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**THE EFFECTS OF INHALED BERGAMOT AND
GERANIUM ESSENTIAL OILS ON RAT BEHAVIOUR**

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of the requirements for the degree
of
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Abstract

The aim of this study was to evaluate the behavioural effects of inhaled bergamot, geranium and a combination of these oils in three novelty evoked tests of anxiety. Sixty adult Hooded Rats (*Rattus norvegicus*), with 10 rats randomly assigned to one of the 6 test groups; three essential oil treated groups, and three control groups. The essential oil groups consisted of bergamot, geranium and a combined group, i.e. the combination of bergamot and geranium oil. The control groups consisted of the odour and vehicle control, with the anxiolytic drug diazepam as a positive control. The behaviour of rats was assessed on the elevated-plus maze, open-field and social interaction test. Diazepam increased open arm entries and the time spent in the open arms, decreased time spent in closed arms and increased the number of head-dips and unprotected stretch-attends in the EPM. In the open-field diazepam increased immobility time, decreased ambulation, increased grooming activity and reduced the amount of time spent exploring the arena. Similarly, diazepam decreased the frequency of separations, sniffs, follows, crawls, passive and active interactions with test partners in the social interaction test. Bergamot, geranium and the combination of the two oils increased total arm entries in the elevated-plus maze. Bergamot increased locomotion and exploratory behaviour in open-field and decreased contact latency and increased passive and active interaction between the rat pairs in the social interaction test. Geranium decrease immobility and increase the time spent rearing in the open-field and also increased active interaction, i.e. partner sniffing and decreased the amount of time the rat pairs spent apart in the social interaction test. The combination of bergamot and geranium oil increased locomotion and the time spent in Zone2, and also increased exploratory behaviour, i.e. the frequency and duration of rears in the open-field. In the social interaction test, contact latency was shortened and active and passive interactions between rat pairs were increased by the combination of essential oils. The present study established that bergamot, geranium, and the combination of the two oils had a stimulating effect in the elevated-plus maze and an anxiolytic effect in the open-field and social interaction tests when inhaled. Furthermore the study also demonstrated that the combining of the oils had a potentiating effect on the anxiolytic properties of the single oils.

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List of Abbreviations

Complementary/alternative medicine	CAM
Contingent negative variation	CNV
Diagnostic and Statistical Manual of Mental Disorders	DSM-III
Electroencephalography	EEG
Elevated plus-maze	EPM
Essential oil	EO
General Practitioner	GP
High anxiety related behaviours	HAB
Hospital Anxiety and Depression Scale	HADS
Low anxiety related behaviours	LAB
Magnetic resonance imaging	MRI
Monoamine oxidase inhibitors	MAOI
Montgomery-Asberg Depression Rating Scale	MADRS
Open-field	OF
Selective serotonin re-uptake inhibitors	SSRI
Social interaction	SI
Tricyclic antidepressants	TCA
Tyler Brief Anxiety Scale	TBAS

Introduction

1.1. Historical overview

1.1.1. The development of contemporary medicine

Throughout the ages people relied on the healing properties of plants to combat the rigors of illness, disease and physical injury. Archaeological discovery of prehistoric cave paintings confirms the use of plants in spiritual and healing rituals. Early man discovered by chance that some of the leaves, berries, and roots that he gathered for food made sick people feel better, and that their juices helped wounds to heal. Initially with only the intention of adding fuel, when the twigs of some bushes or trees were thrown on the fire, the smoke and aroma that they gave off made people drowsy, happy or excited. The “smoking” of patients was one of the earliest forms of medicinal rituals. The use of fumigation with aromatic plants remained a standard medical practice right up to the present century. Until relatively recently French hospitals burnt thyme and rosemary in the wards as a disinfectant (Eisenberg et al., 1998).

The earliest documented medical texts date back to the Egyptians who were using aromatics as far back as 4500 BC for medical and cosmetic purposes, as well as for the embalming of their dead (Eisenberg et al., 1998). Embalmers utilised the natural antiseptic and antibiotic properties in plants, spices and resins to preserve the body after death (Ryman, n.d.). The Ebers Papyrus lists over 800 herbal remedies and prescriptions for various ailments, the plants used included aniseed, cedar, onion, garlic, cumin, coriander, castor oil, grape and watermelon. The ‘Classic Herbal’ text of Indian Ayurvedic medicine (3000 BC) and the ‘Yellow Emperor’s Classic of Internal Medicine’ written by the Chinese emperor Huang Ti (2650 BC), provide further documented examples of the use aromatic oils, spices and herbs around the world (Eisenberg et al., 1998).

Hippocrates (460-370 BC) known as the father of medicine, recommended the burning of aromatic oils to prevent the spread of plague. Due to the lack of coherent records, what was happening medically in Europe between the fall of the Roman Empire and the 10th century is sketchy. However, from the ‘Middle Ages’ and throughout the Tudor era, many forms of plant medicine were utilised by doctors, apothecaries and lay people alike. Little bouquets of aromatic herbs,

known as ‘tussy-mussies’, were carried in public places to ward off infection, especially the plague (Eisenberg et al., 1998). During the Renaissance (1400-1700) explorers like Columbus initiated the development of contemporary medicine as we know it, with the discovery of distant cultures, medical texts and the importation of exotic plants and herbs (The Applied History Research Group, 1997).

By the 17th century the growing new science of experimental chemistry gave rise to new uses for plant extracts in medicine. The spate of witch-burning coincided with this rise in biomedical therapy as the medical establishment wished to suppress the knowledge of the village ‘wise-women’. Scientists continued to research the active ingredients of medicinal plants which remained the mainstay of medicine due to availability and cost effectiveness throughout the 18th and 19th centuries (Eisenberg et al., 1998). Many plant extracts such as caffeine, quinine, morphine and atropine etc., were discovered (Zhang, 2004). With the advent of World War, and the greater demand for quicker more effective medicines, synthetic drugs derived from coal-tar by-products, began to replace natural ingredients (Eisenberg et al., 1998).

1.1.2. The rise in popularity of alternative therapies

The flower-power age of the 1960s and 70s saw a return to more natural or alternative therapies. Interest in alternative therapies has continued to grow since the 1970s. According to a survey conducted by Harvard Medical School in 1997; between 1990 and 1997, the American population made around 627 million visits to alternative medical practitioners, spending an estimated \$27 billion of their own money on alternative therapies and medicines. In contrast, Americans only made 386 million visits to a general practitioner. That is a 47.3% growth in the utilization of alternative therapies since 1990 (Eisenberg et al., 1998). In Australia 57% of the population now use some form of alternative medicine (Bensoussan, 1999). In Europe 46% and 49% of the German and French population respectively, utilize alternative therapies, illustrating the global popularity of alternative medicine (Fisher & Ward, 1994). Between 1991 and 1997 the use of herbal medicine in the United States grew by 380% and the use of vitamin therapy by 130% (Eisenberg et al., 1998).

Similar trends are also reflected in New Zealand; in 1987, 37% of the 463 outpatients with cancer surveyed, sought advice about complementary/alternative medicine (CAM) (Ernst & Cassileth, 1998). A 1990 survey examining the attitude of General Practitioners (GP's) toward CAM revealed, 30% of Auckland GP's practiced some form of CAM and 68.7% referred patients to CAM practitioners (Poynton, Dowell, Dew, & Egan, 2006). Of the 251 children surveyed in 1999, who were admitted to the Starship Childrens Hospital in Auckland with acute illness, 18% received complementary treatments while hospitalised (Armishaw & Grant, 1999).

The resurgence of alternative therapies is partly due to its holistic approach which is focused on disease prevention, by treating the cause through strengthening the body's own natural defences. In contrast conventional medicine is organ specific, relying primarily on surgical or pharmacological interventions, focused on symptom relief rather than prevention (Larsen, 2008). For example, most cancer chemotherapy regimes make use of highly cytotoxic drugs that target proliferating cells. However the non-discriminatory nature of these drugs leads to severe side effects in normal cells with a high proliferative index, i.e. the gastrointestinal tract and the bone marrow.

Another reason for the shift towards alternative therapies would be a general disillusionment with conventional medicine due to the numerous side effects, their ineffectiveness to treat several chronic diseases, microbial resistance, and the high cost of new drugs (Humber, 2002). For example, antipsychotic drugs have been associated with a range of side effects which include Parkinsonism, requiring further drugs to manage the associated muscle tremors. There is still no definitive cure for cancer, and drugs like Herceptin offer little hope because at a cost of \$100,000 US per year (Fleck, 2006) this is unaffordable to the average breast cancer sufferer. The world is plagued by what is termed 'superbugs', virulent strains of the common cold, resistant to many antibiotics (Australian Broadcasting Corporation, 1999).

Hospitals are now the third largest killer in Australia and over one million people are seriously injured in American hospitals every year. Blood infections acquired in American hospitals cause 62,000 fatalities every year and bypass surgery results in 25,000 strokes a year. Two million patients experience adverse drug reactions in hospitals in the United States every year; of these, over 100,000

die making hospital-induced adverse drug reactions the fourth leading cause of death after heart disease, cancer, and stroke (Anderson, 1995; Bates et al., 1995; Cordner, 1995; Lazarou, Pomeranz, & Corey, 1998; Pittet & Wenzel, 1995; Roach, Kanchuger, Mangano, & Newman, 1996).

Although conventional pharmaceuticals work more rapidly than natural therapies, the homeostatic properties of essential oils (Fujiwara, Komori, & Mitsuo Yokoyama, 2002) may provide a viable alternative to conventional pharmaceuticals, without negative side effects and the treat of addiction. Essential oils, specifically monoterpenes, have chemo-preventative properties, i.e. they dilute the harmful consequences of cytotoxic drugs (Morita et al., 2003), they are also effective cancer suppressors in their own right (De Angelis, 2001). The antioxidating properties of essential oils have been found to be effective against heart disease (Graßmann, Hippeli, Spitzenberger, & Elstner, 2005). Oils rich in phenolic constituents such as eugenol and thymol (Naderi, Asgary, Ani, Sarraf-Zadegan, & Safari, 2004), have been effective against atherosclerosis, and lavender has been indicated as having antiplateletic and antithrombotic properties, i.e. decrease platelet aggregation and stop blood from clotting (Ballabeni et al., 2004). Rosemary has demonstrated antidiabetic properties, displaying hyperglycaemic and insulin release inhibitory effects in diabetic rabbits (Al-Hader, Hasan, & Aqel, 1994).

