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## Virgin Coconut Oil Prevents Nicotine Dependence and Relapse

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**Abstract:** Nicotine is an addictive substance with detrimental effects on health. Several measures have been developed to help addicts quit smoking, yet the rate of increase in number of smokers does not seem to have slowed down. The current study was aimed to investigate the effect of Virgin Coconut Oil (VCO) on nicotine dependence and relapse using Conditioned Place Preference (CPP) paradigm in rats. The rats were randomly assigned to one of the following treatment groups: place conditioning without drug treatment before nicotine ( $0.5 \text{ mg kg}^{-1}$ , i.p.); place conditioning with oral VCO at  $5 \text{ mL kg}^{-1}$  or the reference drug diclofenac ( $3.2 \text{ mg kg}^{-1}$ , i.p.) 30 min prior to each nicotine treatment. The conditioning training lasted for 5 days, followed by conditioning test on the following day. In different batch of rats, the conditioning test continued with a 7-day nicotine-free period and the rats were then challenged on the following day with  $0.5 \text{ mg kg}^{-1}$  nicotine intraperitoneally. VCO and diclofenac were given 30 minutes before nicotine challenge to test their effects. Results showed that VCO and diclofenac significantly decreased ( $p < 0.01$ ) the preference to nicotine-paired compartment to those of preconditioning levels in both preference tests. Taken together, results of the present study indicate that VCO prevents nicotine dependence as well as relapse. The results further lay foundation for the development of potent agents for nicotine dependence.

**Key words:** Virgin coconut oil, nicotine, dependence, relapse, rats

### INTRODUCTION

Nicotine, the psychoactive ingredient of tobacco, is an addictive substance with fairly easy accessibility. High prevalence of smoking, which is independent of age and gender, portrays the wide consumption of this addictive substance (Ebisike *et al.*, 2004; Keloglu-Isler and Erdogan, 2007; Naddaf, 2007). Most of components contained in cigarettes have deteriorating effects on health, which include degenerative disorders, such as cardiovascular diseases and cancer (Huang and Chen, 2011; Nourbala *et al.*, 2011). The yearly mortality rate related to tobacco use has been predicted to skyrocket to as much as 10 million in 2025 (West, 2006). As with addictive substances in common, nicotine has rewarding effect (McGehee, 2009) that forces the smokers to stick to smoking habit. Thus, in spite of full consciousness on the devastating consequences of smoking on health, most smokers who attempt to quit smoking do not succeed in long-term (Trofor *et al.*, 2008).

Several attempts have been tried to treat smoking habit and these are directed towards the reduction of the

number of smokers and the ensuing sufferings caused by smoking. The most widely applied modes of treatment are Nicotine Replacement Therapy (NRT) and bupropion (Berrettini and Lerman, 2005). However, the currently applied treatments are considered insufficient and associated with serious adverse events (Wilkes, 2006; Hays and Ebbert, 2010). Moreover, if replacement therapy is the treatment of choice, the smokers are implicitly confronted to dependence on the replacing substance, as is often reported by addicts who receive such a therapy. Alternative therapies with better efficacy and safety features are therefore, still in need.

A number of studies during the past two decades have shown the involvement of neuroinflammatory mechanism in drug dependence. The arachidonic acid cascade, which is an essential component of inflammatory reaction, has been implicated to contribute to the rewarding effect of many addictive substances (Anggadiredja *et al.*, 2003; Yamamoto *et al.*, 2004).

Virgin Coconut Oil (VCO) is the natural, organic and pure coconut oil, which has never been exposed to hydrogenation, heat or long storage and other processing

(Abdurahman *et al.*, 2009; Hamid *et al.*, 2011). In the past few years, empirical as well as experimental data has indicated biological activities of VCO that shows promising medical applications. Thus, Ogbolu *et al.* (2007) have reported anticandidal activity of VCO, which was comparable to that of a synthetic antifungal agent fluconazole. Furthermore, a number of other works have shown VCO activities on the improvement of lipid metabolism related to hyperlipidemia, including that reported by Nevin and Rajamohan (2004). In addition, a recent study has demonstrated hepatoprotective activity of dried- as well as fermented processed VCO (Zakaria *et al.*, 2011). In a recent study (Intahphuak *et al.*, 2010) anti-inflammatory effect of VCO has been demonstrated. This result, together with those showing the involvement of neuroinflammatory mechanism in drug dependence, has become the basis of the present study. The effect of VCO on nicotine dependence as well relapse was investigated in a rat model using Conditioned Place Preference (CPP) paradigm.

