Chapter 4

C. oil: dose dependent structure and functional effect

“Time is swift, it races by; Opportunities are born and die...
Still you wait and will not try - A bird with wings who dares not rise and fly.”

- A. A. Milne -

(Winnie-the-Pooh, 1882-1956)
4.1 Introduction

Plants provide a rich source for natural drug research and development. In recent years, the use of traditional medicine has received considerable attention. The need for basic scientific investigation on medicinal plants using indigenous medical system has become imminent. Recent studies have focused on the effects of dietary antioxidants and polyphenolic compounds from plant sources, such as Ginkgo biloba, curcumin, resveratrol, blueberries, spinach and spirulina reduces ischemia/reperfusion induced apoptosis and cerebral infarction (Wang et al., 2005b). Ginkgo biloba, has been used worldwide as a herbal medicine (McKenna et al., 2001), has been reported to be effective against ischemic brain injury (Kleijnen and Knipschild, 1992; Zhang et al., 2000a), cerebral (or cerebrovascular) insufficiency (Vesper J and KD., 1994), cognitive speed (Kennedy et al., 2000), dementia and Alzheimer’s disease (Le Bars et al., 1997), arterial occlusive disease (Peters et al., 1998) and aging damages (Diamond et al., 2000). The two major constituents of Ginkgo biloba: The terpenoids and the other are flavonoids. However, less information is available on biological action of its chemical constituents.

The major chemical constituents of turmeric rhizome are volatiles; the curcuma oil, and non-volatiles; curcumin.

Some reports already indicate the functionality and utilization of volatile curcuma/turmeric oil, such as insect repellent (Roth et al., 1998), anti-fungous (Apisariyakul et al., 1995), anti-bacterial (Negi et al., 1999), and anti-carcinogenic (Aratanechemuge et al., 2002). There is almost a complete absence of studies which address the potential of the neuroprotective effect of Curcuma oil except the recent report (Rathore et al., 2007). Recently the chemistry of major constituents of C.oil isolated from C. longa L. rhizome has been reported (Jain et al., 2007). In the present study, the effects of Curcuma oil extracted from C. longa (short name: herbal medicament or HM, US patent No – 6991814, grant date-31/1/2006) were explored by occlusion of middle cerebral artery model of stroke in rat. In this chapter the dose response of C.oil was evaluated on the volumes of infarct and edema; neurological deficits and spatial learning are explored in the ischemic challenged rats. In rodents, the sensorimotor cortex, the striatum, and the hippocampus are predominantly damaged after ischemic insult. Therefore, three corresponding behavioral tests were chosen;
water-maze test to assess spatial learning and memory for function of the hippocampus (Morris et al., 1982), rota-rod test for a motor cortical dysfunction (Bogo et al., 1981), and open-field for behavioral test for cerebral structural integrity (Daenen et al., 2001; Prior et al., 2004).

4.2 Results

4.2.1 Neurological examination

The rats in the ischemia group showed significant neurological deficits (Median 2.8; 95% CI 2.69-2.85) including hemiplegia and postural abnormalities, such as forelimb flexion, extreme body twisting, and circling behavior. In contrast, hemiplegia and postural abnormalities were milder in the C.oil-treated rats. The scores were (Median 1.85; 95% CI 1.735-1.957), (Median 1.65; 95% CI 1.553-1.75) and (Median 1.26; 95% CI 1.797-1.335) for the groups treated with 125, 250 and 500 mg/kg C.oil, respectively (Fig.1). With MK801, a reference compound, used showed (Median 1.0; 95% CI 0.936-1.178) with dose. C.oil significantly reversed the decrease of neurological scores caused by the MCAo. In contrast, MK-801 had no beneficial effect on sensorimotor function. The 250 mg/kg C.oil-treatment significantly reversed the degree of neurological deficit scores caused by MCAo. The C.oil treatment group showed a significant reduction of ischemia-induced motor impairment compared with the ischemia group.

![Neurological deficit scores](image-url)

**Fig.1:** Effect of MK 801 and different doses of C.oil on neurological deficits score after 24hrs. Sham operated group, ischemia with no treatment, ischemia with MK 801 (1mg/Kg); ischemia with C.oil (125 mg/Kg); ischemia with C.oil (250 mg/Kg);
ischemia with C.oil (500mg/Kg) given i.p. Number of animals used were five in each group *P<0.001 as compared with the ischemic group.

4.2.2 Dose response of C.oil in MCAo model

The extent of infarction caused by MCAo was quantified in ischemic and C.oil-treated rats. Coronal sections (2 mm) were obtained by slicing the brain from the rostral extremity of the frontal cortex and staining with TTC. TTC stained viable brain tissue as red and the infarcted regions did not take up TTC stain so remained white in colour. The infarcted area extended from the core regions (caudate putamen, parietal cortex and temporal cortex) to the peripheral regions (penumbra).

Attenuation of the infarct volume was observed with ascending doses of C.oil 125 to 500mg/Kg by intra peritoneal route. In the 250 and 500-mg/kg C.oil-treated group, the infarct volume was significantly smaller (147.49 ±38; 99.88 ± 33 mm³; P<0.05) compared with that of the ischemic group (284.79 ± 24.4 mm³). The group that received 125 mg/kg C.oil also had a smaller infarct volume than did the control group (208.86 ± 42 mm³), but the difference was not statistically significant. C.oil showed a dose related effect on the infarct volume (Fig.2). It was found that pretreatment with MK 801, a reference compound, at 1mg/Kg caused a significant protective effect against MCAo (176.36 ± 37.0). C.oil had a better effect to MK-801 at reducing the volume of infarct at the doses tested.