1.2. Stress, anxiety and depression

Stress is a leading precursor to diseases like anxiety and depression (Cohen, Kessler, & Underwood Gordon, 1995; Wilkinson & Marmot, 2003). Cohen, Kessler, & Underwood Gordon (1995), define stress as an, “environmental demand that exceeds the adaptive capacity of an organism, resulting in psychological and biological changes that may place a person at risk of disease”. Conditions associated with long term stress also include diabetes, high blood pressure, cardiac arrest, stroke anorexia, obsessive compulsive disorder, alcohol and drug abuse, and hyperthyroidism (National Institute of Child Health and Human Development, 2004).

When we experience stress the thalamus, believed to act as a translator or processor of the information received from the brain, activates the hypothalamus. The hypothalamus in turn activates; the endocrine system, responsible for the

release of extracellular signalling molecules/hormones; and the autonomic nervous system, which influences heart rate, digestion, respiration rate, etc. (Jerajani, 2006). Effectively, the stress hormones released by the endocrine system bath the immune cells in molecules which tell them to stop fighting (Wein, 2000). The dysregulation of the neuroendocrine and immune function is thought to be associated with some psychosomatic and psychological disorders, i.e. chronic stress makes the body vulnerable to, anxiety, depression, infection, colds and flu (Komori, Fujiwara, Tanida, Nomura, & Yokoyama, 1995).

Anxiety and depression can have cognitive, affective, physiological, and behavioural implications. Examining the effects of anxiety; at a cognitive level, stimuli or situations can be construed as threatening; affectively a person may feel apprehensive, tense or uneasy; and physiologically, autonomic arousal prepares the body for flight or fight and in extreme case freezing or immobility. Correspondingly, examining the effects of depression; cognitively, negative events are internalised; affectively a person will have a low mood, feel guilty, worthless or suicidal; and physiologically a person may experience weight gain/loss, insomnia/hypersomnia and psychomotor agitation or retardation (Carr, 2006).

In Australia, stress related disorders like anxiety/depression are the biggest non- fatal drain on the national disability budget (Mathers, Vos, & Stevenson, 1999). Paradoxically, despite the over-whelming growth in popularity of complementary/alternative therapies, compared to contemporary medicine there is insufficient empirical evidence to support their efficacy as a treatment for stress and depression. This is disturbing if one considers that only 50 % of Australians who suffer from depression seek evidence-based professional help, relying instead on alternative interventions (Andrews & Sanderson, 2000). More surprisingly, in a national survey only 29% of the Australian population regard anti-depressants as likely to be helpful. On the other hand, 57% of the population regarded vitamins, minerals, tonics or herbal medicines as likely to be helpful (Andrews & Sanderson, 2000). In New Zealand, of the 1043 patients surveyed who accessed the Emergency Room (ER) at Waikato Hospital, between December 2004 and January 2005, 38.1% utilized some form of CAM. Of the 397 CAM users 7% used St John Wort as a treatment for depression (Nicholson, 2006).

Alarming, there is no evidence to support the effectiveness of ginkgo biloba, natural progesterone, selenium or glutamine; all qualitatively popular self-help interventions for depression. Of the vitamins examined, only folate (vitamin B₉) showed any promise as an antidepressant (Jorm, Christensen, Griffiths, & Rodgers, 2002). Conversely, research demonstrates that essential oils offer a potential natural alternative to conventional medicine for several psychiatric (Zhang, 2004) and physiological disorders (Edris, 2007), which further reiterates the need for more empirical research into the possible therapeutic benefits of essential oils.

Needless to say not everyone who experiences a traumatic event or other stressors will develop anxiety and/or depression. Vulnerability to the negative effects of stress would depend on environmental and predisposing factors (Barlow, 2000). Environmental and predisposing factors would include; illness or injury, abuse, loss, violence, low socio-economic status and genetic vulnerability, inhibited temperament, low self-esteem and an external locus of control (Carr, 2006).

1.2.1. Comorbidity of anxiety and depression

Current diagnostic manuals allow for the diagnoses of major depressive disorder and one or more anxiety disorders on Axis I; and for the classification of mixed anxiety disorder NOS, defined as a mixture of anxiety and depressive symptoms (American Psychiatric Association, 2000). Earlier versions of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) however, identified anxiety and depression as two distinctly separate disorders (Levine, Cole, Chengappa, & Gershon, 2001). This distinction has resulted in research focused on anxiety and depression separately, until recently it was believed the drugs used to treat depression could not be used to treat anxiety and visa versa (Levine, Cole, Chengappa, & Gershon, 2001). However, the high rate of comorbidity, and emerging evidence, indicating a shared serotonergic and noradrenergic dysfunction, suggesting anxiety and depression may share common neurobiological pathways (Argyropoulos, Sandford, & Nutt, 2000; Jack, 1996), has resulted in revisions to their diagnostic criteria.

1.3. Psychopharmacology

Second-generation antidepressants, such as selective serotonin re-uptake inhibitors (SSRI), e.g. paroxetine, fluvoxamine, fluoxetine, and sertraline, have superseded psychotropic drugs, like monoamine oxidase inhibitors (MAOI) and tricyclic antidepressants (TCA) in popularity, due to the side effects associated with them. Antidepressants work by inhibiting the re-uptake of the neurotransmitters norepinephrine and serotonin by the neurons. In the central nervous system, serotonin plays an important role as a neurotransmitter in the modulation of anger, aggression, body temperature, mood, sleep, sexuality, appetite, and metabolism, as well as stimulating vomiting (Jack, 1996).

Despite increasing focus on the use of antidepressants and other agents for the treatment of anxiety related disorders, benzodiazepines have remained a mainstay of anxiolytic pharmacotherapy due to their speed and robust efficiency (Argyropoulos, Sandford, & Nutt, 2000; Schatzberg, Cole, & DeBattista, 2007; Stevens & Pollack, 2005; Uhlenhuth, Balter, Ban, & Yang, 1999a).

Benzodiazepines have a continuum effect, between its depressant and stimulant properties, on the central nervous system via the modulation of the GABA_A receptor, the most prevalent inhibitory receptor within the brain. As such the sedating properties of benzodiazepines are useful for the short-term treatment of severe anxiety (Bulach, Myles, & Rusznak, 2005; McKernan et al., 2000).

Benzodiazepines are usually administered orally for the treatment of anxiety and at low doses for depression (Petty, Trivedi, Fulton, & John Rush, 1995).

Occasionally lorazepam or diazepam may be given intravenously for acute anxiety (Uhlenhuth, Balter, Ban, & Yang, 1999b). Contra-indicators of benzodiazepines include, drug interactions, psychomotor retardation, memory impairment, disinhibition (indiscretionary or antisocial behaviours normally suppressed by social restraints), depression/emotional blunting, and addiction (Longo & Johnson, 2000).

1.4. Essential oils

Alterations in brain chemistry due to anxiety/depression provide a popular rationale for a psychopharmacology treatment approach. However, as Homes (1998) points out, social, environmental, psychological influences as well as nutrition may also be important mediating factors for anxiety/depression.

Considering the function of the amygdala and the fact that the olfactory system provides a more direct route to the hypothalamus than either, the intravenous or intrathecal route (an injection into the vein or spinal canal, respectively), there is a strong justification for research examining the psychological benefits of essential oils through inhalation (Clark, 1983).

From an evolutionary perspective, the sense of smell was vital to the survival of primitive men, for detecting danger and familiarizing with an unfamiliar world (Barnham & Broughan, 2002). This innate survival instinct is still evident today, when a baby is born because his eyesight is poor, he uses his sense of smell to recognise his mother and seek nourishment by detecting the pheromones a mother secretes from her breast, as well as the sweet odour of her milk (Ryman, n.d.; Worwood, 1995).

Anatomically, research demonstrates that axons (nerve fibres) of the sensory neurons in the nose, project from the vomeronasal organ to the olfactory bulb, and from there to the hypothalamus via the amygdala (Scott & Chafin, 1975; Zald & Pardo, 1997). The primary role of the amygdala is the formation and storage of memories associated with emotional events (Amunts et al., 2005).

Based on human evolution and the structure of the olfactory system, it is believed that physiological and psychological imbalances, produced through chronic stress, can be normalized through the inhalation of essential oils without the side effects associated with conventional psychotropic drugs (Fujiwara, Komori, & Mitsuo Yokoyama, 2002; Komori, Fujiwara, Tanida, Nomura, & Yokoyama, 1995).

Essential oils offer a reasonable alternative to the psychopharmacological approach for the treatment of anxiety/depression because they have both a direct physiological influence, due to their chemical properties and permeability (molecular size), and an indirect psychological influence, due to their hedonic properties (see section 1.4.3) (Broughan, 1998; Holmes, 1998).

Aromatherapy has become synonymously associated with essential oils. The term aromatherapy was first coined in 1928 by Gattefossé, a French chemist working in his family's perfumery business, after discovering by accident that lavender was able to rapidly heal a severe burn on his hand and help prevent scarring (Gattefossé, 1993). Most flowers, seeds, barks, grains, roots and resins as well as leaves contain essential oils.

Essential oils can be obtained from plants in a number of ways (Ryman, n.d.).

- ❖ *Maceration* - flowers or petals are spread on a tray lined with fat or vegetable oil, the essential oil is then separated from the fat with solvent and purified.
- ❖ *Distillation* - Steam is passed over leaves or flowers, possibly in a vacuum or under pressure, so the essential oil within them vaporise. When the steam is cooled the essential oils condense and because they are not water soluble, they separate and can be easily collected.
- ❖ *Dissolving* - volatile solvent like alcohol used to extract essential oils from gums and resins.
- ❖ *Pressing* - commonly used to squeeze oils from rinds and peels for fruit.

1.4.1. Chemical constituents

The properties of essential oils are based on their chemical profile. There are two groups of chemical constituents of significant therapeutic value (Table 1); hydrocarbons, which are made up of terpenes; and oxygenated compounds, which include esters, aldehydes, ketones, alcohols, oxides and phenols (Dunning, 2006; Ryman, n.d.; Sellar, 1992). The acids, lactones, sulphur, and nitrogen compounds are the constituents that fall outside of these two main groups.

Quantitative differences between the neurotropic effects of lavender oil and its constituents on the central nervous system, suggest the sedating activity of the oil is not mediated solely by any specific constituents, but rather by the combination of the constituents on the whole (Atanassova-Shopova & Roussinov, 1970). Atanassova-Shopova and Roussinov, (1970) suggest the difference between the 'whole' oil and its individual constituents may be the potentiating effect of the combined constituents, by reducing the toxicity of the terpenes and alcohols. Gattefosse also found that essential oils were more effective in their totality than either their synthetic substitutes or any of their isolated ingredients (Gattefossé, 1993).