## MATERIALS AND METHODS

**Subjects:** Male Wistar rats weighing approximately 200 g, supplied from Animal Laboratory of School of Pharmacy Institute of Technology Bandung, were used. All animals were experimentally naive. Procedures for animal handling and treatment have been evaluated by the medical ethical committee of Hasan Sadikin General Hospital of Bandung. Experiments were conducted between August 2010 and April 2011.

**Drugs:** Nicotine [(-)-nicotine di(+)-tartrate salt] was purchased from Sigma Chemical. Sodium diclofenac was a generous gift from Dexa Medica (Jakarta, Indonesia). The drugs were dissolved in physiological saline and injected intraperitoneally (i.p.) at a volume of 1 mL kg<sup>-1</sup> body weight. VCO, obtained from School of Life Sciences and Technology, Institute of Technology Bandung, was given orally at a dose of 5 mL kg<sup>-1</sup>.

**Apparatus:** The place conditioning apparatus consisted of three distinct compartments (separated by guillotine doors). The overall dimensions of the apparatus were 30×30×70 cm (H×W×L). The center compartment of 10×10 cm dimension was gray with a smooth PVC floor. The choice compartments were 30 cm long. One compartment was all black with a stainless steel grid rod floor consisting of 4.8 mm rods, placed 16 mm on center, while the other was all white with a 0.25×1.25 cm stainless steel mesh floor. The apparatus had clear acrylic lid for animal loading.

**Place conditioning training:** On day, 1 rat were injected with either nicotine (0.5 mg kg<sup>-1</sup>) or saline and immediately confined to one of the choice compartment for 30 min. The specific drug-paired chamber for each animal was randomly assigned, to give unbiased nature of this CPP design. Four hours later, the rats were injected with the alternate condition: rats receiving drug in the morning session received saline in the afternoon session and viceversa Rate then immediately confined to the opposite choice compartment for 30 min. This procedure was repeated for the next four days (day 2-5) so that each rat received a total of five drug and five saline sessions. The sequence of drug injections differed across and within rats on these conditioning days to eliminate any potential order effects. The treatment condition was altered each day so the rats did not receive the same treatment condition at the same time each day.

**Preference test:** On day, 6 rats were tested for post conditioning side preference. Following a 5 min acclimation period in the center gray compartment, the guillotine doors were lifted and rats were allowed to access to all three compartments for 15 min. The times spent in both compartments were recorded. Preference scores, the index of rewarding property in CPP paradigm, were then determined by dividing sojourn time in the drug-paired compartment to the total time spent in both drug-paired and saline-paired compartments during test session, using the following simple formula:

$$\text{Preference score} = \frac{\text{Sojourn time in drug-paired compartment (sec)}}{\text{Total time spent in all compartments (sec)}}$$

VCO (5 mL kg<sup>-1</sup>) was administered orally to rats in test group 30 min prior to each nicotine injection during conditioning training. In different test group, instead of VCO, the reference drug diclofenac at 3.2 mg kg<sup>-1</sup> was given intraperitoneally.

**Relapse test:** Following conditioning test, the rats in this batch entered a 7-day drug free period. On the following day, they were challenged with nicotine at 0.5 mg kg<sup>-1</sup> intraperitoneally. VCO (5 mL kg<sup>-1</sup>) was administered orally to rats in test group 30 minutes prior to nicotine challenge. In different test group, the reference drug diclofenac at 3.2 mg kg<sup>-1</sup> was given intraperitoneally.

**Statistical analysis:** Data were expressed as preference score, which was calculated dividing sojourn time (sec) in drug-paired compartment by total sojourn time(sec) in both drug-paired and saline-paired compartments. One-way Analysis of Variance (ANOVA) was used to compare

means of preference score and Fisher's PLSD was used for post hoc test (Anggadiredja *et al.*, 2003), using Statview statistical software.

