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H_2O_2-Induced Oxidative Stress, AChE inhibition and mediated brain injury attenuated by *Thymus algeriensis*

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Abstract

The aim of the present study is to evaluate the acetylcholinesterase (AChE) inhibition, the antioxidant enzyme activities and malondialdehyde (MDA) levels induced by the hydrophobic fractions of *Thymus algeriensis* (HFTS) growing in Tunisia. The obtained results showed that Hydrogen peroxide (H_2O_2), an oxidative stress inducer, act by decreasing body weight and brain weight of rats. Moreover, we found higher MDA levels in the group treated with H_2O_2 ($P<0.05$) and a significantly lower activity of catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and superoxide dismutase (SOD) levels, as well as a reduction of reduced glutathione (GSH) activity in the brain tissues of the H_2O_2-treated rats than the control groups ($P<0.05$), whereas rat receiving HFTS with H_2O_2 decline MDA levels in the brain.

In contrast, HFTS demonstrated neuroprotective effects in rat brain. Overall exposure to HFTS prior to hydrogen peroxide induced marked dose-dependent increase in radical oxygen species (ROS) scavengers ($P<0.05$) levels was accompanied with statistically significant decrease in MDA levels ($P<0.05$) in HFTS-exposed groups when compared with the control.

Notably, the activity of AChE was affected by exposure to natural compounds; levels were significantly lower in HFTS-treated rats, or the combination of HFTS and low/high dose of H_2O_2. Furthermore, histopathological analysis showed that brain injuries occurred at high doses of H_2O_2 administered alone or with low dose of HFTS, whereas, high dose of essential oil (EO) markedly alleviated neurone degeneration.

The obtained results suggest that HFTS alleviates neuroinflammation by acting as an AChE inhibitors (AChEIs) and attenuates H_2O_2-induced brain toxicity.

Keywords: H_2O_2, *Thymus algeriensis*, brain, MDA, AChE, Antioxidant enzymes, non-enzymatic antioxidants

Introduction

Traditionally used by Tunisians as alternative medicine, various native plants from Jebel Orbata (Gafsa, Tunisia) are an important botanical source of pharmacological research, due to their chemical composition, including secondary metabolites (Guesmi et al., 2017). Dietary antioxidants have had mixed success in protecting against damage and disease (Veal et al., 2007). There is a growing interest in uses of natural antioxidants as a protective strategy against the brain problem. Lamiaceae species were found to be rich in phenolic acids as active constituents that significantly contribute to their neuroprotective properties (Dastmalchi et al., 2007). Worldwide, *Thymus* polar extracts are an attractive target for the screening of health-promoting properties for possible industrial applications in food, cosmetics, or pharmaceutical industries, among others (Alfonso et al., 2017). The antiradical scavenging ability of plants is attributed to the nature of their components and to a high amount of phenolic compounds (Guesmi et al., 2017). Dietary natural antioxidants intake, including, vitamins, phenolic compounds, terpenes, proteins, coenzyme Q, and nitrogen compounds act as a free radical scavenging molecules by binding to redox metals to prevent free radical generation (Uttara et al., 2009).

H_2O_2, as a model of endogenous oxidant from environmental pollutants (Sattayasai et al., 2013), leads to tumorigenesis by activating MAP kinases (Reuter et al., 2010) and nuclear transcription factors (NF-κB, AP-1, and p53) that possibly lead to upregulation of death proteins or production of inhibitors of survival proteins leading to apoptosis and cell death (Price et al., 1998; Lowe et al., 1993; Tu et al., 2016). The toxicity of hydrogen peroxide, largely mediated by hydroxyl radicals ($OH^{\cdot}$) generated by the Fe^{2+}-catalyzed Fenton reaction (Veal et al., 2007) may inflict alteration to brain molecules. Brain was most vulnerable to oxidation due to limited antioxidant system (Vega-Naredo et al., 2005) that, in turn, the main target for drug effect and adverse effects because it contain the highest number of receptors (Herz, 1998). The brain is believed to be particularly vulnerable to the damaging effect of H_2O_2 (Jeon et al., 2009). Free radicals attack glial cells and neurons, which are post-mitotic cells and therefore, they are particularly sensitive to free radicals, leading to neuronal damage (Uttara et al., 2009). Brain contains high level of polyunsaturated fatty acids that are more susceptible to radical oxygen species (ROS) attack (Praticò, 2002).

The antioxidant system, including a variety of enzymes, namely, SOD, CAT, enzymes from the thioredoxin system (e.g., thioredoxins, thioredoxin peroxidases, and peroxiredoxins),

glutathione system enzymes (includes glutathione, glutathione reductase, peroxidase, and transferase), and vitamins such as E and C (Meher and Mishra, 2017) help to regulate the ROS thus generated (Uttara et al., 2009). The major antioxidant enzymes involved in the catalytic breakdown of peroxides are catalase, glutathione peroxidase, and thioredoxin peroxidase (peroxiredoxins) (Veal et al., 2007).