The homogeneous nature of essential oils could account/provide another rationale for research regarding their viability as a treatment for anxiety and depression.

Table 1

The major chemical constituents of essential oils, their reported properties and examples of essential oils that contain the particular chemical in their composition.

Major chemical constituents	Example essential oils	Reported therapeutic properties
Terpenes		
Monoterpenes <i>Almost all essential oils contain terpenes. They are particularly prevalent in citrus oils except Bergamot.</i>	Lemon, Orange, Juniper, Neroli, Pine, Rosemary, Melissa, Rose, Eucalyptus, Frankincense, Tea-Tree, Lavender, Geranium (<i>Sabinene</i>)*	Analgesic Anti-bacterial Anti-septic Anti-viral Bactericidal Stimulant**
Sesquiterpenes	Black Pepper, Tea-tree, Cedarwood, Camomile, Basil, Clary Sage, Ylang-ylang, Ginger, Melissa, Rosemary, Lavender	
Diterpenes <i>Not common due to their molecular weight.</i>		
Triterpenes & tetraterpenes <i>More common in herbs as sterols, steroids and saponins carotenoids.</i>		
Alcohols		
<i>Considered the most therapeutically beneficial</i>		Anti-fungal Hepatic Vaso-decongestant Sedative**
Monoterpenols	Camphor, Rosemary, Marjoram, Sandalwood, Lavender, Geranium (<i>Geraniol</i> , <i>Citronellol</i> , <i>Linalool</i> , <i>Myrtenol</i> , <i>Terpineol</i>)* Bergamot (<i>Linanol</i> , <i>Nerol</i> , <i>Terpineol</i>)*	
Sesquiterpenols		
Phenols	Jasmine, Patchouli, Vetiver, Camomile, Geranium (<i>Eugenol</i>)*	Very stimulating to the immune system Sedative in large doses
Aldehydes <i>Sedating when inhaled</i>	Oregano, Thyme, Aniseed, Fennel, Nutmeg, Vanilla, Tarragon, Clove, Geranium (<i>Citral</i>)*	Anti-pyretic Anti-inflammatory Vaso-dilator Sedative**
Ketones	Lemon verbena, Melissa, Citronella, Cinnamon, Geranium (<i>Methone</i>)*	Analgesic Mucolytic Sedative
Esters	Aniseed, caraway, Dill, Hyssop, Sage, Bergamot (<i>Linalyl acetate</i>)*	Calming Anti-spasmodic Sedative** <i>Regulates sympathetic nervous system</i>
Oxides	Citronella, Lavender, Lemon, Melissa, Rose, Bergamot*	Expectorant

* Indicates essential oils of interest to this study.

** Indicates properties of interest to this study

1.4.2 Oil combining

In aromatherapy, rarely are single oils utilized, but rather a combination or blend of oils are mixed with a carrier oil, usually grape seed, olive or peanut oil. Considering the potentiating effect the combined constituents in essential oils have (Gattefossé, 1993), it stands to reason the combination of different essential oils could provide a similar potentiating effect.

In a study by Lemon (2004), patients suffering from depression were massaged fortnightly over a 12 week period. Four drops, from a range of 9 essential oils, chosen because of their therapeutic properties for relieving anxiety and depression, were selected according to the presenting physical and psychological symptoms at the time of treatment, e.g. anxiety, depression, headaches and sleep problems. Bergamot, clary sage and lemon oil were selected for their uplifting, euphoric properties, and lavender because of its versatility. Roman chamomile and geranium were selected for their soothing, calming properties, and rose, sandalwood and jasmine oil for their sedating properties. Patients demonstrated significant improvements on the Montgomery-Åsberg Depression Rating Scale (*MADRS*), the Tyler Brief Anxiety Scale (*TBAS*) and the Hospital Anxiety and Depression Scale (*HADS*).

In another study Komori et al., (2006) demonstrates the combined effect of sandalwood, juniper berry, rose and orris, prolonged pentobarbital induced sleep time in rats by 15 %, a 10 % compared to the individual oils. Caution should be taken however, to ensure the oils combinations are homologous in their affect and not counteractive to each other, i.e. either stimulating or sedating but not both (Hongratanaworakit, 2004).

Following this chain of thought, most essential oils studies have focused on the effects of individual oils and/or its component parts, however few have considered the combined effects of two or more essential oils.

1.4.3. Human research

In humans, essential oils are absorbed into the body via the nose/mouth or the skin (Worwood, 1995). Scientific analysis of, perspiration, faeces, urine, breath (Worwood, 1995), blood and tissue samples, have revealed that inhaled essential oil components have been detected in the skin, lungs, olfactory bulb and the brain (Buchbauer, Jirovetz, Jager, Plank, & Dietrich, 1993; Inouye, 2003).

Electroencephalography (EEG), measuring the electrical activity of the brain (Diego et al., 1998; Lorig, Herman, Schwartz, & Cain, 1990); magnetic resonance imaging (MRI), which highlight activated parts of the body and brain (Poellinger et al., 2001); heart rate and blood pressure (Hongratanaworakit, 2004); electrodermal activity, the resistibility of the skin to a mild electric shock (Jellinek, 1998); and psychometric tests have been utilized to demonstrate that odours can influence cognition and mood (Moss, Cook, Wesnes, & Duckett, 2003).

In general it appears that; floral, spicy and minty odours are stimulating, producing increases in contingent negative variation (CNV is a measure of activity in the motor cortex the brain leading up to voluntary muscle movement); while citrus, herbal and woody odours are sedating, producing decreases in CNV. However, neroli (produced from the blossom of the bitter orange tree) which contains both floral (geraniol) and citrus (linalool) components, can either be stimulating or sedating depending on whether it is perceived as either citrus or floral (Torii, 1997). Similarly, essential oils may also be either stimulating or sedating depending on necessity (Sakamoto, Minoura, Usui, Ishizuka, & Kanba, 2005), e.g. lavender oil may be either stimulating or sedating due to the monoterpenes and alcohols in its chemical composition. To elaborate, Yerkes-Dodson law contends that when task demand or arousal exceeds a maximum threshold additional stimulation would impair performance, however Degel & Koster (1999) reported that lavender, which was expected to impair performance due to its stimulating properties, improved performance which suggests its sedating rather than its stimulating properties must have been utilized.

In a study by Diego et al. (1998); lavender produced an increase in beta power, suggesting increased drowsiness; and rosemary showed decreases in frontal alpha and beta power, suggesting increased alertness. Cognitively, the stimulating properties of cinnamon improved memory performance (Zoladz & Raudenbush, 2005), while lavender and rosemary, enhanced the speed and accuracy of math computation (Diego et al., 1998). Further more, patients exposed to lavender or orange aroma showed a mood improvement and a reduction in anxiety while waiting for dental treatment (Lehrner, Marwinski, Lehr, Jöhren, & Deecke, 2005). The sedating properties of lavender have also

proven beneficial to sleep disorders, promoting deep sleep (Goel, Kim, & Lao, 2005; Komori et al., 2006).

There is evidence to suggest the psychological effects of odours in human studies are mediated by their hedonic valence (Moss, Howarth, Wilkinson, & Wesnes, 2006). Hedonic valence is a conditioned response influenced by exposure, experience, culture, beliefs, expectation (Ayabe-Kanamura et al., 1998; Broughan, 2005; Kirk-Smith, van Toller, & Dodd, 1983) and gender (Fitzgerald et al., 2007). Barnham and Broughan, (2002) tested the influence of hedonics on emotion and task performance of children between the ages of 2 and 11, in pleasant and unpleasant odour conditions. The results reveal no significant age difference in performance in the unpleasant condition, suggesting an innate/evolutionary reaction to unpleasant odours. Older children responded more favourably than younger children in the pleasant odour condition however, indicating a more hedonic/learned response to pleasant odours based on exposure and experience. Odours are experienced within the context of environmental situations i.e., if an odour is experienced in a situation or environment that is emotionally charged or pleasant/relaxing, it may have either a stimulating or relaxing effect, respectively (Jellinek, 1998). Thus odours acquire hedonic values through pairing with emotionally significant events (Kirk-Smith, van Toller, & Dodd, 1983) which can also result in a placebo effect (Jellinek, 1998; Knasko, Gilbert, & Sabini, 1990).

Due to the hedonic influence of odours in humans, it may be argued that animal studies provide a more accurate prediction of the pharmacological/physiological effect of essential oils because one can safely assume that any changes in behaviour are unlikely to be cognitively mediated (Broughan, 1998).

1.4.4. Animal research

The sedatory and stimulating effects of essential oils, found in human studies, have been mirrored in animal studies. Lavender and camomile, rosemary and peppermint, demonstrated sedating and stimulating effects on dogs housed in rescue shelters, respectively (Graham, Wells, & Hepper, 2005). When exposed to the ambient odour of lavender, dogs with a history of travel-induced excitement spent significantly more time resting and sitting, and less time moving and

vocalizing (Wells, 2006). Umezu, (1999) demonstrated that rose oil exhibits similar anti-conflict properties to diazepam with mice, in the Vogel/Geller conflict test. In another study, lemon odour demonstrated antidepressant properties by significantly reducing the total immobility time of rats in a forced-swim test and significantly decreasing the total locomotor activity in an open-field test (Komori, Fujiwara, Tanida, Nomura, & Yokoyama, 1995).

In her study of animals and their responses to essential oils, Ingraham (2004) observed that animals have an innate ability to choose the plants or aromas that would best respond or provide for their needs. Interestingly, herbivores and carnivores indicate a distinct preferential difference to essential oils. Ingraham observed that oils were ingested for internal ailments and inhaled for emotional problems. Animals showed a particular dislike for essential oils that contained cineole, e.g. rosemary, eucalyptus or tea-tree (manuka), except where these plants were part of their natural habitat. Animals also tended to avoid oils with monoterpenes with the exception of bergamot. These observations would suggest that careful consideration should be given in the utilization of essential oils in animal studies (Ingraham, 2004).

In addition to the oils used in animal studies, careful consideration should also be given to subject selection because this can have a significant influence on test results. It has been proposed that different strains or breeds of rats could exhibit different levels of anxiety (Ramos, Berton, Mormède, & Chaouloff, 1997; Shepard & Myers, 2008). Studies reveal significant differences in anxiety and fear related behaviour of juvenile, adolescent and adult rats in the elevated plus maze (Imhof, Coelho, Schmitt, Morato, & Carobrez, 1993) and social interaction test (Garau, Marti, Sala, & Balada, 2000). Korte and de Boer (2003) report gender differences with regards to stress levels and stress response i.e. the amount of time spent in the open arm of elevated plus maze.

