Anti-inflammatory and anti-pyretic properties of *Spirulina platensis* and *Spirulina lonar:* A comparative study

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Abstract: *Spirulina spp.* is a blue-green algae belongs to the family of Oscillatoriaceae, which having diverse biological activity. The aim of this current study was to evaluate and compare the anti-pyretic and anti-inflammatory activity of *Spirulina platensis*/SP and *Spirulina lonar*/SL extracts. In the anti-pyretic study, the ability to reduce the rectal temperature of rats induced pyrexia with 2g/kg Brewer's Yeast (BY) was performed. Rats were dosed either 2 or 4 mg/kg SP or SL. Rectal temperature was taken every hour for 8 hours. Results shown that there were significant dose-dependent ($p<0.05$) reduction of both treatments. However, SP treatment revealed faster reduction in rectal temperature. For anti-inflammatory activity, the reduction in the volume of paw edema induced by Prostaglandin E_2 (100 IU/rat intraplantar) was measured. Rats were dosed orally with 2 or 4 mg/kg SP or SL. The paw edema was measured every 30 minutes for 4 hours using plethysmometer. Results had shown a significant dose dependent reduction in diameter of paw edema ($p<0.05$). The finding suggests that SP and SL extracts have anti-pyretic and anti-inflammatory properties. However, SP was found to be more effective than SL as anti-pyretic and anti-inflammatory agent.

Keywords: *Spirulina platensis*; *Spirulina lonar*; anti-pyretic; Brewer's Yeast; anti-inflammation; Prostaglandin E_2

INTRODUCTION

Spirulina platensis (SP) and *Spirulina lonar* (SL) belong to the family of Oscillatoriaceae. Both SP and SL are blue-green algae that contain balanced proteins and various essential nutrients. It contains one of the highest protein content of 70% where 18 out of 22 essential amino are available. Therefore, SP and SL are popular vegetarian source of complete protein (Somchit *et al.*, 2007). SP and SL have also been reported to be a rich natural source of carotenoids and other micronutrients (Mathew *et al*., 1995). Their color is derived from the green pigment of chlorophyll and the blue from the protein phycocyanin. SP is widely available in South East Asia (Somchit *et al.*, 2007) and SL is mainly in India (Kuriakose and Kurup, 2010).

In vitro and *in vivo* studies have demonstrated several properties of SP, including anticancer (Schwartz and Sklar, 1986; Zhang *et al*., 2011), antioxidant (Dartsch, 2008), antiviral (Murugan and Radhamadhavan, 2011), anti-allergy (Belay, 2002), immune-enhancing (Qureshi *et al*., 1996), liver- protecting (Vadiraja *et al.*, 1998), blood vessel-relaxing (Gur *et al.*, 2013), and blood lipid-lowering (Colla *et al.*, 2008) effects. SP is rich in dietary g-linolenic acid (GLA), which is also act as an anti-inflammatory compound (Belay, 1993). The amount of phycocyanin in SP and SL varies up to 15% of the dry weight. Phycocyanin has been reported as a potent anti-oxidant and anti-inflammation (Romay *et al*., 1998, Gonzalez *et al*., 1999, Gur *et al.*, 2013) and even has liver-protective effects (Vadiraja *et al*., 1998). One previous study had demonstrated a selective inhibition of cyclooxygenase-2 (COX2) by C-phycocyanin, a biliprotein from SP (Reddy *et al*., 2000). However, research on SL has not been reported extensively as SP. Kuriakose and Kurup (2010), reported hepatoprotective activity of SL due to its anti-oxidant activity. SL also contains similar phytochemicals as SP. Therefore; the objective of this present investigation was to evaluate the anti-pyretic and anti-inflammatory properties of SP and SL in rats.

MATERIALS AND METHODS

Materials

SP was cultivated by University Industri Selangor and SL from the National Centre for Conservation and Utilization of Blue-Green Algae (NCCUBGA), New Delhi, India. SP and SL were dried and extracted using distilled water as solvent in a Sox let apparatus as described previously (Somchit *et al.*, 2003). Male Spargue Dawley rats with bodyweight of 180 to 200 g were purchased from Institute of Medical Research, Kuala Lumpur, Malaysia. They were kept in rat polypropylene cages with wood shavings as bedding in 12 h light/dark cycle. The animals were adapted to laboratory conditions for 7 days prior to the experiments and were given feed and tap water *ad*

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libitum. The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulation followed in this institute.

Anti-inflammatory assessment

4 groups of rats (n=6/group); positive control (Group 1), were given Prostaglandin E_2 (100 IU/rat), while for negative control (Group 2) rats were given normal saline intraplantar at the hind left paw. Group 3 and 4 were treated with 2mg/kg and 4mg/kg SP orally for 4 consecutive days before administration of Prostaglandin E_2 (100 IU/rat) intraplantar. Group 5 and 6 received 2 mg/kg and 4 mg/kg SP orally for 4 consecutive days before administration of Prostaglandin E_2 (100 IU/rat) intraplantar. Paw volume was measured every 30 min for 6 hrs (Somchit *et al.,* 2003 and Somchit *et al.,* 2004).