**RESULTS**

As depicted in Fig. 1, following a 5-day period of conditioning training, the preference score of rats receiving repeated nicotine treatment increased significantly, reaching a value of  $0.516 \pm 0.195$ , compared to

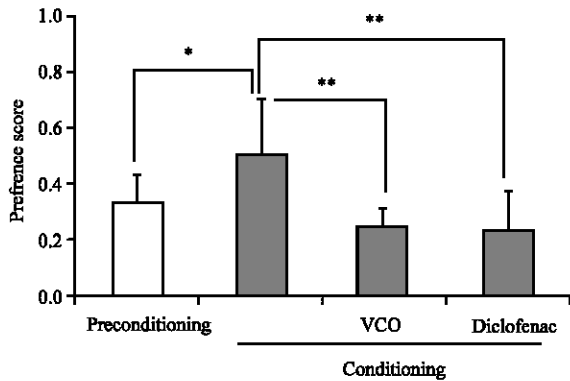


Fig. 1: Effects of VCO on the of nicotine dependence in rats as tested with conditioned place preference (CPP) paradigm. VCO (5 mL kg<sup>-1</sup>, p.o.) or diclofenac (3.2 mg kg<sup>-1</sup>, i.p) was given 30 min before each nicotine (0.5 mg kg<sup>-1</sup>, i.p.) injection during the 5-day period of preference training. \*, \*\*p<0.05 and 0.01, respectively (one-way ANOVA followed by Fisher's PLSD)

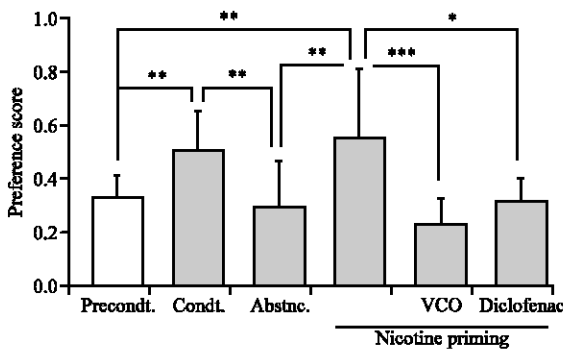


Fig. 2: Effects of VCO on nicotine relapse in rats as tested with conditioned place preference (CPP) paradigm. VCO (5 mL kg<sup>-1</sup>, p.o.) or diclofenac (3.2 mg kg<sup>-1</sup>, i.p) was given 30 min before nicotine (0.5 mg kg<sup>-1</sup>) challenge delivered after a 7-day period of nicotine abstinence. \*, \*\*, \*\*\*p<0.05, 0.01, and 0.001, respectively followed by Fisher's PLSD)

the pre-conditioning training score of  $0.332 \pm 0.097$ . Figure 1 further revealed significant effect of VCO as well as diclofenac treatments, which decreased the preference score to  $0.233 \pm 0.138$  and  $0.243 \pm 0.066$ , respectively ( $F(3,26) = 5.509$ ,  $p = 0.0046$ , one-way ANOVA, post hoc Fisher's PLSD).

Figure 2 describes that when the rats were made nicotine-free during a 7-day period of abstinence, the preference score was significantly decreased to a preconditioning-training level of  $0.291 \pm 0.175$ . The score was again increased significantly (to as high as  $0.553 \pm 0.257$ ) when the rats were challenge with nicotine on the following day after the abstinence period. Figure 2 further describes, treatment with VCO or diclofenac before nicotine challenge resulted in significant decrease in preference score to  $0.317 \pm 0.084$  and  $0.227 \pm 0.099$ , respectively, ( $F(5,54) = 5.571$ ,  $p = 0.0003$ , one-way ANOVA, post hoc Fisher's PLSD).

**DISCUSSION**

Result which showed the increase in preference score in rats treated repeatedly with nicotine corroborates the rewarding effect of nicotine. The same observation has been reported in early study by Shoaib *et al.*( 1994) which showed that CPP was produced in subjects having nicotine history with a dose range of 0.4-0.8 mg kg<sup>-1</sup>, within which the current experimental dose (0.5 mg kg<sup>-1</sup>) was used. This dose has also been used in previous CPP study (Risinger and Oakes, 1995; Grabus *et al.*, 2006). Larger dose of nicotine of as high as 2 mg kg<sup>-1</sup> used in CPP experiment, on the other hand, produced conditioned place aversion, instead of preference (Le Foll and Goldberg, 2005).