Acetylcholinesterase (AChE), as a key enzyme in the cholinergic nervous system (Mathew and Subramanian, 2014), hydrolyzes acethylcholine (ACh) to choline and acetate, thereby terminating the effect of this neurotransmitter at cholinergic synapses (Vladimir-Knežević et al., 2014). Treatment of human neuroblastoma cells with H_2O_2 modified AChE isoform profile and reduced its available amount and/ or cellular death by apoptosis (Garcimartín et al., 2017). Numerous phytoconstituents and promising plant species have been reported as AChEIs (Deka et al., 2017).

Essential oils of *T. algeriensis* are very heterogeneous mixtures and presented several chemotypes in Tunisia and Algeria and a subsequent variability in their quality.

HFTS has been reported to possess bioactive compounds like Campher, 1,8-Cineol, Linalol, 4-Carvomenthenol, Terpinen-4-ol, Bornyl acetate, Viridiflorol and p-cymen; that protect against oxidative stress in rats, through the mechanisms by which HFTS protects gastric tissues, testes, liver and kidney from oxidative stress induced by different chemicals by increasing antioxidant defense enzyme activities and reducing MDA levels (Guesmi et al., 2014, 2016a, 2016b).

In present work, the chemical composition of leaf HFTS was characterized by GC/MS. As part of our work on characterizing aromatic and medicinal plants that grow spontaneously in Tunisia, we report here the first studies on the neuroprotective effects of HFTS. Furthermore, the present report was conducted to explore *in vitro* and *in vivo* protective effects of this specie against H_2O_2. We investigated whether this essence can act as an antiradical agent by evaluating the brain antioxidant enzyme levels and abolishing the AChE inhibition, resulting in the increase of AChE levels in the brain. Additionally, we provide evidence that this specie reduce the damage induced by H_2O_2, that triggered the number of degenerated neurons. Moreover, we demonstrated that oxidative stress could affect body and brain weight changes, acetylcholinesterase inhibition, brain lipid peroxidation status, antioxidant defense enzyme levels and neuroprotective property of terpenes extracted from HFTS in the brain oxidative stress status of rats assessed by histopathological analysis.

Materials and methods

Reagents

All reagents, including Ellman's reagent, GSH, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), H_2O_2, thiobarbituric acid (TBA), 2,4-dinitrochlorobenzene (CDNB), and Tris-HCl buffer were purchased from Sigma (St. Louis, MO, USA), Fluka Chemie (Buchs, Switzerland), and Merck (Nottingham, UK).

Plant Material

The aerial parts of HFTS were collected from the Mount Orbata, Tunisia (Fig.1). HFTS was extracted with a Clevenger apparatus and dried over Na_2SO_4. Purified EO was stored at 4 °C and freshly diluted as needed *in vivo* experiment.

Animal model

We conducted experiments using thirty-six healthy male Sprague Dawley rats, averaging 6-8 weeks old. Rats were maintained in the polypropylene cages for 7 days and handled under room temperature (20° ± 25°C) and 12 h light/dark cycle, with relative humidity of 55% ± 10%, and were allowed *ad libitum* to access to standard pellets and tap water obtained from the animal laboratory of the Pasteur Institute of Tunis. The Medical Ethical Committee for the Care and Use of animal experiments of Pasteur Institute of Tunis and the Faculty of Sciences of Bizerte, University of Carthage, Tunisia, approved all animal protocols (Ethic# LNSP/Pro 152012) governing the experiments.

Experimental procedures

The rats were treated in six groups as follows and were exposed to chemicals with an oral dose for 15 days: control (C), low dose Hydrogen peroxide (LD H_2O_2) (0.1mmol/L), high dose Hydrogen peroxide (HD H_2O_2) (1 mmol/L), HFTS (180 mg/kg per day dissolved in normal saline), HFTS combined with Hydrogen peroxide (HFTS + LD H_2O_2) (180 mg/kg per day and 0.1 mmol/L, respectively), and HFTS combined with Hydrogen peroxide (HFTS + HD H_2O_2) (180 mg/kg per day and 1 mmol/L, respectively). Animals in these two latter groups receiving both agents were treated with HFTS 1 h prior to H_2O_2 administration in animals.