Anti-pyretic assessment

Rats (n=6/group) were divided into; Positive control (Group 1) which was treated with Brewer's Yeast (2g/kg) ip and negative control (Group 2) was given with distilled water. Groups 3 to 6 were treated with SP or SL extracts (2 mg/kg and 4 mg/kg for 4 days orally) before administration of Brewer's Yeast (2g/kg) ip. Rectal temperature was taken every 30 min for 6 hrs. The detailed method described by Bruguerolle *et al.*, 1994 and Zakaria *et al.,* 2006.

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation (SD) and analyzed statistically using one-way analysis of variance (ANOVA). The statistical difference between treatments groups were calculated by the use of Duncan Test. Result was considered significantly at $p<0.05$.

RESULTS

Anti-pyretic Test

Results for the effects of the aqueous extracts of SP and SL on temperature increased induced by Brewer's Yeast (BY) are displayed in fig. 1. There had significant statistical effect on pyrexia for extract of 2mg/kg SP, 4mg/kg SP, 2mg/kg SL and 4mg/kg SL as compared to the negative and positive control rats. Fig. 2 shows mean rectal temperature among groups in 7 hours. For the results between 2mg/kg and 4mg/kg of *SP* extract had no significant effect on pyrexia induced by BY. Besides that, the results of the 2mg/kg *SL* and 4mg/kg SL extract also were showed no statistical significant difference. All these result had exhibited no dose-dependent pattern in anti-pyretic test.

The results showed statistical significant difference between 2mg/kg *SP* and SL extract. Meanwhile, for the results between 4mg/kg *SP* and 4mg/kg *SL* were showed no significant difference on the pyretic rats.

Anti-inflammatory Test

Fig. 3 demonstrate the results for the effect of the SP and SL extracts on reduction of increased in edema induced by formal in 5%. As shown in fig. 4, the graph shows the mean paw edema between the treatment group and control group in 240 minutes. The extract in the entire treatment group demonstrated significant anti-inflammatory effects against formal in 5% induced inflammation as compared to control group.

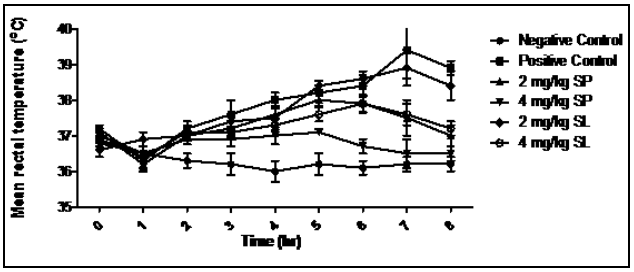

Fig. 1: Mean rectal temperature of rats
Values are Mean ± sd. (n=6/group). NC (negative control) given distilled water, PC (positive control) given Brewer's Yeast 2g/kg, SP (*Spirulina platensis*), SL (*Spirulina lonar*)

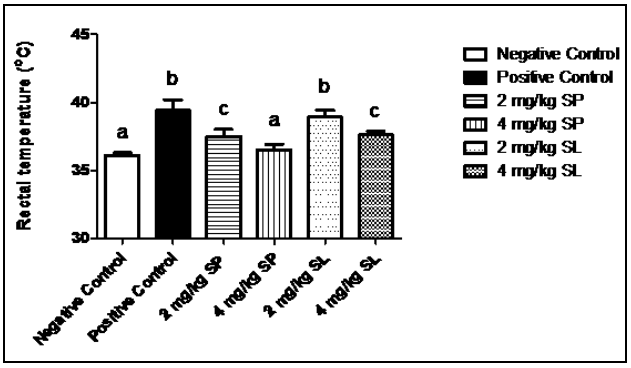

Fig. 2: Rectal temperature at 7 hours
Values are Mean ± sd. (n=6/group). Mean with different alphabet differ significantly ($p<0.05$) using ANOVA and Duncan Post Hoc Test). SP (*Spirulina platensis*); SL (*Spirulina lonar*).

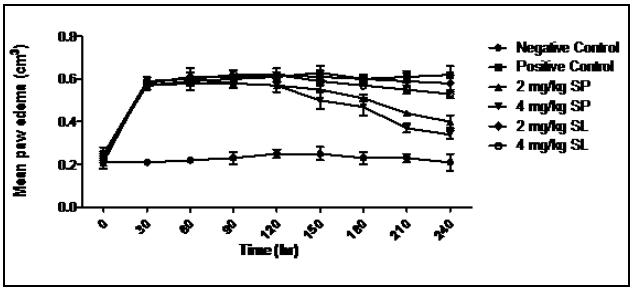

Fig. 3: Mean paw edema of rats
Values are Mean ± sd. (n=6/group). NC (negative control) given distilled water, PC (positive control) given Brewer's Yeast 2g/kg, SP (*Spirulina platensis*), SL (*Spirulina lonar*)

In this test, the results demonstrated no statistical significant between 2mg/kg and 4mg/kg of each extract. The results were showed no dose-dependent pattern in all

the treatment group of both SP and SL. However, significant difference was seen between extract of 2mg/kg of *SL* and *SP*. Besides that, in the concentration of 4mg/kg of *SP and SL* extract were showed a significant anti-inflammatory effect against edema-induced formal in 5% in rats when compared to controls.

