In vivo anti-inflammatory response and bioactive compounds' profile of polyphenolic extracts from edible Argan oil (*Argania Spinosa L.)*

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Introduction. Nowadays, natural food ingredients and specific natural anti-inflammatory compounds, which have benefits on the human health receive more attention from the scientific community. Several studies were conducted on the anti-inflammatory effects of Mediterranean plants and foods (Hodge et al 2018, Conforti et al. 2008). The anti-inflammatory potential was studied in order to indicate natural, safe and effective ingredients for food diets and pharmaceutical applications. The phenolic compounds from plants exhibit a high anti-inflammatory action against chronic inflammatory diseases (Recio et al. 2012).

Aim. The present work aims assessing the anti-inflammatory effects by testing polyphenolic extracts resulting from two different edible Argan oils (HP and MP) on two types of edema induced in Wistar rat. The chemical composition and bioactive molecules of HP and MP were also quantified and discussed. This work presents and discusses the first study about the *in vivo* anti-inflammatory potential of polyphenolic extracts from edible Argan oils.

Materials and Methods. In this study the polyphenolic components were extracted from the edible Argan oils respecting the experimental protocol from Pirisi et al. (2000). The evaluation of the polyphenolic-extract anti-inflammatory activity of edible Argan oil (*Argania spinosa L.*) on paw oedema was made by experimental trials using two stimulus: chemical stimul (Winter et al. 1963) or mechanical stimul (Riesterer and Jaques 1970).

After the stimuli, all Wistar rats fasted during 18 hours before the anti-inflammatory test and received 5 ml water with gastric gavages. The right hind paw is considered as control without treatment. The anti-inflammatory activity was expressed as percentage inhibition (%) of oedema thickness in treated animals vs the

control group:

% of inhibition = (mean [VL - VR] Control - mean [VL - VR] Treated) / [VL - VR] Control × 100

With VR the oedema volume of the right paw, and VL the oedema volume of the left paw.

In the present study, anti-inflammatory activity was tested using the carrageenan induced paw oedema assay, according to Winter et al. (1963) and Ferreira et al. (1971). For each polyphenolic extract (HP or MP) is a group of six male Wistar rats was applied as control and reference groups (n=6 for each group). Injection of 0.05 ml 1% carrageenan suspension in 0.9% NaCl into the left paw and under the sub-plantar aponeurosis was made (Nguemfo et al 2007), 1 hour after the oral administration of the polyphenolic compounds from the Argan oils at 300 and 500 mg/kg or of reference drug indomethacin at 20 mg/kg (Kaushik et al 2012). The volume variation was measured using a plethysmometer LE7500 (Ugo Basile, Italy) after the carrageenan injection and after at 90, 180 and 360 min.

Results and Discussion. Induction of paw edema by carrageenan is a method extensively used to verify the anti-inflammatory activity of many substances. The edema, which is a result of carrageenan injection in the left foot, is a biphasic event (Vinegar et al. 1969). The first phase, which occurs between 0 and 150 min after the injection of carrageenan, has been attributed to serotonin, bradykinin and histamine on vascular permeability (Panthong et al. 2007, Vinegar et al. 1969, Yonathan et al. 2006). Authors have declared that serotonin and histamine are released during 1h30min, whereas bradykinin is released during 2 h3 0 min after carrageenan injection (Mequanint et al. 2011, Panthong et al. 2007). The second phase, which occurs between 2h30min and 6 h post-carrageenan injection, contains the secretion of prostaglandins (Gomes et al. 2008, Mequanint et al. 2011). Carrageenan is sensitive to cyclo-oxygenase and not to lipoxygenase inhibitors and is used to test the effect of nonsteroidal anti-inflammatory drugs which first inhibit the cyclooxygenase implicated in prostaglandins synthesis. It has been reported that the inhibition of carrageenan-induced inflammation stops after 360 min (Bounihi et al. 2013). The injection of carrageenan into the sub-plantar tissue and experimental

trauma of the left foot induced edema development in the control groups, which peaked (0.57±0.014 and 0.71±0.014 ml, respectively) in paw volume,180 min after the induction. This confirms that carrageenan injection and experimental trauma provokes an acute inflammatory reaction into the left hind paw. Polyphenolic extracts from Argan oils (HP and MP) at 300 and 500 mg/kg, p.o., reduced the edema in the first and second phases of carrageenan-inflammation (significance $p<0.05$). Both HP and MP effects on carrageenan-edema were dose- and time dependent; 180 min after carrageenan administration, HP and MP extracts at 500 mg/kg showed a good inhibition activity with a peak effect of 72% for HP and 47% for MP. This effect from HP was not statistically different ($p < 0.05$) from that of the reference drug indomethacin at a dose of 10 mg/kg with an inhibition value of 72.63%. In the trauma-induced edema, the polyphenolic extracts of Argan oil (HP or MP) decreased edema in the different phases of the inflammatory response. The effect of HP and MP on experimental trauma was also dose dependent with a significant and higher inhibition (56% for HP and 44% for MP after 180 min) produced at 500 mg/kg. The effect is in accordance to that caused by indomethacin (20 mg/kg, 84.08% inhibition). The inhibition of paw volume during the inflammation by polyphenolic extracts from the two Argan oil (HP and MP), was also reported by Masresha et al. (2012).

Conclusions. The polyphenolic extracts from edible Argan oil HP and MP, thus have an anti-inflammatory activity by inhibiting the secretion of inflammatory mediators; histamine and serotonin furthermore suppressing cytokine and prostaglandin (Bounihi et al. 2013). However, the real mechanism of this anti-inflammatory action of the polyphenolic extracts from the edible Argan oils used in the present study is yet not clear and more investigation should be established. The anti-inflammatory potential of the traditional edible Argan oil was found better than that of the oil obtained by mechanical pressing. This result directly indicates the effect of the preparation process on the therapeutic activity of the edible Argan oils. Further investigation should be done in order to characterize and isolate the bioactive molecules responsible for the anti-inflammatory potential.