1.5. Animal models of anxiety and depression

Kalueff and Tuohimaa, (2004) describe the criterion of animal models in terms of face, predictive and construct validity. Face validity as they describe it, reflects “phenomenological similarities” between animal models and human pathology. However, as Smith (1965) points out, even when animal models demonstrate face, predictive and/or construct validity, behaviours observed in

animals, may have different meaning to similar human behaviours because humans are verbal. According to Kalueff and Tuohimaa, (2004) predictive validity is based on the ability to postulate a drug's effectiveness on humans, based on its effects in animal models. Finally, the similarity of the theoretical rationale behind the pathology in humans and animals determines construct validity.

Mc Kinney and Bunney (as cited in (Overall, 2000), outline five basic criterion for animal models to mirror human symptomology of stress related disorders.

- ❖ The symptoms of the condition in animals should be reasonably analogous to those seen in humans.
- ❖ There should be behavioural changes in both model and patient that can be objectively evaluated.
- ❖ Independent observers should agree on the objective criteria for drawing conclusions about the subjective state.
- ❖ The treatment modalities effective in reversing the human condition should have the same effect on the animal condition.
- ❖ The model should be reproducible by other investigators.

Animal models of anxiety and depression may be *measured*, i.e. based on changes in animal pathology, or *induced* through drugs, gene mutation, brain lesion/stimulation or stress (Kalueff & Tuohimaa, 2004). To elaborate, anxiogenic and anxiolytic drugs may be utilized to manipulate behavioural responses to different test conditions (Pellow & File, 1986; Rodgers & Dalvi, 1997; Wilson, Burghardt, Ford, Wilkinson, & Primeaux, 2004). Stress induced models include among others, Porsolt's forced-swim test (Frazer & Morilak, 2005; Porsolt, Anton, Blavet, & Jalfre, 1978), foot-shocking (Pijlman & van Ree, 2002) and novel environments (Montgomery, 1955; Montgomery & Monkman, 1955), to name but a few.

Novelty evoked stress models, based on the exploratory, social, defensive, and conditioned behaviours of animals (Wall & Messier, 2001), produce two affective dimensions, the activated component (agitation, anxiety) or the inhibitory/withdrawal component (sleep, appetite, and motor activity), i.e. depression (Frazer & Morilak, 2005). These behaviours can be manipulated

through early weaning, single/group housing conditions (Ferdman, Murmu, Bock, Braun, & Leshem, 2007; Newport, Stowe, & Nemeroff, 2002), selective breeding i.e., breeding of high or low anxiety related behaviours (Henniger et al., 2000; Langraf & Wigger, 2002; Landgraf & Wigger, 2003), exposure to predatory odours (Korte, DeBoer & Bohus, 1999; Korte & De Boer, 2003) or restraint (Albonetti & Farabollini, 1992; Korte, De Boer & Bohus, 2003). Korte, De Boer and Bohus (1999) maintain the presence of predatory odours and restraint conditions activates the defensive behaviours of animals and has a potentiating effect on test results.

For ethical reasons and ease of use, only drug induced and novelty evoked models of anxiety and depression were considered in this study. These models include, the elevated plus-maze (Pellow, Chopin, File, & Briley, 1985), the open-field (Hall, 1934) and the social interaction test (File & Hyde, 1978). Overall, (2000) suggests that novelty evoked animal models of anxiety and depression are more homologous to human symptomology because they tap into the instinctive behavioural instincts associated with survival, i.e. they measure the natural behaviours or responses of animals to novel environments or stimuli.

1.5.1. Novel environments and animal behaviour

In 'prey' animals, survival is an innate evolutionary instinct which covers the need to feed, propagate and avoid danger. In a novel environment this survival instinct produces a dichotomy between approach and avoidance (Montgomery, 1955; Montgomery & Monkman, 1955), i.e. a conflict between defensive behaviours (flight, fight or freeze) (Blanchard, Yudko, Rodgers, & Blanchard, 1993); and risk assessment (the need to explore or assess potential danger, food sources etc.).

From a research perspective the dichotomy between fear and risk assessment (approach/avoidance) behaviours, allows researchers to investigate the effectiveness of anxiolytic and anxiogenic drugs, i.e. drugs that reduce or increase levels of anxiety respectively (Arakawa, 2007). Behaviour analysis of rats selectively bred for high (HAB) and low (LAB) anxiety related behaviours, provides an accurate illustration of the defensive behaviours to expect from rats exposed to novel environments (Table 2) (Landgraf & Wigger, 2002; Salome et al., 2002).

Table 2

Principal behavioural profiles in experimental models of anxiety and depression (taken from Kalueff & Tuohimaa, 2004).

Behavioural Indices	Anxiety	Depression
General locomotion	+	-
Exploration	-	-
Self-grooming	+ (frequency)	+ (duration)
Immobility	+ (freezing)	+ (despair)*
Defecation/Urination	+	?
Aggression	+	+
Self-aggression	0	+
Transitions between behaviours	+	-
Risk assessment	+ or - **	-

(+) increased; (-) decreased; (?) unclear or inconsistent effects; (0) no effects; *in Porsolt's swim and tail suspension tests; **depending on the model

1.5.2. The elevated plus-maze model

The elevated-plus maze (EPM) model consists of a plus shaped maze with two open and two closed arms elevated above the floor (Figure 1). The design of the maze exploits the natural fear of rodents to avoid bright open elevated places and initiates a conflict between their defensive and risk assessment behaviours (Henniger et al., 2000).



Figure 1. Rat in open-arm of Elevated-plus Maze

Open arm exploration is affected by the levels of anxiety, thus one would expect anxiolytic and anxiogenic drugs to increase or decrease open arm exploration, respectively (Pellow, Chopin, File, & Briley, 1985; Pellow & File, 1986). Other risk assessment behaviours also include; head dipping, i.e. leaning over the edge of the open arms; and 'stretch attend postures', in which the rodent stretches forward on front paws then retracts back without moving the hind paws (Rodgers & Dalvi, 1997). Risk assessment behaviours may take place from the safety of the centre or closed arms of the maze (protected) or occur on the exposed open arm of the maze (unprotected) (Dawson & Tricklebank, 1995). Increased duration and frequency of these behaviours in the open unprotected arms of the EPM is indicative of lower levels of anxiety (Kalueff & Tuohimaa, 2004).

Past studies have reported the anxiolytic effects of diazepam in the EPM are characterised by an increase in the number of entries and time spent in the open arms (Drapier et al., 2007) as well as a reduction in total entries (Pellow & File, 1986) in the maze. Diazepam also has an anxiolytic effect on risk assessment behaviours, by decreasing protected and increasing unprotected risk assessment behaviours (Dawson & Tricklebank, 1995). In the EPM, selective serotonin reuptake inhibitors (SSRIs), e.g. fluoxetine, cause a decrease in the number of entries and time spent in the open arms, however the number of total entries remains unaffected (Brocco, Dekeyne, Veiga, Girardon, & Millan, 2002; Drapier et al., 2007). Similarly, amphetamines cause a decreased ratio of entries and time spent in the open arms, with out a reduction in total entries in the EPM (Kask, Schiöth, Mutulis, Wikberg, & Rågo, 2000).

The EPM has been utilized to examine the anxiolytic properties of essential oils. Mice injected intra-peritoneally with lemon verbena (Vale, Matos, de Lima, & Viana, 1999) and woundwort (Rabbani, Sajjadi, & Zarei, 2003), displayed a significant increase in the number of open arm entries and time spent on the open arms of an EPM. Similarly, mice treated with an oral dose of bitter orange showed significant increases in open arm entries and duration of exploration in the EMP (Carvalho-Freitas & Costa, 2002). Further more, exposure to lavender odour (Bradley, Starkey, Brown, & Lea, 2007; Bridges & Starkey, 2004) and rose (Bradley, Starkey, Brown, & Lea, 2007) had an anxiolytic effect on gerbils in EPM.

1.5.3. *The open-field model*

The open-field (OF) may be a circular, square or rectangular enclosure; however in this study a square arena was utilized (Figure 2). Changes to the size of the open-field affects moderate but not significant behavioural changes, as such, Eilam (2003) suggests that behaviours elicited in a small open-field are representative of general patterns of behaviour to novel environments.



Figure 2. Single rat in Zone1 of open-field

Like the EPM, the OF also exploits the dichotomy between the natural tendency of rodents to avoid bright open spaces and their need to explore novel environments. When exposed to an open-field rodents establish a home base in one of the corners from which they explore their environment, preferring to move along the walls of the OF (Haimovici, Wang, Cohen, & Mintz, 2001). Exploratory behaviours in the OF include, sniffing, and rearing, i.e. the rodent lifts forepaws off the ground and stretches vertically on hind legs (Prut & Belzung, 2003). Levels of anxiety and depression can be determined by measuring changes in ambulation and exploration activity (Komori, Fujiwara, Tanida, & Nomura, 1995; Vécsei & Widerlöv, 1990). Increased ambulation and exploration, as well as an

increase exploration of central more exposed parts of the OF are indicative of an anxiolytic effect (Prut & Belzung, 2003).

Other useful measures of anxiety and depression in the OF include, immobility (freezing) and grooming behaviour (Kalueff & Tuohimaa, 2004). Increased immobility in the OF is characteristic of increased levels of anxiety and depression (Fromm, Heath, Vink, & Nimmo, 2004; Homberg et al., 2002). In rodents grooming plays an important role in behavioural adaptation to stress, stress-coping and de-arousal (Spruijt, Van Hooff, & Gispen, 1992). Grooming is sensitive to various stressors, psychotropic drugs and genetic manipulations. Anxiolytics like diazepam have demonstrated a reduction in rat grooming behaviour (Moody, Merali, & Crawley, 1988).

The anxiolytic effect of diazepam in the OF causes an increase in locomotor and exploratory (rears and sniffs) behaviours as well as in increased exploration of the more exposed central parts of the arena; the ratio of grooming is reduced as well as the time spent immobile. Similarly, SSRIs have also demonstrated an anxiolytic effect by increasing the ambulation and exploration behaviours in the OF (Prut & Belzung, 2003).

Examining the anxiolytic properties of essential oils in the OF, lemon verbena (Vale, Matos, de Lima, & Viana, 1999) and ginger (Felipe et al., 2008) have demonstrated a significant reduction in the number of rears and grooming incidence in mice. Similarly, thistle demonstrated an anxiolytic effect in mice by increasing total locomotion and ambulation into the centre of the OF (Ambavade, Mhetre, Tate, & Bodhankar, 2006).