Body weight was recorded daily for 15 days. After completion of the experiment, rats were killed by cervical dislocation and the whole brain collected from animals was dissected, and a

portion of the brain tissue were fixed in 10% buffered formaldehyde for histopathological overview after staining with hematoxylin and eosin (H & E). A part of the brain were extracted with rotary homogenizer in phosphate buffer (0.1 mol/L, pH = 7.4). At the end of the experimental period, brain samples obtained after centrifugation at 8000 × g for 15 min were taken from all rat for histopathological examination and evaluation of antioxidant enzyme (GSH, GPx, GST, CAT, and SOD), MDA and AChE levels.

Biochemical Analysis

Protein estimation

Protein content of brain homogenates was measured by the method of Lowry et al. (1951) using crystalline bovine serum albumin (BSA) as a standard graph. Assays were done in triplicate.

Assessment of lipid profile

The experiment was carry out using the TBARS assay to evaluate lipid peroxidation. TBA reacts with MDA to produce a stable chromogen that can be quantified by either spectrophotometry or HPLC. Assays were done in triplicate.

Assessment of antioxidant defense enzymes

Biochemical parameters like enzymatic and non-enzymatic antioxidants were done as described previously. SOD was assayed by the method of Marklund et al. (1985). GST was determined using the method of Habig et al. (1974). CAT was estimated according to the method of Takahara et al. (1960). GPx was assayed according to the method of Hafeman et al. (1973). GSH was estimated according to the method of Sedlak and Lindsay (1968). Assays were done in triplicate.

***In vitro* acetylcholinesterase activity**

The inhibitory effect of HFTS on AChE activity was investigated using the colorimetric method of Ellman et al. (1961). Essays were carried out on a spectrophotometer. Briefly, 10 µL of brain sample from each group was mixed with 250 µL of phosphate buffer (0.1 mM, pH 8.0) and 20 µL of AChE solution (0.02 U/ml). The mixture was incubated for 5 min at 30ºC. Then DTNB (10 mM; 20 µL) was added as substrate to initiate the reaction. A blank was also prepared by replacing the brain tissues with 160 µL of phosphate buffer (0.1 mM,

pH 8). The yellow product of this reaction was measured at 412 nm and the enzyme activity was monitored and expressed in µmol/min/mg protein. Experiments were carried out in triplicate.

Pathohistologic examination

Histology assessment was performed using brain tissues from all the groups. Formalin solution (10%) was used to fix tissue samples rinsed with 0.9% saline solution. Sections of brain were obtained and processed. Tissues were stained with Hematoxylin and eosin (H&E) stains.

Neuronal damage was semi-quantitatively scored by a pathologist, who was blinded to study design, by assessing the percentage of neurons that showed atrophy, and thickening of neuron as follows; 0, none; 1+, ,5%; 2+, 5–15%; 3+, 16–25%; 4+, 26–35%; 5+, 36-45%; 6+, 46–55%; 7+, 56–65%; 8+, 66–80%; of neurons.

Statistical Evaluation

Experiments were performed in six animals per group (n = 6). Data were reported as Mean ± SEM (n=3 independent experiments for each bar) and one-way analysis of variance (ANOVA), followed by Bonferroni's test for multiple comparison or Dunnett's multiple range test were used to assess the difference between treated groups and control group. The statistical significance was set at $P<0.05$.

Results

Body weight gain, toxicity and mortality

Results seems to show that orally administration of HFTS alone at a single dose of 180 mg/kg/per day induced body weight gain, also show no mortality, and no sign of toxicity during 15 days of experiment. In the animals exposed to H_2O_2, we observed a significantly ($P < 0.05$) body weight decrease compared to control (Fig.2A).

***In vitro* effect of HFTS on endogenous antioxidant status**

Results obtained here showed that administration of H_2O_2 significantly diminished non-enzymatic antioxidant GSH level and the activities of SOD, CAT, GPx, and GST concomitantly with marked elevation in MDA levels in brain of the treated groups compared to control and to those of the thyme-treated group (Table 1). After HFTS coadministration with high H_2O_2 doses, we showed lower antioxidant enzyme levels. However, administration

of HFTS alone, repairing the increase of MDA level (P<0.05) but increased the activity of antioxidant enzyme in the tissues of rat compared with the control groups (P<0.05).

AChE inhibition

Figure 2B depicts that acute intoxication of rats with H_2O_2 induced significant (P<0.05) increase of the levels of AChE brain toxicity markers in a dose-dependent manner (Fig.2B), while the pretreatment with HFTS at 180 mg/kg body weight, reduced stress-induced brain toxicity, prevented the increases of this parameter and reversed this change to control values. Likewise, exposure of rats for 15 days to HFTS alone was even better compared to that of combination with H_2O_2 by protecting the brain from oxidation.

