ABSTRACT

Many medicinal plants including *Gingko biloba* (GB) and their phytocompounds are known to play an important role in treating Central Nervous System (CNS) disorders like Alzheimer’s and Parkinson’s diseases etc. Its standardised extract EGB761 is a distinct product with well-marked contents including 24% flavone glycosides: mainly Quercetin, Kaempferol and Isorhamnetin which are especially considered to be the most pharmacologically active components responsible for treating various CNS disorders. In spite of its known potential for treating CNS disorders, medicinal usage of GB are limited majorly due to its low reach to the brain, low bioavailability, early degradation in both gut and systemic circulation, and rapid elimination from the body. The present study was conducted to formulate a suitable formulation of EGB761 which can be delivered through an appropriate route to overcome the above said difficulties.

Two of the finest approaches which are being actively used to combat with these challenges are developing nano/micro sized formulations and targeting the appropriate delivery route. There is a wide acceptance for nano/microemulsion system (ME) as drug carrier systems so. ME was chosen as a formulation to be developed through intranasal route which is also reported to be appropriate to deliver drugs directly to the brain through olfactory route.

To fulfill the objectives of this study EGB761 was characterized though various qualitative and quantitative methods before developing the ME for the same. EGB761 was found to have 0.83 ± 0.005 ng/mg of Quercetin, 0.51 ± 0.041ng/mg of Kaempferol and 0.37 ± 0.06 ng/mg of Isorhamnetin in the given sample of EGB761 by LCMS estimation.

Various excipients (oil, surfactant and co surfactants) like Capryol 90, Olive oil, Castor oil, Linseed oil; Plurol oleique, Tween 80, Tween 20, Cremophor RH40, Transcutol P; Ethanol, Propanol and Methanol were screened for their ability to solubilise maximum amount of EGB761. The excipients which were found to have maximum comparative solubility (Isopropyl Myristate (IPM), Tween 20 and Ethanol) were titrated by water titration method for their ability to make a stable ME. They were found to make appropriate ME system and were then evaluated for their thermodynamic stability and other parameters like – pH, conductivity, density and viscosity ME having S_{mix} ratio of 1:1 was found to be stable and also had optimum pH (6.7 ± 0.01), conductivity (22 ± mS/m) and viscosity (38 ± 1.01 cP) and density (1.16± g/mL). The ME having S_{mix} ratio of 1:1 was then chosen and was named as GBME. The selected formulation was further
characterized for its size, zeta potential and PDI index. These parameters were found to be
259.8 nm (average particle size), 0.186(PDI) and zeta potential as -9.87mv for GBME.
The size and shape of GBME particles was further verified with TEM which was found to
be spherical and in nanometric size range (84 nm to 260 nm). It was further characterized
by FT-IR and it was found that the extract was internalized to the droplets of ME system.
Further, EGB761 and GBME were compared for their antioxidant ability by DPPH and
ABTS method and GBME was found to have better (ABTS = 89.2 ± 0.78%, DPPH = 94.6
± 0.04%) antioxidant activity than EGB761.

GBME was further investigated for its safety and protective activity *in vitro*, on two cell
lines; RPMI2650 and NB41A3 by MTT assay, LDH assay, NO synthase activity and lipid
peroxidation and then *in vivo* on swiss albino mice model with initial observations of
food, and water intake and weight gain for 14 days which found to safe on animal model.

GBME was tested on Alzheimer’s model of mice by oral as well as intranasal route for its
therapeutic potential and was compared with EGB761 and the standard drug Reminyl.
After the treating the animals with of 20 µl of GBME (60 mg/ml) for 7 days continuously,
it was found that GBME was able to reduce the beta amyloid level in the mice brain
hippocampal region in comparison to other controls, which was verified with immuno
blotting and histochemical analysis using rabbit anti-Amyloid -β 42 antibody. It was also
found to improve the cognitive dysfunctioning of the Alzheimer’s model after treatment.

GBME was also investigated for its stability after storing it for a year using its antioxidant
potential as a parameter. It was found that GBME was more stable than EGB671 as it
retained 95.8 ± 0.49% antioxidant potential (by ABTS assay) than EGB761.

So, it can be concluded from this study that GBME has been successfully developed for
intranasal application and was able to reduce beta amyloid level as well as also could
improve on cognitive functions. Further pharamacokinetics and pharamacodynamics
studies are required to investigate the amount of drug reached to the brain and its
elimination profile from the body.