1.5.4. The social interaction model

Social interaction (SI) between two rodents is observed in the OF apparatus described in section 1.5.3. Once again the fear/risk assessment dichotomy is vital to the success of this model of animal anxiety and depression, with rats showing less socialisation under more aversive conditions, e.g. bright open spaces (File & Hyde, 1978).

As outlined by Sala-Roca, Marti-Carbonell, Garau, Darbra and Balada, (2002), there are four groups of dependant measures in the social interaction model. The four groups consist of, active social behaviour (proximity and following, sniffing, social grooming, crawling over and under, escaping and

avoidance, passive social behaviour (passive interactions), activity/mobility (ambulation and exploration), and stress related behaviours (freezing and self-grooming). File and Hyde (1978) suggest the more time a pair of test subjects spend in active social interaction the less anxious they are.

Research suggest that diazepam has an anxiolytic effect on social interaction by increasing active and passive interaction (Cheeta et al., 2001). Amphetamines and SSRI antidepressants demonstrate anxiogenic effects by reducing social interaction, however chronic administration of SSRIs produces an anxiolytic effect on social behaviour (File & Seth, 2003).

The odour of orange essential oil increased the social interaction between rats, demonstrating a decrease in anxiety compared to the control subjects (Leite et al., 2008).

1.6 Aims

Taken together, research suggests that the anxiolytic/antidepressant effects of essential oils can be detected when studying novelty evoked exploration in rodents. To date, animal research has focused on the most commonly used essential oils, such as lemon, rose and lavender, whilst others have been less well studied. However, there are other oils such as bergamot and geranium, which are reported to improve anxiety and depression in humans (Lemon 2004), which have not yet been tested in animals. Furthermore, both of these oils contain linalool and linalyl acetate (Sellar, 1992), two of the major constituents of lavender, which have sedative effects in mice (Buchbauer, Jirovetz, Jager, Dietrich, & Plank, 1991).

Therefore, the overall aim of this study was to evaluate the behavioural effects of inhaled bergamot, geranium and a combination of these oils in three novelty evoked tests of anxiety; the elevate-plus maze, open-field and social interaction test. A second aim was to compare the effects of these oils with the known anxiolytic diazepam.

Method

2.1 Animals and housing

Sixty Hooded Rats (*Rattus norvegicus*), bred at the University of Waikato were used in this study. They were adult males over 120 days of age with an average weight of 492.9g at the time of testing.

The rats were housed in pairs in standard rodent cages sized (38x25x20 cm). The cages consisted of a plastic tray like bottom covered in dry non-treated wood shavings for bedding, and a wire cage top with a food hopper seated through a hole in the roof of the cage. The rats were kept under a 12-h reverse light cycle, in a temperature controlled environment of (23 ± 1 °C). Food and water were available at libitum, except during the period the subjects were being tested. Food and water was replenished daily. Where cages were unduly soiled or wet they were cleaned and fresh bedding provided, otherwise cages were cleaned every 4 days in accordance with the ethical principles of animal husbandry stipulated by the University of Waikato. The study received ethical approval from the University of Waikato Animal Ethics Committee.

2.2 Study design

Ten rats were randomly assigned to one of six groups, three essential oil (EO) treated groups, and three control groups. The EO treated groups consisted of the bergamot, geranium and combined treatment group. The combined group consisted of an equal combination of bergamot and geranium oil. The control groups consisted of the odour control, vehicle control and diazepam control treatment groups.

Each group was tested in each of three animal tests of anxiety and depression, the elevated plus-maze (EPM), the open-field (OF) and the social interaction (SI) test.

2.3 Apparatus

The EMP consisted of a black painted wooden maze in the shape of a plus sign raised 50cm from the floor. The maze arms where 50x10cm in length with closed (protected) arms enclosed with sides 40cm in height with open roof. The

open end of the closed arms and similarly the open (unprotected) arms were positioned opposite each other extending outward from a central platform 10x10cm² (Figure 1). In addition to the normal laboratory lighting, 100W anglepoise lamps were utilized to illuminate each open arm. The lamps were positioned in such a way as to leave the closed arms in shadow.

Open-field activity and social behaviour were tested in a 100x100x600cm square Perspex box lined on the outside with black PVC plastic. A 5x5 grid was taped to the floor of the open field dividing the arena into 25 squares (20x20cm²), effectively creating 3 concentric zones, the central (Zone1), middle (Zone2) and outer (Zone3) zones. The outer zone was situated proximal to the wall of the open-field (see Figure 2). The floor of the arena was covered with clear PVC for ease of cleaning. In addition to the normal laboratory lighting, 100W anglepoise lamps were placed on opposite side of the OF, positioned to remove any shadows.

Diazepam was given at a dose of 2mg/kg at a volume of 1ml/kg by intra-peritoneal (i.p.) injection (Pamlin supplied at a concentration of 5mg/ml by Newstead Veterinary Practice). Diazepam was diluted to the appropriate concentration with sterile water (Water for injection, Baxters, NZ). Vehicle controls received an intra-peritoneal (i.p.) injection of sterile water (1ml/kg). The injections were given 30mins prior to testing with the rats in a restraint tube.

2.4 Tests procedure

Each group of rats were tested on different days to prevent all the rats being exposed to the EOs. Each group of rats were placed in the experimental room 24 hours before testing began. During this time, rats in the essential oil groups were exposed to appropriate odour, whilst the odour, vehicle control and diazepam groups were left undisturbed. The essential oils were permeated into the air via two electric insect repellent vaporisers placed at opposite ends of the testing room, at a dosage of two drops of essential oil per vaporiser every two hours.

Testing was conducted between the hours of 9:00 and 14:00 with the laboratory lights on, during the nocturnal phase of the animal's circadian cycle. All the rats were placed in a restraint tube and weighed half an hour prior to testing. Rats in the vehicle control and diazepam groups received an i.p. injection whilst in the restraint tube and all animals were returned to their home cages prior

to testing. The behavioural tests were conducted 48 hours apart in the following order: EPM, OF then SI tests. Each group of rats remained in the experimental room until all the tests were completed. The 48 hour period between tests consisted of 24hr rest period plus a 24hr exposure period for the EO groups; the control groups effectively receiving a 48hr rest period.

In the EPM trials, the rats were placed on the centre platform facing an open arm and allowed to explore for 5 min. Test subject that fell off the apparatus were replace in the centre square of the maze facing an open arm.

In the trials where the open-field was utilized the test subjects were place in the centre (Zone1) of the arena facing away from the experimenter and allowed to explore for 5 min. A similarly process was conducted for the SI trials, except two unfamiliar (non-related) rats, i.e. from two different home cages, were placed in the open-field arena simultaneously,

The apparatus was wiped down with a dilute solution of the cleaning detergent used to clean the cages, then dried with paper towel between each trial. After each test group had participated in the three sets of trial, the rats were returned to their home room and the testing room was cleaned in preparation for the next group of rats. The test sessions were recorded with a Panasonic Camcorder model PV-GS19 suspended above the testing arena and the rats were monitored via a portable television.

2.5 Behavioural measures

Spatiotemporal measures in the EPM included the frequency of entries and the duration (measured in seconds) of time spent in the open and closed arms and centre square of the maze. Percentage measures were calculated as the proportion of entries made in relation to the total entries. The scored behaviours included, the frequency of head-dips and stretch-attends. Stretch-attend were further differentiated as protected or unprotected depending on where they occurred in the maze. Protected stretch-attends occurred in the centre square or close arm of the maze, unprotected stretch-attends occurring in the open arm of the EPM. Anxiolytic response in the EPM would be represented by an increase in frequency and time spent in the open arms of the EPM (Pellow, Chopin, File, & Briley, 1985; Pellow & File, 1986), as well as an increase of risk assessment behaviours,

i.e. head-dips and unprotected stretch-attends, in the maze (Rodgers & Dalvi, 1997).

Spatiotemporal measures in the OF included the frequency of entries into each zone and the time spent in each of the 3 zones. Behavioural measures included the total frequency and duration of immobility, grooming, sniffing and rearing. Furthermore the total number of line crossings and the total mobility were also measured. Increased locomotion and exploration of the more central zones (1&2) of the arena represent an anxiolytic response in the OF (Komori, Fujiwara, Tanida, & Nomura, 1995; Vécsei & Widerlöv, 1990). An increase in exploratory behaviour, i.e. rears and sniffs and a decrease in immobility and grooming are also indicative of an anxiolytic response in the OF (Prut & Belzung, 2003).

Behavioural measure in the social interaction test included frequency and duration of time apart, in passive contact and in active interactions. All forms of bodily contact which did not involve active interaction with test partner were defined as passive contact/interaction. Active interaction included sniffing, following, fighting and crawling (over or under) test partner. A further measure included contact latency, i.e. the length of time it took for the rats to interact from the beginning of the trial. Percentage measures were calculated as the proportion of behaviour in relation to the total frequency of all the measured behaviours. An anxiolytic response in the SI test was as increase in passive and active interaction (File & Hyde, 1978; File & Seth, 2003)

A program called Hindsight 1.5, a computer assisted behavioural scoring program (ScottWeiss, University of Leeds) was used to score the video tapes of the rats in each of the tests.

2.6 Statistical analysis

Statistical analyses were conducted using SPSS for Windows version 11. For analysis of the EPM and OF results, odour and vehicle control groups were compared by independent group t-test. Similarly, diazepam and the control groups were also compared by independent group t-test. One-way between-groups ANOVAs were conducted to determine whether there were any significant differences in the behaviour measures between the combined control group and the EO groups. When ANOVAs were significant post-hoc tests were conducted to further investigate the effect. Dunnett's t-tests were used to compare the treatment

groups to the control, whilst Bonferroni corrected t-tests were used to compare between the treatment groups when required.

Considering that individual rat behaviour is influenced by its partner, it is suggested that the social behaviours of the rats be measured as a unit rather than separately (Genn, Tucci, Marco, Viveros, & File, 2004). The combined assessment of test partners effectively reduced the sample size making it imperative to utilize non-parametric techniques for analysis. SI results; Mann-Whitney U test was used for pair-wise testing of odour and vehicle control groups, and the diazepam and control groups. Kruskal-Wallis tests were conducted to determine whether there were any significant differences in the behaviour measures between the combined control group and the EO groups. Were an effect was found, Mann-Whitney U-tests were used for pair-wise testing of control and each of the EO groups, bergamot and combined group, and geranium and the combined group to further investigate the effect. Faulty recording made the first two test pairs in the odour control difficult to score, consequently they were excluded from the analysis.