Brain Histopathology

Microscopic examination of brain tissue of rats orally treated with HFTS alone showed no pathological change and normal histoarchitecture and led to significantly reduced neuronal damage, whereas, vascular congestion, oedema, and few lymphocytes were detected in the brain of rat group treated with either 0.1 mM or 1 mM H_2O_2 once daily for 15 days in a dose-dependent manner (Fig. 3A), that triggers neurodegeneration. Photographs of brain tissues show that orally-pretreatment with HFTS revealed a marked decrease of the tissue damage in brain injury condition induced by H_2O_2. The semi-quantitative analysis of histologic injury showed a significant decrease in the score damage of the brain tissue after combination of both low dose of H_2O_2 and HFTS-treated animals when compared to H_2O_2 group (Fig. 3B).

Discussion

Oxidative stress (OS), associated with the formation of ROS, plays an important role in the pathogenesis of various deleterious processes and diseases in human, such as inflammation, immunosuppression, aging and cancerogenesis (Svobodová et al., 2006). OS can induce radical mediated damage to cellular biomembranes resulting in lipid peroxidation (Hsu and Stedeford, 2010). Sufficient production of ROS presumably overwhelms cellular antioxidant defenses, leading to toxic levels of oxidants and injury from their effects on cellular macromolecules (Xu et al., 1997). ROS are particularly active in the brain and neuronal tissue as the excitatory amino acids and neurotransmitters, whose metabolism is factory of ROS, which are unique to the brain and serve as sources of oxidative stress (Uttara et al., 2009). There is a strong correlation between ROS damaging cells *via* oxidative stress and brain diseases (Yoo and Kang, 2009). H_2O_2, the most toxic of the ROS involved in many diseases,

is generated from nearly all sources of oxidative stress, can diffuse freely in and out of many kinds of cells and tissues (Jeon et al., 2009) and may damage all of the major classes of biological macromolecules in the cells through direct oxidation of lipids, proteins, and nucleic acids (Repetto et al., 2012). ROS is known to destroy cells by amplifying the lipid peroxidation of a cell membrane (Yoo and Kang, 2009). Lipid peroxidation can lead to the generation of a variety of oxidized products including reactive electrophiles, such as epoxides and aldehydes, which are capable of modifying DNA, protein, and other macromolecules, include MDA, 4-hydroxy-2-nonenal (HNE), 2-propenal (acrolein), and isoprostanes, which can be measured as an indirect index of oxidative stress (Dalle-Donne et al., 2006). MDA, as a mutagen and a genotoxic agent that may contribute to the development of human cancer (Feron et al., 1991) and is a physiologic ketoaldehyde which can combine with free amino groups of proteins, producing MDA-modified protein adducts (Dalle-Donne et al., 2006). Exposure of reactive oxygen to proteins produces denaturation, loss of function, cross-linking, aggregation and fragmentation of connective tissues as collagen (Repetto et al., 2012).

The deleterious chemical effects of H_2O_2 molecules can be divided into the categories of direct activity, originating from their oxidizing properties, and indirect activity in which they serve as a source for more deleterious species, such as $OH^{\cdot}$ and hypochlorous acid (Kohen and Nyska, 2002) in the presence of transition metal ions (Lee et al., 2002). H_2O_2 can potentially reach the nucleus to interact with DNA (Termini, 2000). Increased levels of H_2O_2 in cells can result in OS and cause cellular damage (Veal et al., 2007), and, this, could lead to aberrant cell cycle re-entry into G0/G1 phase through several pathways (Ismail et al., 2012). Once across the cell membrane, the antioxidant defenses of the cells counteract H_2O_2 and the subsequently-produced ROS, but reach a threshold and eventually succumb to severe oxidative damage, leading to cell death (Law et al., 2014). The catalytic reduction of hydrogen peroxide by antioxidant enzymes such as GPx involves the oxidation of catalytic thiol goups on selenocysteine (she) or cysteine (SH) residues (Veal et al., 2007).

By scavenging free radicals that are produced during neurodegeneration, antioxidants have been shown to provide neuroprotection against the cell death associated with ischaemia-reperfusion injury (Saleh et al., 2017), and thus through its anticholinesterase properties by directly inhibiting the enzyme in the active site or by preventing free radicals to target neurotransmitter by their neutralization or moreover by increasing the hippocampal antioxidant enzymes (SOD, CAT and GPx) activities (Popović et al., 2017). Natural products