The rewarding characteristic of nicotine was further confirmed when the drug was re-introduced to the abstinent rats previously exposed to repeated nicotine. Such an observation has been reported in a number of earlier works by Biala and Budzynska (2006, 2008), Fattore *et al.* (2009), Biala *et al.* (2009) and Budzynska *et al.* (2009). In clinical setting, this phenomenon of relapse has been a main hurdle in the treatment of drug addiction. Thus, addicts who have been drug-free, even for years, may still resume the drug taking habit when they are exposed to the drug or drug cues.

The present results showed that pretreatment with VCO prevent the development of nicotine dependence, as shown by the decrease in preference score in rats receiving VCO prior to every nicotine dose. VCO also prevented the increase in preference score in abstinent rats challenged with nicotine injection, indicating a blocking effect of VCO on relapse to nicotine.

It was only recently demonstrated that VCO has analgesic, antipyretic as well as antiinflammatory effects (Intahphuak *et al.*, 2010). This range of pharmacological activities are the characteristics of non-steroidal antiinflammatory drugs, whose effects are known to interfere with the arachidonic acid cascade. In the past several years, the involvement of this endogenous metabolic pathway in the phenomena of dependence and relapse of addictive substances has been shown. Thus, prostaglandin, an end product of the cyclooxygenase pathway of the arachidonic acid cascade, has been shown to prevent the expression of withdrawal signs in mice made dependent on the cannabinoid agent delta-9 tetrahydrocannabinol (THC, a psychoactive ingredient of marihuana). This was confirmed by the withdrawal-inducing activity of diclofenac in mice repeatedly treated with delta-9 THC (Anggadiredja *et al.*, 2003). Using withdrawal expression as indicator, it was further found that the signs were expressed when a group of cannabinoid-naive mice were treated consecutively with diclofenac and a cannabinoid receptor antagonist (Anggadiredja *et al.*, 2005). With regard to relapse, a previous work with methamphetamine further corroborate the essential role of the arachidonic acid cascade. Using self-administration paradigm, in which rats were trained to actively deliver methamphetamine, it was found that diclofenac prevented methamphetamine-seeking behavior following the challenge of a small dose of this addictive substance in the drug-abstinent rats (Anggadiredja *et al.*, 2004a).

The aforementioned works were also the demonstration of cross talk between the arachidonic acid cascade and endocannabinoid system. This endogenous interaction has also been demonstrated by Yamaguchi *et al.* (2001, 2004). Recently, Merritt *et al.* (2008) have reported that endogenous cannabinoid modulated nicotine CPP, corroborating the essential of cannabinoid-prostanoid interaction in rewarding property of psychoactive drugs and nicotine in particular.

Diclofenac was used in present study as reference substance for VCO effect on the rewarding property of nicotine. This was based on previous results which demonstrated the effectiveness of diclofenac in ameliorating dependence and reinforcing properties of several psychoactive drugs. Judging from the present results which showed that the effect of VCO was comparable with that of diclofenac, one may hypothesize that VCO interfered with the arachidonic acid cascade machinery. One possible explanation might reside in the common lipid nature of VCO (Dayrit *et al.*, 2008) and the arachidonic acid (Morrow and Roberts, 2001). Thus, it

might be that lipid components of VCO served as the source for arachidonic acid synthesis, whose downstream processes might eventually led to silencing effect on the preference to nicotine-paired CPP compartment.

The approach applied in the present study is closely related with learning-memory aspect, because in the development of dependence the rats were let to learn to associate drug to certain cue, in this case the drug-paired compartment of CPP apparatus. Indeed, as indicated in the previous study by Anggadiredja *et al.* (2004b), it is the memory that perpetuate the engagement between drug and drug users. Matsumoto *et al.* (2004) has indicated the role the arachidonic acid cascade in memory formation. Taking into consideration the possibility of VCO to interfere with the arachidonic acid cascade, one may further hypothesize that it also plays a role in the learning and memory aspects of drug dependence.

Taken together, while still at the very early stage, results of the present study are in favor with the evidence reported so far on interaction among dependence, memory and the arachidonic acid cascade metabolic pathway. VCO, whose preventive effects on nicotine dependence and relapse was demonstrated in this study, might have an essential role in the modulation of the arachidonic acid cascade by acting as a source of fatty acid.

## CONCLUSION

The present work demonstrated, for the first time, preventing effects of VCO on nicotine dependence as well as relapse, as studied using conditioned place preference paradigm in rat model. The results may further lay foundation for the development of agent that can help addicts quit smoking and for combating drug addiction in general.