Results

The results are presented in three sections, each addressing one of the three novelty evoked models of anxiety utilized in this study. Firstly, within each section a comparison will be made between the odour and vehicle control, to determine whether they can be pooled. Secondly, the diazepam group will be compared to the control group to illustrate the effect of an anxiolytic on rat behaviour and establish a baseline for comparison for the anxiolytic properties of the essential oils. The EO groups will then be compared to the control to determine if they had a significant effect on rat behaviour and whether the combining of the oil had a potentiating or additive influence on the anxiolytic effectiveness of the oils. Finally, a comparison will be made between the effects of the EO groups and the diazepam group

Sample size constraints prohibited the exclusion of behavioural outliers; to this extent all animals were included in the analysis. Further more, it should be noted that only significant ($p < 0.05$, $p < 0.01$) and marginally significant ($p < 0.1$) results will be presented.

3.1. Elevated-plus Maze

Many studies (Dawson & Tricklebank, 1995) have differentiated between protected and unprotected risk assessment behaviours, this distinction will only be applied to stretch-attend and not head-dip behaviour because it was difficult to establish a clear distinction between the protected stretch-attend and the protected head-dip behaviours, i.e. all protected risk assessment was considered to be stretch-attend behaviour.

In order to determine whether the control groups could be pooled, an independent group t-test was conducted between the odour and vehicle control groups. This analysis revealed the vehicle controls made marginally fewer [$t(18)=1.56$, $p < 0.1$] protected stretch-attends than the odour control group. Considering there were no other statistically significant variables in any of the behaviours measured between the odour and vehicle controls, the controls were pooled for any subsequent analyses. These data are summarised in Table 3.

Table 3.

Behaviour of odour and vehicle controls in elevated-plus maze

Behaviour	Odour Control	Vehicle Control
Closed arm frequency	6.6±1.0	7.0±0.7
Open arm frequency	1.1±0.5	1.2±0.5
Centre frequency	8.1±1.1	8.4±0.8
Closed arm duration	179.6±20.1	187.0±15.9
Open arm duration	11.3±6.2	25.3±10.7
Centre duration	109.1±17.6	87.7±9.8
% Closed arm	42.1±2.7	42.9±2.6
% Open arm	6.9±2.7	6.5±2.7
% Centre	51.1±0.5	50.6±0.4
Head-dip frequency open arm	1.0±0.6	1.5±0.7
Head-dip duration open arm	3.6±2.4	3.5±1.4
Unprotected stretch-attend frequency	1.4±0.7	2.1±1.0
Unprotected stretch-attend duration	6.0±3.1	17.7±8.0
Protected stretch-attend frequency	6.2±1.1	4.3±0.5\$
Protected stretch-attend duration	41.6±7.6	30.4±5.1
Total entries	15.8±2.2	16.6±1.5

Data are represented as means ± S.E.M. Duration is measured in seconds (s). Sample size – n=10 odour & vehicle control groups. Level of significance - \$= p<0.1 compared to odour control.

To determine if 2mg/kg dose of diazepam altered risk assessment behaviour on the elevated-plus maze (EPM), an independent group t-test was conducted between the pooled control and diazepam groups. These data are summarised in Table 4. Compared to the control group, the diazepam group made significant more open arm entries [$t(28)=2.33$, $p<0.05$] of marginally longer duration [$t(28)=1.92$, $p<0.1$], spent marginally more time in the centre [$t(28)=1.51$, $p<0.1$] and significantly less time in the closed arm [$t(28)=2.25$, $p<0.05$] of the EPM. Analysis of the behavioural measures revealed the diazepam group made significantly more unprotected stretch-attends [$t(28)=1.49$, $p<0.01$], as well as significantly more [$t(28)=2.33$, $p<0.5$] and longer [$t(28)=2.33$, $p<0.05$] head-dips than the control group (see Fig 3). Proportionate to the total number of entries, the diazepam group made significantly less closed arm entries [$t(28)=2.39$, $p<0.5$] and more open arm entries [$t(28)=2.08$, $p<0.05$]. On the whole diazepam increased open arm entries and the time spent in the open arms and centre square of the EPM; decreased time spent in closed arms and increased the number of head-dips and unprotected stretch-attends.

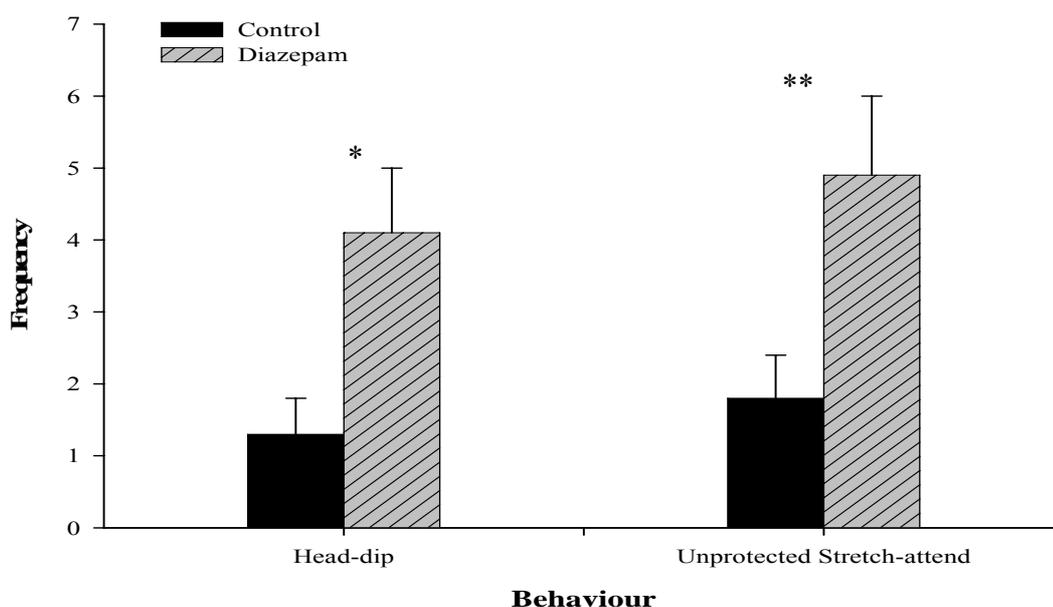


Figure 3. Frequency of exploratory behaviour on EPM for control & diazepam groups over a 5 minute test session. Data are represented as mean ± S.E.M. Level of significance- ** $p < 0.05$, * $p < 0.01$.

One-way between-groups ANOVAs were conducted to determine whether there were any significant differences in the behavioural measures between the combined/pooled control group and the EO groups. When ANOVAs were significant, post-hoc tests were conducted to further investigate the effect. Dunnett's t-tests were used to compare the treatment groups to the control, whilst Bonferroni corrected t-tests were used to compare between the treatment groups when required.

Firstly a series of one-way ANOVAs were conducted to investigate the behavioural effects of bergamot and a combination of bergamot and geranium compared to control rats. The ANOVAs revealed significant differences among these groups for closed arm [$F(2,37) = 3.48$, $p < 0.05$], centre square [$F(2,37) = 3.72$, $p < 0.05$] and total [$F(2,37) = 3.80$, $p < 0.05$] entries; marginally significant differences were found for the number of entries [$F(2,37) = 2.27$, $p < 0.1$] and time spent [$F(2,37) = 2.07$, $p < 0.1$] in the open arm, and the frequency [$F(2,37) = 2.47$, $p < 0.1$] and duration [$F(2,37) = 2.12$, $p < 0.1$] of unprotected stretch-attends. Dunnett's t-test comparisons of the groups indicate the bergamot group made

significantly more close arm entries ($p < 0.05$) and marginally more centre square entries ($p < 0.1$) than the control group.

Secondly, a series of one-way ANOVAs were conducted to investigate the behavioural effects of geranium and a combination of bergamot and geranium compared to control rats. The ANOVAs revealed significant differences among these groups for closed arm [$F(2,37)=6.48, p < 0.01$], centre [$F(2,37)=6.17, p < 0.01$] and total [$F(2,37)=6.39, p < 0.01$] entries, and the frequency of head-dips [$F(2,37)=2.17, p < 0.1$].

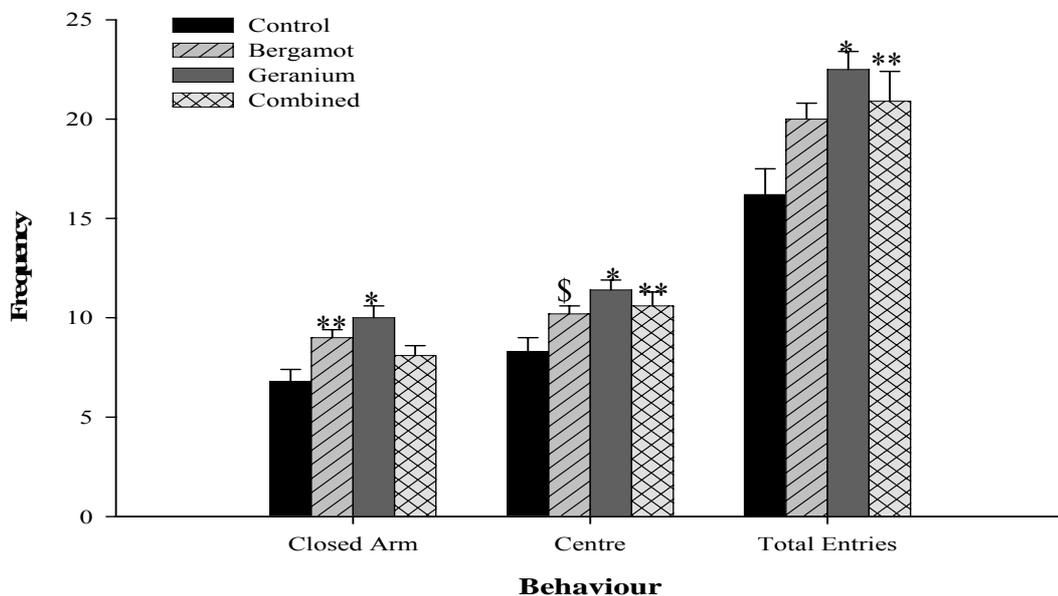


Figure 4. Frequency of exploratory behaviour on EPM for control & essential oil groups over a 5 minute test session. Data are represented as mean \pm S.E.M. Levels of significance - \$ $p < 0.01$, ** $p < 0.05$, * $p < 0.1$ compared to control group.