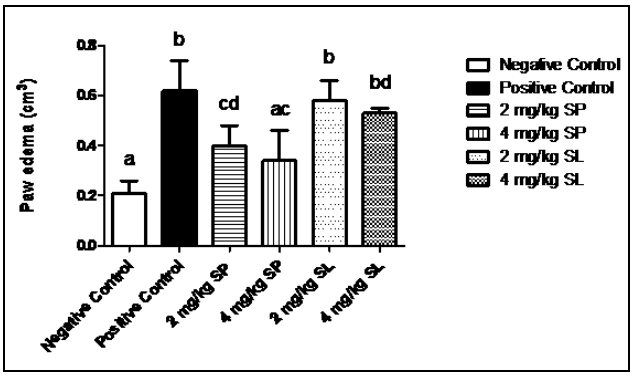

Fig. 4: Mean paw edema at 240 minutes
Values are Mean ± sd. (n=6/group). Mean with different alphabet differ significantly ($p < 0.05$) using ANOVA and Duncan Post Hoc Test). SP (*Spirulina platensis*); SL (*Spirulina lonar*).

DISCUSSION

Based on the result obtained, extract of SP and SL have anti-pyretic activity, where the reduction effect on the increased in rectal temperature induced by in the entire treatment group as compared to control group. From statistical analysis, there are significant differences ($p<0.05$) between 2mg/kg of *SP* and SL extracts. From this result, SP was found to contain the more potent anti-pyretic activity as compared to SL. When there is infections, cytokines will be released that can directly act on hypothalamus, causing the release specific prostaglandins. Prostaglandins, in turn, reset the hypothalamic thermostat to a higher temperature causing the body to initiating heat-promoting mechanisms. Most commercial drug such as non-steroidal anti-inflammatory drug (NSAID) such as diclofenac, ibuprofen or mefenamic acid has the therapeutic actions, to reduce inflammation and pyrexia (Litalien and Jacqz-Aigrain, 2001). However, these NSAIDs may produce adverse reactions such as hepatotoxicity (Somchit *et al.,* 2004). As also other commercial synthetic drugs cause severe liver toxicity (Somchit *et al.,* 2002). Therefore, natural remedies have been a popular alternative.

Acute inflammation is characterized by its short duration (from few minutes to several days) and the main characteristics are infiltration of newtrophils and exudation. Several studies have demonstrated by injection of formal in, the paw will develop edema rapidly due to acute inflammation (Lee and Jeong, 2001; Sulaiman *et al.,* 2008).

The anti-inflammatory test, reduction of formal in-induced edema in rats is one of the most suitable test procedures to screen anti-inflammatory agents. Injection of formal in intraplantarly into left hind paw of rats produced localized inflammation and pain. The nociceptive effect of formal in is biphasic, an early neurogenic component followed by a later tissue mediated response (Wheeler-Aceto and Cowan, 2001).

The formalin injection will result in a progressive biphasic behavioral change like licking and biting of the injected paw in rats (Lee and Jeong, 2001). Statistical analysis revealed the extracts in the entire treatment group were showed significant differences as compared to control rats. From this result, both SP and SL have anti-inflammatory effect, which the reduction in diameter of left hind paw edema induced by 5% formal in. Each concentration of 2mg/kg and 4mg/kg between SP and SL extract was showed significant differences. This result was demonstrated that SP was found to be more effective than SL act as an anti-inflammatory agent, where to reduce the paw edema induced by 5% formal in. The exact mechanism of anti-inflammation induced by SP and SL is yet to be determined. But, inhibition of certain specific cofactors of inflammation can be the key (Ahmad *et al.,* 2006). The present findings in these studies depict that SP was found to be the more potent and effective act as an anti-pyretic and anti-inflammatory agent as compared to SL. This might due to the higher amount of characteristic constituents' likes high protein content, vitamins (especially B12 and β-carotene), fatty acid composition, linolenic acid (especially gamma-linolenic acid), phycocyanin and others, which present in SP. Indeed, phycocynin has been documented to reduce inflammation and accelerate wound healing (Gur *et al.,* 2013).

In anti-pyretic study, 2 SP and SL reduced rectal temperature induced by in rats. In anti-inflammatory test, both extracts also reduced the left hind paw edema induced by formal in 5%. We can conclude that extract of SP more potent than SL. Indeed, more studies must be performed to further investigate the exact mechanism of anti-inflammation and anti-pyretic properties of SP and SL.

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