have already proven to be promising sources of useful AChEIs (Mathew and Subramanian, 2014). Plant-derived EO and lipid-soluble bioactive compounds have gained attention for biological roles due to their higher bioavailability compared to water-soluble bioactive compounds (Bak et al., 2012). This is consistent with the effect of flavonol content of some species on brain lipid peroxidation (Popović et al., 2017). That observation can be supported by the previous *in vivo* studies about neuroprotective effect of orientin on hydrogen peroxide□induced apoptosis in SH□SY5Y cells brain (Law et al., 2014). Phytochemicals are able to target cerebral blood flow, free radical scavenging, anti-inflammation, inhibition of amyloid-β neurotoxicity, glucoregulation and interaction with other neurotransmitters (such as γ-aminobutyric acid) and signalling pathways (e.g. *via* kinase enzymes) (Wightman, 2017).
No reports regarding the medicinal use of thyme on brain protection have been described. Few studies have characterized HFTS showing a rich composition with approximately 35 compounds, including monoterpenes mainly p-cymene, γ-terpinene, and variable contents of carvacrol and/or thymol as major components (Guesmi et al., 2014). These data suggested that carvacrol is a high potent neuroprotector on cerebral I/R injury and may act on membrane receptors to modulate intracellular signal pathways (Yu et al., 2012). Moreover, it was reported that the compounds like linalool, myrcene, and eucalyptol can reduce the damage caused by H_2O_2 to cells (Gautam et al., 2014). It has been shown that the administration of H_2O_2-induced cell cytotoxicity to human neural cells IMR32 cell line, and this was protected by *Hippophae rhamnoides* (L.) (Shivapriya et al., 2015).

The major antioxidant enzymes that can eliminate the H_2O_2 include catalase, glutathione peroxidase and peroxiredoxins (Phaniendra et al., 2015), and loss in antioxidant capacities results in an intrinsic accumulation of MDA (Wang et al., 2011). Hydrogen peroxide reacts readily with Fe^{2+}, and consequently the Fe^{2+}-containing cofactors found in some proteins are potentially susceptible to oxidation (Veal et al., 2007). At low level of H_2O_2, Fe^{2+} induces lipid peroxide decomposition, generating peroxyl and alkoxyl radicals and favoring lipid peroxidation (Repetto et al., 2012).

Acetylcholinesterase is critical for the function of the peripheral and central nervous systems (Jia et al., 2017) and rapidly degrades Ach in the periphery and the brain (Kalb et al., 2013). It plays an essential role in cognitive functions (Baraldi et al., 2013). In this study, we demonstrated the mechanism of brain neurotoxicity of H_2O_2 as an AChE inducers. It was found that H_2O_2 induced AChE inhibitory activity. The exposure of rats to HFTS for 15 days,

however, show a significant increase of AChE inhibition, that occurred in brain. These findings confirm the studies done by Kindl et al., showing that many *Thymus* species growing in Croatia exhibited anti-AChE activity in a dose dependent manner. This data coincides with previous observations that administration of H_2O_2 to human neuroblastoma SH-SY5Y cell line modifies the isoform profile of AChE and its activity, which was strongly increased from 1 μM to 1000 μM of H_2O_2 (Garcimartín et al., 2017).

This inhibition induced by bioactive compounds can help to enhance the level of the neurotransmitter acetylcholine in the neuronal synapse and then preventing the alteration of neuronal function (Coulibaly et al., 2011). Various plants in nature and phytochemical substances have demonstrated AChE inhibitory activity and thus could be beneficial in the treatment of neurodegenerative disorders such as Alzheimer Disease (Vladimir-Knežević et al., 2014). AChEIs protect cells from free radical toxicity and β-amyloid-induced injury, and increased production of antioxidants (Tabet, 2006).

Increase in MDA levels, and AChE reduction; decline in protein sulfhydryl and ROS scavenger activity observed in brain tissues of neurotoxic rats were recovered by the administration of HFTS, which significantly reversed these changes. These brain biomarkers correlated with histopathology signs. These findings agree with the study of Siddique et al. (2009) who reported that the administration of H_2O_2 to human peripheral blood lymphocytes cell lines increased the concentration of MDA in dose-dependent manner.

Competing Interests:
The authors declare that they have no competing interests.

Authors' Contributions
GF conceived and designed the study; GF and BH performed the animal experiments and histological assessment; GF drafted the manuscript; GF performed the statistical analysis and revised the manuscript. All authors read and approved the final manuscript.