Dunnett's t-test comparisons of the groups indicate the geranium group made significantly more closed arm ($p < 0.01$), centre ($p < 0.01$) and total ($p < 0.01$) entries than the control group. The combined group made significantly more centre square ($p < 0.05$) and total ($p < 0.05$) entries than the control group. On the whole, bergamot increased closed arm and centre square entries; geranium increased closed arm, centre and total entries; and the combination of bergamot and geranium increased the entries into the centre of the maze and the total entries made (Fig 4). These data are summarised in Table 4.

Table 4.
Behaviour measures of test groups in elevated-plus maze

Behaviour	Combined Control	Diazepam (2mg/kg)	Bergamot	Geranium	Combined
Closed arm frequency	6.8±0.6	7.4±1.8	9.0±0.4**	10.0±0.6*	8.1±0.5
Closed arm duration	183.3±12.5	126.1±26.0**	178.7±11.8§	187.2±13.4°	170.7±12.4§
Open arm frequency	1.2±0.3	2.9±0.8**	0.8±0.2°°	1.1±0.4°	2.2±0.7
Open arm duration	18.8±6.2	40.7±10.9§	9.7±3.3°°	12.5±5.3°°	33.7±11.1
Centre frequency	8.3±0.7	10.7±1.9	10.2±0.4§	11.4±0.5*	10.6±0.7**
Centre duration	98.4±10.1	133.2±25.9§	111.6±11.2	100.4±9.4	95.6±3.7
% Closed Arm	42.5±1.8	29.0±5.3**	45.0±1.4°°	44.4±1.6°°	39.7±2.6§
% Open Arm	6.7±1.9	15.0±4.3**	4.0±1.2°°	5.0±1.7°°	9.4±2.6
% Centre	50.8±0.3	56.0±4.9	51.0±0.4	50.7±0.4	50.9±0.5
Head-dip frequency	1.3±0.5	4.1±0.9*	0.9±0.4°	0.7±0.4°	2.8±1.1
Head-dip duration	3.5±1.3	11.2±3.8**	2.3±1.2°°	1.2±0.7°°	5.4±2.1
Unprotected stretch-attend frequency	1.8±0.6	4.9±1.1**	1.2±0.4°°	1.6±0.7°°	3.8±1.3
Unprotected stretch-attend duration	11.8±4.4	15.8±4.5	5.9±1.9	8.5±3.3	22.3±7.0
Protected stretch-attend frequency	5.3±0.6	4.8±0.9	5.6±0.7	5.1±0.9	6.0±0.6
Protected stretch-attend duration	36.0±4.6	25.8±6.4	37.4±3.9	26.6±5.6	32.8±3.6
Total entries	16.2±1.3	21.0±3.8	20.0±0.8	22.5±0.9*	20.9±1.5**

Data are represented as means ± S.E.M. Duration is measured in seconds (s). Sample size - n=20 control; n=10, diazepam, bergamot, geranium & combined groups. Levels of significance - §= p<0.1, **= p<0.05, *= p<0.01 compared to control group; §= p<0.1, °°= p<0.05, °= p<0.01 compared to diazepam.

Finally, in order to make a comparison between the anxiolytic effects of diazepam and the EOs on rat behaviour in the EPM, independent group t-tests were conducted. Only the behavioural measures where the diazepam demonstrated anxiolytic effects compared to the control rats were included in this analysis. Compared to the diazepam group, the bergamot group made significantly fewer open arm entries [$t(18)=2.44$, $p<0.05$], and spent marginally more time in closed arm [$t(18)=1.84$, $p<0.1$] and significantly less time in the open arm [$t(18)=2.72$, $p<0.05$]. The bergamot group also made significantly fewer head-dips [$t(18)=3.28$, $p<0.01$] of shorter duration [$t(18)=2.20$, $p<0.05$], and fewer unprotected stretch-attends [$t(18)=3.07$, $p<0.05$]. Proportionate to the total number of entries, the bergamot group made significantly more closed arm entries [$t(18)=2.90$, $p<0.05$] and significantly fewer open arm entries [$t(18)=2.48$, $p<0.05$]. Similarly, the geranium group made marginally fewer open arm entries [$t(18)=1.99$, $p<0.1$], and spent marginally more time in closed arm [$t(18)=2.09$, $p<0.1$] and significantly less time in the open arm [$t(18)=2.33$, $p<0.05$]. The geranium group also made significantly fewer head-dips [$t(18)=3.42$, $p<0.01$] of shorter duration [$t(18)=2.54$, $p<0.05$], and fewer unprotected stretch-attends [$t(18)=2.50$, $p<0.05$]. Proportionate to the total number of entries, the geranium group made significantly more closed arm entries [$t(18)=2.75$, $p<0.05$] and significantly fewer open arm entries [$t(18)=2.19$, $p<0.05$]. Independent group t-test indicates, the only difference between the diazepam and combined group, was that the combined group spent marginally more time in the closed arm [$t(18)=1.55$, $p<0.1$] than the diazepam group, and proportionate to total arm entries, the combined group made marginally more closed arm entries [$t(18)=1.80$, $p<0.1$]. On the whole, bergamot and geranium and the combination of the two oils increased the time spent in the closed arm, decreased the number of open arm entries and the time spent in the open arms, reduced the frequency and duration of head-dips and reduced the frequency of unprotected stretch-attends compared to control. Compared to diazepam, the combination of bergamot and geranium increased the time spent in the closed arm of the maze. These data are summarised in Table 4.

3.2. Open-field

In order to determine whether the control group could be pooled, an independent group t-test was conducted between the odour and vehicle control groups. This analysis revealed the vehicle controls spent significantly less time in Zone1 (centre of arena) [$t(18)=2.50$, $p<0.05$] than the odour control group. However, considering there were no other statistically significant in any of the behaviours measured between the odour and vehicle controls the two groups were pooled for any subsequent analyses. These data are summarised in Table 5.

Table 5.
Behaviour of odour and vehicle controls in the open-field test

Behaviour	Odour Control	Vehicle Control
Frequency zone 1	1.5±0.3	1.2±0.2
Frequency zone 2	3.6±0.9	2.8±0.3
Frequency zone 3	3.1±0.6	2.6±0.3
Duration zone 1	6.0±1.1	2.7±0.7*
Duration zone 2	8.3±2.6	7.8±1.4
Duration zone 3	285.7±2.5	289.5±1.7
Total line frequency	44.2±6.5	44.0±4.4
Total immobile frequency	2.0±0.7	2.5±0.6
Total immobile duration	12.5±6.8	23.8±10.1
Total grooming frequency	0.5±0.2	0.6±0.2
Total grooming duration	5.2±2.4	3.5±1.3
Total sniffing frequency	40.7±4.7	38.8±3.8
Total sniffing duration	171.2±7.5	179.7±11.2
Total rearing frequency	12.3±2.2	9.8±1.3
Total rearing duration	13.4±2.5	11.6±1.8
Total ambulation	97.7±9.9	81.4±9.3

Data are represented as means ± S.E.M. Duration is measured in seconds (s). Sample size - n=10, odour & vehicle control. Levels of significance - *= $p<0.05$ compared to odour control.

To determine if 2mg/kg dose of diazepam altered exploratory behaviour in the open-field (OF), an independent group t-test was conducted between the combined control and diazepam groups. Compared to the control group, the diazepam group spent marginally less time in Zone2 (between centre and outer zone of arena) [$t(18)=1.82$, $p<0.1$] compared to the Control group. Overall the diazepam group expressed significantly longer periods of immobility [$t(18)=3.38$, $p<0.01$], groomed marginally more frequently [$t(18)=1.89$, $p<0.1$], sniffed for marginally shorter periods [$t(18)=1.84$, $p<0.1$], made marginally fewer rears [$t(18)=1.73$, $p<0.1$] and were marginally less mobile [$t(18)=1.87$, $p<0.1$] than the control group. On the whole, the analysis illustrates diazepam increased

immobility time, decreased ambulation, increased grooming activity and reduced the amount of exploration in the OF (Fig5). These data are summarised in Table 6.

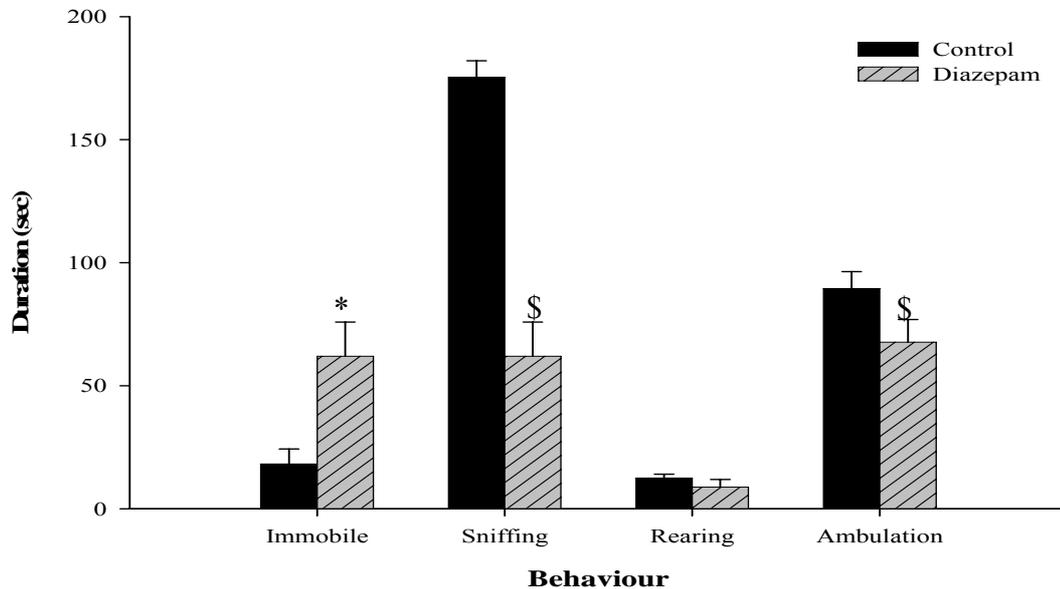


Figure 5. Duration of exploratory behaviour in open-field (OF) for control & diazepam groups over a 5 minute test session. Data are represented as mean \pm S.E.M. Level of significance - \$ $p < 0.1$, * $p < 0.01$.