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## REFERENCES

- Abdurahman, H.N., F.S. Mohammed, R.M. Yunus and A. Arman, 2009. Demulsification of virgin coconut oil by centrifugation method: A feasibility study. *Int. J. Chem. Technol.*, 1: 59-64.
- Anggadiredja, K., T. Yamaguchi, H. Tanaka, Y. Shoyama, S. Watanabe and T. Yamamoto, 2003. Prostaglandin E2 attenuates SR141716A-precipitated withdrawal in tetrahydrocannabinol 1- dependent mice. *Brain Res.*, 966: 47-53.

- Anggadiredja, K., K. Sakimura, T. Hiramita and T. Yamamoto, 2004a. Naltrexone attenuates cue-but not drug-induced methamphetamine seeking: A possible mechanism for the dissociation of primary and secondary reward. *Brain Res.*, 1021: 272-276.
- Anggadiredja, K., M. Nakamichi, T. Hiranita, H. Tanaka, Y. Shoyama, S. Watanabe and T. Yamamoto, 2004b. Endocannabinoid system modulates relapse to methamphetamine seeking: Possible mediation by the arachidonic acid cascade. *Neuropsychopharmacology*, 29: 1470-1478.
- Anggadiredja, K., T. Yamaguchi, H. Tanaka, Y. Shoyama, S. Watanabe and T. Yamamoto, 2005. Decrease in prostaglandin level is a prerequisite for the expression of cannabinoid withdrawal: A quasi abstinence approach. *Brain Res.*, 1066: 201-205.
- Berrettini, W.H. and C.E. Lerman, 2005. Pharmacotherapy and pharmacogenetics of nicotine dependence. *Am. J. Psychiatry*, 162: 1441-1451.
- Biala, G. and B. Budzynska, 2006. Reinstatement of nicotine-conditioned place preference by drug priming: Effects of calcium channel antagonists. *Eur. J. Pharmacol.*, 537: 85-93.
- Biala, G. and B. Budzynska, 2008. Calcium-dependent mechanisms of the reinstatement of nicotine-conditioned place preference by drug priming in rats. *Pharmacol. Biochem. Behav.*, 89: 116-125.
- Biala, G., B. Budzynska, and N. Staniak, 2009. Effects of rimobant on the reinstatement of nicotine-conditioned place preference by drug priming in rats. *Behav. Brain Res.*, 202: 260-265.
- Budzynska, B., M. Kruk and G. Biaza, 2009. Effects of the cannabinoid CB1 receptor antagonist AM 251 on the reinstatement of nicotine-conditioned place preference by drug priming in rats. *Pharmacol. Rep.*, 61: 304-310.
- Dayrit, F.M., O.E. Buenafe, E.T. Chainani and I.M. de Vera, 2008. Analysis of monoglycerides, diglycerides, sterols and free fatty acids in coconut (*Cocos nucifera* L.) oil by 31P NMR spectroscopy. *J. Agric. Food Chem.*, 56: 5765-5769.
- Ebisike, K., O.O. Ayejuyo, J.A. Sonibare, O.A. Ogunkunle and T.V. Ojumu, 2004. Pollution impacts of cigarette consumption on indoor air quality in Nigeria. *J. Applied Sci.*, 4: 623-629.
- Fattore, L., M.S. Spano, G. Cossu, M. Scherma, W. Fratta and P. Fadda, 2009. Baclofen prevents drug-induced reinstatement of extinguished nicotine-seeking behaviour and nicotine place preference in rodents. *Eur. Neuropsychopharmacol.*, 19: 487-498.
- Grabus, S.D., B.R. Martin, S.E. Brown and M.I. Damaj, 2006. Nicotine place preference in the mouse: Influences of prior handling, dose and strain and attenuation by nicotinic receptor antagonists. *Psychopharmacology*, 184: 456-463.
- Hamid, M.A., M.R. Sarmidi, T.H. Mokhtar, W.R.W. Sulaiman and R.A. Aziz, 2011. Innovative integrated wet process for virgin coconut oil production. *J. Applied Sci.*, 11: 2467-2469.
- Hays, J.T. and J.O. Ebbert, 2010. Adverse effects and tolerability of medications for the treatment of tobacco use and dependence. *Drugs*, 70: 2357-2372.
- Huang, R.Y. and G.G. Chen, 2011. Cigarette smoking, cyclooxygenase-2 pathway and cancer. *Biochim. Biophys. Acta*, 1815: 158-169.
- Intahphuak, S., P. Khonsung and A. Panthong, 2010. Anti-inflammatory, analgesic and antipyretic activities of virgin coconut oil. *Pharm. Biol.*, 48: 151-157.
- Keloglu-Isler, E. and I. Erdogan, 2007. Opinions of Turkish university students on cigarette smoking at schools. *J. Applied Sci.*, 7: 2762-2768.
- Le Foll, B. and S.R. Goldberg, 2005. Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacology*, 178: 481-492.
- Matsumoto, Y., T. Yamaguchi, S. Watanabe and T. Yamamoto, 2004. Involvement of arachidonic acid cascade in working memory impairment induced by interleukin-1 beta. *Neuropharmacology*, 46: 1195-1200.
- McGehee, D.S., 2009. Nicotine's allure. *Neuron*, 10: 564-565.
- Merritt, L.L., B.R. Martin, C. Walters, A.H. Lichtman and M.I. Damaj, 2008. The endogenous cannabinoid system modulates nicotine reward and dependence. *J. Pharmacol. Exp. Ther.*, 326: 483-492.
- Morrow, J.D. and L.J. Roberts, II, 2001. Lipid-Derived Autacoids. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, Goodman, L.S., J.G. Hardman, L.E. Limbird and A.G. Gilman (Eds.). 10th Edn., McGraw-Hill, New York, USA., ISBN-13: 9780071354691, pp: 669-685.
- Naddaf, A., 2007. The social factors implicated in cigarette smoking in a Jordanian community. *Pak. J. Biol. Sci.*, 10: 741-744.
- Nevin, K.G. and T. Rajamohan, 2004. Beneficial effects of virgin coconut oil on lipid parameters and *in vitro* LDL oxidation. *Clin. Biochem.*, 37: 830-835.
- Nourbala, M.H., E. Nemati, Z. Rostami and B. Einollahi, 2011. Impact of cigarette smoking on kidney transplant recipients: A systematic review. *Iran J. Kidney Dis.*, 5: 141-148.