Abbreviations
CAT: Catalase;
CDNB: 2,4-Dinitrochlorobenzene;
DTNB: 5,5'-dithiobis-(2-nitrobenzoic acid);
eNOS: endothelial nitric oxide synthase;
EO: essential oil;
GSH: Reduced glutathione;
GPx: Glutathione peroxidise;

GSH: Glutathione;
GST: Glutathione S-transferase;
LPO: Lipid peroxidation;
MDA: Malondialdehyde;
ROS: Reactive oxygen species;
SOD: Superoxide dismutase;
TBARS: Thiobarbituric acid reactive substances
U: Unit

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Tables

Groups	Total protein (µg/ml)	CAT (µmol H_2O_2 Consume/min/m protein)	SOD (U/mg of protein)	GPx (µmol GSH/mg protein/ml)	GSH (µmol/mg protein)	GST (nmol of CDNB conjugate formed/min/mg protein)	LPO (nmol MDA/mg protein)
Normal group	268.75±25.70*	12.68± 0.42*	55.75±4.50*	16.37±0.04*	5.41±0.02*	25964±34.60*	193.67± 15.26*
0.1 mmol/L H_2O_2	90±7.50$^{*\#}$	3.84±0.00$^{*\#}$	23.25±0.04$^{*\#}$	13.2±0.08$^{*\#}$	1.22±0.02$^{*\#}$	10605±86.72$^{*\#}$	2812.06± 23.14$^{*\#}$
1 mmol/L H_2O_2	72.5±92.70$^{\#}$	1.18±0.50$^{\#}$	15.83±0.30$^{\#}$	12.8±0.08$^{\#}$	0.75±0.01$^{\#}$	8652±45.80$^{\#}$	3648.47±33.22$^{\#}$
HFTS (180mg/kg)	206.25±5.00$^{*\#}$	9.42±0.00$^{*\#}$	71.11±7.00$^{*\#}$	27±0.65$^{*\#}$	6.14±0.18$^{*\#}$	23096±19.06$^{*\#}$	262.3± 8.16$^{*\#}$
HFTS+0.1mmol/L H_2O_2	123.75±6.20$^{*\#\S}$	6.42±0.50$^{*\#}$	31.32±1.00$^{*\#}$	15.8±0.32$^{*\#}$	2.66±0.04$^{*\#}$	13571±98.80$^{*\#}$	433± 26.28$^{*\#}$
HFTS +1 mmol/L H_2O_2	83.75±0.50$^{*\#}$	3.84±9.00$^{*\#}$	25.86±2.30$^{*\#}$	14.2±0.00$^{*\#}$	2.24±0.34$^{*\#}$	10250±88.90$^{*\#}$	743.57± 41.12$^{*\#}$

Table 1. Effect of HFTS on brain ROS scavengers, and MDA levels in rat.

CAT: catalase; SOD: superoxyde dismutase; GPx: Glutathion peroxydase; GSH: reduced glutathion; GST: Glutathion S-transferase; LPO: lipid peroxidation. *Significant when compared to the high dose H_2O_2 groups (1 mmol/L H_2O_2); #Significant when compared to the control group. § Significant when to the low dose H_2O_2 groups (0.1 mmol/L H_2O_2); Mean values of 3 independent experiments have been plotted. significant di□erence at $P<0.05$ (ANOVA followed by Dunnett's test) compared with normal control and toxicated groups.

Figure legends

Fig. 1. Recoveries of major bioactive compounds identified in the hydrophobic fractions (HFTS) (Campher, (a) 1,8-Cineol, (b) Linalol, (c) 4-Carvomenthenol, (d) Terpinen-4-ol, (e) Bornyl acetate, (f) Viridiflorol, (g) p-cymen, (h)) (Upper parts) and methanolic extract of *Thymus algeriensis* (METS) (Tyrosine, (a) Flavone, (b) Vanillin, (c) (+)-Catechine hydrate, (d) Rutin, (e)) (Lower parts) with neuroprotective effects.

Fig.2. A. Effect of HFTS on body weight in rats. **B.** Inhibition of AChE activity by HFTS. Ctrl: Control, LD H_2O_2: Low dose treatment with hydrogen peroxide (0.1 mmol/L), HD H_2O_2: High dose treatment with hydrogen peroxide (1 mmol/L), HFTS: hydrophobic fraction of *Thymus algeriensis*. Mean values of 3 independent experiments have been plotted. **Significant Value was at $P<0.05$.

Fig.3. A. Histological examination of H&E-stained section of brain tissues of normal control (a) and experimental group (b, c, d, e, f) of rats. B. Quantitative analysis of neuronal damage was assessed by semi-quantitatively injury scoring (0-8) of H&E staining results. **Significant Value was at $P<0.05$. N: Neuron; CV: Cytoplasmic Vacuolization; NGC: Normal Glial Cells; GC: Glial Cells; DN: Degenerated Neuron; INF cells: Inflammatory cells. Magnification: ×40.

