Mangetthepi M, Baissac Y, Fernandez-Alvarez J, Gros R, Roye M, Leconte A, Gomis R, Ribes G. 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. *Diabetes* 1998, *47*:206-10.

43. Thakran S, Siddiqri MR, Baquer NZ. Trigonella foenum graecum seed powder powder protects against histopathological abnormalities in tissues of diabetic rats. *Molecular and cellular biochemistry* 2004, *266*:151-9.

44. Skandhan KP, Rajanariprasad A. Estrogen in milk and plants. *Medical Hypotheses* 2005, *64*:429-430.

45. Schreihof DA. Phytoestrogens as neuroprotectants. *Drugs of today* (Barcelona, Spain: 1998) 2009, *45*:609-27.

46. Baquer NZ, Kumar P, Taher A, Kale R, Cowak S, McLean P. Metabolic and molecular action of Trigonella foenum-graecum (fenugreek) and trace metals in experimental diabetic tissues. *Journal of Biosciences* 2011, *36*:383-396.

47. Rao PU, Sesikaran B, PS R, Naidu AN, Rao W, Ramachandran EP. Short term nutritional and safety evaluation of fenugreek. *Nutrition Research* 1996, *16*:1495-1505.

48. Gong Q-H, Wu Q, Huang X-N, Sun A-S, Shi J-S. Protective effects of Ginkgo biloba leaf extract on aluminium-induced brain dysfunction in rats. *Life sciences* 2005, *77*:190-8.

49. Głoweksi J, Ivensen LL. REGIONAL STUDIES OF CATECHOLAMINES IN THE RAT BRAIN-I. *Preparative Ultracentrifuge.* 10*th* edition (London, England: 2000)

50. Wilcox TJ, Kawada K, Shimada T, Mori M. The hypocholesterolemic and antioxidative effect of dietary diosgenin and trace metals in experimental diabetic tissues. *Japanese Journal of Biomedical Science* 2011, *10*:358-365.

51. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods in enzymology* 1978, *52*:302-10.