- Ogbolu, D.O., A.A. Oni, O.A. Daini and A.P. Oloko, 2007. *In vitro* antimicrobial properties of coconut oil on *Candida* species in Ibadan, Nigeria. *J. Med. Food*, 10: 384-387.
- Risinger, F.O. and R.A. Oakes, 1995. Nicotine-induced conditioned place preference and conditioned place aversion in mice. *Pharmacol. Biochem. Behav.*, 51: 457-461.
- Shoaib, M., I.P. Stolerman and R.C. Kumar, 1994. Nicotine-induced place preferences following prior nicotine exposure in rats. *Psychopharmacology*, 113: 445-452.
- Trofor, A., V. Esanu and E.I. Frasila, 2008. Extreme solutions for those who do not succeed to quit smoking. About smokeless tobacco and harm reduction. *Pneumologia*, 57: 105-108.
- West, R., 2006. Tobacco control: Present and future. *Br. Med. Bull.*, 77-78: 123-136.
- Wilkes, S., 2006. Bupropion. *Drugs Today (Barc)*, 42: 671-681.
- Yamaguchi, T., T. Kubota, S. Watanabe and T. Yamamoto, 2004. Activation of brain prostanoid EP3 receptors via arachidonic acid cascade during behavioral suppression induced by  $\Delta^8$ -tetrahydrocannabinol. *J. Neurochem.*, 88: 148-154.
- Yamaguchi, T., Y. Shoyama, S. Watanabe and T. Yamamoto, 2001. Behavioral suppression induced by cannabinoids is due to activation of the arachidonic acid cascade in rats. *Brain Res.*, 889: 149-154.
- Yamamoto, T., K. Anggadiredja and T. Hiramita, 2004. New perspectives in the studies on endocannabinoid and cannabis: A role for the endocannabinoid-arachidonic acid pathway in drug reward and long-lasting relapse to drug taking. *J. Pharmacol. Sci.*, 96: 382-388.
- Zakaria, Z.A., M.S. Rofiee, M.N. Somchit, A. Zuraini and M.R. Sulaiman *et al.*, 2011. Hepatoprotective activity of dried- and fermented-processed virgin coconut oil. *Evid. Based Complement Alternat Med.*, 2011: 1-8.