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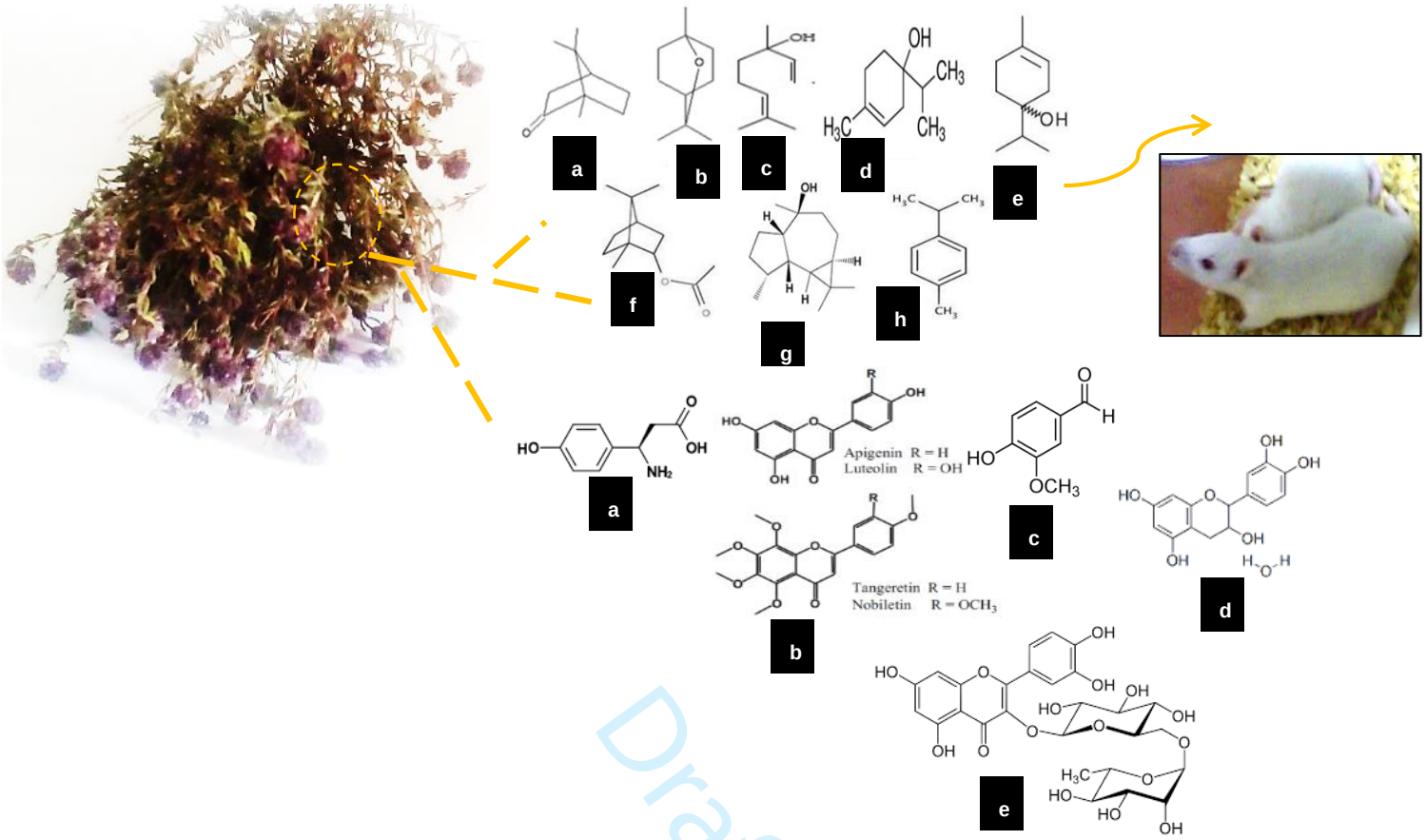
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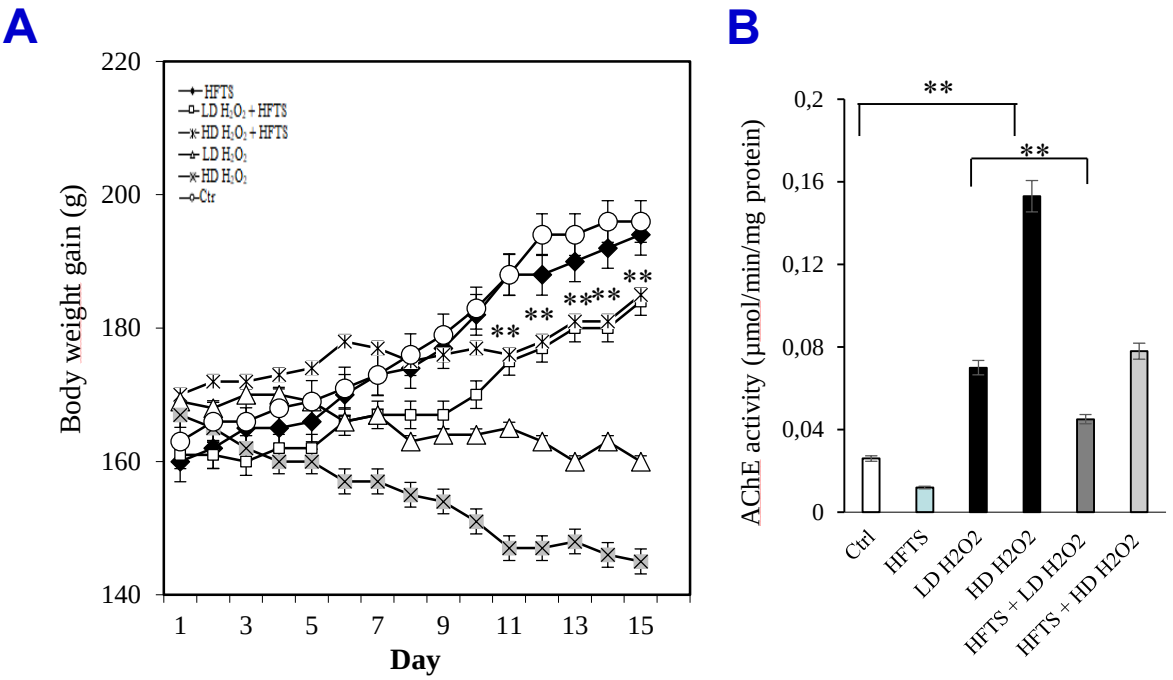