![Fig.2: Effect of MK 801 and different doses of C.oil on volume of infarct after TTC staining. Sham operated group; ischemia with no treatment, ischemia with MK 801 (1mg/Kg); ischemia with C.oil (125 mg/Kg); ischemia with C.oil (250 mg/Kg); ischemia with C.oil (500 mg/Kg); p- compared with the ischemic group.](image-url)
C. oil (500mg/Kg) given i.p. Number of animals used were five in each group *\( P<0.05; **P<0.01 \) as compared with the ischemic group.

4.2.3 Effect of C. oil on the Rota rod test

The mean value of the time spent on the spindle or latency to fall in pre MCAo rats was \( (119 \pm 2.01) \) which was taken as base line value. The reduction in average time spent on the rotarod by the MCAo rats (*Pvalue<0.05) when compared with the sham operated animals after 24 h was highly significant. Average time spent on rotarod was significantly improved in the C. oil treated group \( (97.05 \pm 3.29) \) in comparison to ischemic animals \( (65.05 \pm 4.59) \) (Fig.2).

![Fall Latency Graph](image)

**Fig.3:** Bar chart showing the fall latency on an accelerating Rotarod from sham operated, I/R (24h of reflow) and C. oil pretreated -I/R (24h of reflow) rats. Time [in sec] expressed as Mean ± S.E.M. (n=5). Effects of C. oil I/R vs. I/R rats, *** \( P<0.001 \).

4.2.4 Effect of C. oil on the water maze test

Untreated and C. oil treated rats showed reduction in the latency time to reach platform. Significant decrease in latency time occurred on the day 1, day 2 and day 7 of I/R in C. oil treated group respectively. The water-maze data for latency on postoperative days 1, 2 and 7 are presented in **Fig.4**. There was a significant group effect in the water-maze latency (*\( P<0.001 \)) to reach the platform. This behavior was further confirmed by the infarct volume in all the groups of rats and C. oil significantly decreases the infarct volume as compared to ischemic group of rats on day 7 (*\( P<0.001 \)).
Chapter 4

Fig.4: Effect of C. oil on ischemia-induced deficit in learning and memory behavior by water maze test in the sham operated, ischemic, C. oil treated group. C. oil treatment resulted in significant improvement in latency time to reach the platform when compared to ischemic group.

![Latency time (in sec)]

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**Fig.4:**

- **Day 1:**
  - **Sham:** median 2.55;
  - **Ischemia:** 2.10 (95% CI 1.97-2.33)
  - **C. oil:** 2.35 (95% CI 2.15-2.55)

- **Day 2:**
  - **Sham:** 2.20 (95% CI 2.00-2.40)
  - **Ischemia:** 2.30 (95% CI 2.10-2.50)
  - **C. oil:** 2.10 (95% CI 1.90-2.30)

- **Day 7:**
  - **Sham:** 2.00 (95% CI 1.80-2.20)
  - **Ischemia:** 1.80 (95% CI 1.60-2.00)
  - **C. oil:** 1.60 (95% CI 1.40-1.80)

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**Fig.5:** Effect of C. oil on neurological deficits score after day 7; sham operated group, ischemia, C. oil treated group. Number of animals used were five in each group. 

*P*<0.001 as compared with the ischemic group.

![Neurological deficits score]

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**Fig.5:**

- **Sham:** median 1.49 (95% CI 1.30-1.69)
- **Ischemia:** median 2.55 (95% CI 2.30-2.80)
- **C. oil:** median 2.10 (95% CI 1.90-2.30)
4.3 Discussion

In the present study, the therapeutic potential of C.oil on the MCAo model of stroke in rats was observed. The results of the present study demonstrate that administration of different doses of C.oil before MCA occlusion reduces the volume of ischemic brain infarct that develops subsequently. The data presented confirms that C.oil has a dose-dependent neuroprotective effect in ischemic rats. MCAo caused significant impairment in motor performance, which was evident from the increased neurological deficit score and the poor performance on the rotarod. This could be because of the neuronal damage in the territory supplied by the middle cerebral artery, i.e. the caudate nucleus, the putamen, and the striatum, areas that regulate motor coordination. The present results are similar to previous reports, which have also shown significant neurological deficit at 24 hours after MCAo (Tatlisumak et al., 1998).

The treatment with C.oil reduced the failure in the spatial memory function in the water maze test, suggesting that this agent is capable of improving learning and memory function impaired by sustained cerebral ischemia. The retention test was conducted on day 7 after the operation to examine whether the effect of treatment may persist. Notably, C.oil-treated rats revealed a significant shortening of the escape latency in the retention test. This finding suggests that C.oil is capable of exerting a persistent effect on learning and memory function or of preventing the loss of spatial navigation ability due to ischemic damage in animals. Furthermore, at the first trial in the contraposition test on day 7 where the platform had been moved to the opposite quadrant, C.oil shortened the escape latency of the rats,
suggesting that the overall process, including search strategy, of the C.oil treated animal may be superior to that of the untreated animal. The shortened escape latency at the fourth trial in the contraposition test predicted better acquisition of short-term memory in the C.oil-treated rats. The question arises as to the nature of the mechanism underlying the C.oil-mediated improvement of learning and memory function by C.oil ischemia-induced animals.

C.oil is recently been reported to offer neuroprotection due to its antioxidant and anti-inflammatory properties (Rathore et al., 2007). Further studies are warranted to pursue the interesting lead emerging from the present results to exploit the full therapeutic potential of C.oil in Cerebro vascular disease.