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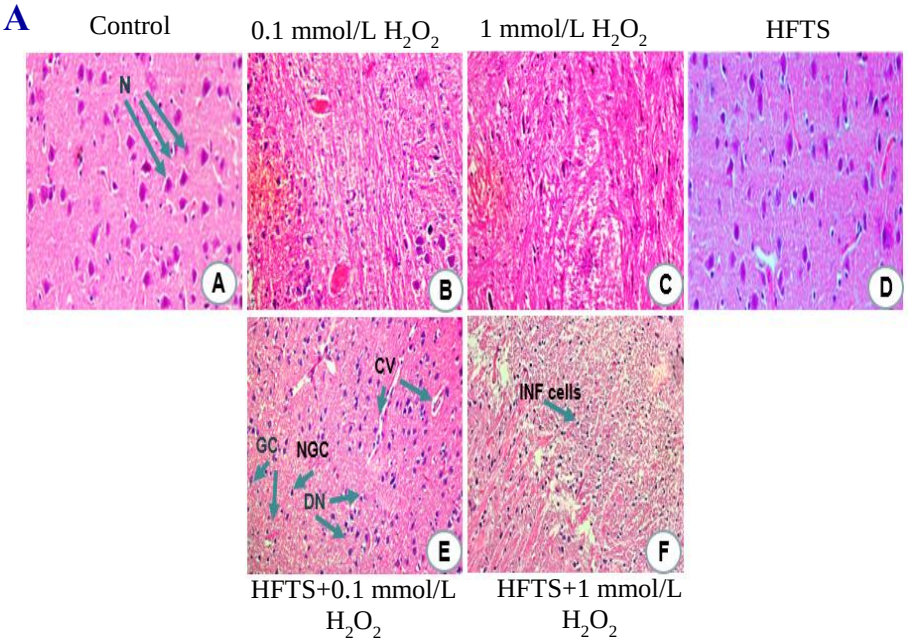
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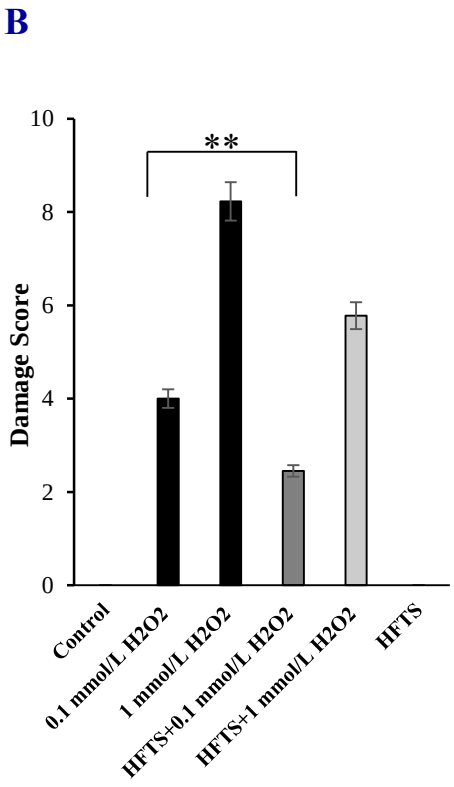
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