One-way between-groups ANOVAs were conducted to determine whether there were any significant differences in the behaviour measures between the combined control group and the EO groups. When ANOVAs were significant post-hoc tests were conducted to further investigate the effect. Dunnett's t-tests were used to compare the treatment groups to the control, whilst Bonferroni corrected t-tests were used to compare between the treatment groups when required.

Firstly a series of one-way ANOVAs were conducted to investigate the behavioural effects of bergamot and the combination of bergamot and geranium compared to the control rats. The ANOVAs revealed significant differences among these groups for number of entrances into Zone2 [$F(2,37)=3.19$, $p < 0.05$] and Zone3 (outer zone) [$F(2,37)=3.92$, $p < 0.05$] and marginally significant differences in the time spent in Zone1 [$F(2,37)=2.14$, $p < 0.1$] and Zone2 [$F(2,37)=2.54$, $p < 0.1$]. Other significant differences across the test groups include; the number of line-crossings [$F(2,37)=3.74$, $p < 0.05$], and immobile frequency

[F(2,37)=4.94, $p<0.05$], with marginally significant differences for the time spent immobile [F(2,37)=2.36, $p<0.1$]. Significant group differences were found for time spent sniffing [F(2,37)=3.54, $p<0.05$], rearing [F(2,37)=5.20, $p<0.05$], and ambulation around the arena [F(2,37)=3.95, $p<0.05$], with marginally significant differences for the number of rears made [F(2,37)=2.61, $p<0.1$]. Dunnett's t-tests indicated the bergamot group had marginally fewer episodes of immobility ($p<0.1$), spent significantly less time sniffing ($p<0.05$) and significantly more time rearing ($p<0.05$), and spent significantly more time ambulating/exploring the arena ($p<0.01$) compared to controls.

Secondly, a series of one-way ANOVAs were conducted to investigate the behavioural effects of geranium and a combination of bergamot and geranium compared to control rats. The ANOVAs revealed significant differences among the groups for the number of entries into Zone2 [F(2,37)=3.83, $p<0.05$] and Zone3 [F(2,37)=6.08, $p<0.01$] and the amount of time spent in Zone2 [F(2,37)=4.39, $p<0.05$], with marginally significant differences for the amount of time spent in Zone3 [F(2,37)=2.44, $p<0.1$]. Other significant differences across the test groups included; the number of line-crossings [F(2,37)=4.24, $p<0.05$], immobile frequency [F(2,37)=6.59, $p<0.01$], with marginally significant differences in the number of grooming episodes [F(2,37)=2.97, $p<0.1$]. Marginally significant differences were also found for the number of rears made [F(2,37)=2.25, $p<0.1$], with significant differences in the time spent rearing [F(2,37)=4.99, $p<0.05$]. Ambulation time [F(2,37)=2.99, $p<0.1$] was also significantly different between the groups. Dunnett's t-tests indicated that the geranium group, had significantly fewer episodes of immobility ($p<0.05$) and spent significantly less time immobile ($p<0.05$), had marginally more grooming sessions ($p<0.1$), and spent marginally more time rearing ($p<0.1$) compared to the control rats.

When the combined EO group was compared with the control group, Dunnett's t-test indicated that the combined group entered Zone2 ($p<0.05$) and Zone3 ($p<0.05$) significantly more, and spent significantly more time in Zone2 ($p<0.05$). Furthermore, the combined group, made significantly more line crossings ($p<0.05$); experienced significantly fewer episodes of immobility ($p<0.01$) and spent marginally less time immobile ($p<0.1$), made marginally more rears ($p<0.1$) and spent significantly more time rearing ($p<0.01$). The combined group also spent significantly more time ambulating around the arena ($p<0.5$).

Further analysis with Bonferroni post-hoc comparisons revealed the geranium group made significantly fewer entries into Zone2 ($p<0.05$) and Zone3 ($p<0.01$), and spent significantly less time in Zone2 (0.05), and made significantly fewer line crossings ($p<0.05$), and than the Combined group.

On the whole; bergamot decreased immobility and sniffing and increased rearing and overall ambulation/exploration of the open-field; geranium decreased immobility and increased grooming and rearing; and the combination of bergamot and geranium increase the line crossing, decreased immobility, and increased rearing and general ambulation/exploration of the arena (Fig 6). These data are summarised in Table 6.

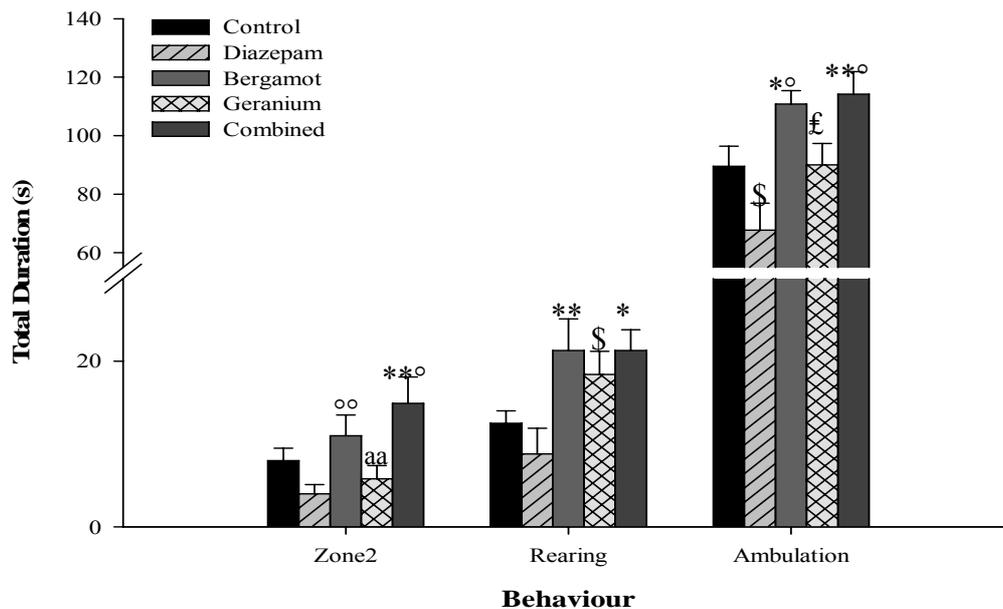


Figure 6. Duration of exploratory behaviour in open-field (OF) for control & diazepam groups over a 5 minute test session. Data are represented as mean \pm S.E.M. Levels of significance - \$= $p<0.1$, **= $p<0.05$, *= $p<0.01$ compared to control group; aa= $p<0.05$ compared to combined group; £ $p<0.1$; °°= $p<0.05$, °= $p<0.01$ compared to diazepam.

Table 6.
Behaviour of test groups in the open-field

Behaviour	Combined Control	Diazepam (2mg/kg)	Bergamot	Geranium	Combined
Frequency zone 1	1.4±0.2	1.5±0.3	1.3±0.2	1.5±0.2	1.8±0.4
Frequency zone 2	3.2±0.5	3.1±0.8	3.3±0.4	2.6±0.6 ^{aa}	5.2±0.9**
Frequency zone 3	2.9±0.3	2.8±0.7	3.0±0.4	2.0±0.4 ^a	4.6±0.7**
Duration zone 1	4.4±0.8	10.3±6.2	2.2±0.3	3.9±1.0	3.0±0.8
Duration zone 2	8.0±1.5	4.0±1.1\$	11.0±2.5 ^{oo}	5.8±1.6 ^{aa}	14.9±3.2 ^{**o}
Duration zone 3	287.6±1.5	285.7±6.0	286.8±2.7	290.3±2.4	282.1±3.8
Total line frequency	44.1±3.8	51.9±6.8	50.2±2.6	42.3±3.6 ^{aa}	58.6±3.1**
Total immobile frequency	2.3±0.5	2.5±0.6	0.9±0.5\$	0.6±0.3**	0.3±0.2*
Total immobile duration	18.2±6.1	62.0±13.9*	10.1±4.7 ^o	15.8±9.3 ^{**oo}	0.8±0.4\$ ^o
Total grooming frequency	0.6±0.2	1.1±0.3\$	1.1±0.3	1.4±0.3\$	0.9±0.3
Total grooming duration	4.4±1.4	6.9±2.0	7.0±2.5	6.8±1.7	7.5±2.8
Total sniffing frequency	39.8±3.0	35.3±4.1	38.4±2.8	38.5±2.6	45.9±2.3
Total sniffing duration	175.4±6.7	154.5±8.9\$	150.8±5.2**	169.0±6.2	156.1±8.6
Total rearing frequency	11.1±1.3	7.0±2.1\$	15.5±2.4 ^{oo}	13.7±2.0 ^{oo}	15.7±1.8\$ ^o
Total rearing duration	12.5±1.5	8.8±3.1	21.3±3.8**	18.4±2.8\$	21.3±2.5*
Total ambulation	89.5±6.9	67.7±9.2\$	110.8±4.6* ^o	90.0±7.3£	114.2±7.7 ^{**o}

Data are represented as means ± S.E.M. Duration is measured in seconds (s). Sample size - n=20 control; n=10, diazepam, bergamot, geranium & combined groups. Levels of significance - \$= p<0.1, **= p<0.05, *= p<0.01 compared to control group; aa= p<0.05, a= p<0.01, compared to combined group; £p<0.1; °= p<0.05, °= p<0.01 compared to diazepam.

Finally, in order to make a comparison between the anxiolytic effects of diazepam and the EOs on rat behaviour in the OF, independent group t-tests were conducted. Only the behavioural measures where the diazepam demonstrated significant differences compared to the control rats were included in this analysis. Compared to the diazepam group, the bergamot group spent significantly more time in Zone2 [$t(18)=2.49$, $p<0.05$], spent significantly less time immobile [$t(18)=3.55$, $p<0.01$], made significantly more rears [$t(18)=2.66$, $p<0.05$] and spent significantly more time ambulation/exploring the arena [$t(18)=4.19$, $p<0.01$]. Similarly, the geranium group spent significantly less time immobile [$t(18)=2.77$, $p<0.05$], made significantly more rears [$t(18)=2.36$, $p<0.05$] and spent marginally more time ambulation/exploring the open-field [$t(18)=1.90$, $p<0.1$]. T-tests between the diazepam and combined group indicate, the combined group spent significantly more time [$t(18)=3.20$, $p<0.01$] in Zone2, was immobile for a significantly shorter period of time [$t(18)=3.45$, $p<0.01$], made significantly more rears [$t(18)=3.19$, $p<0.01$], and spent significantly more time ambulating/exploring the open-field [$t(18)=3.88$, $p<0.01$]. On the whole it appears bergamot and the combination of bergamot and geranium increased the time spent in Zone2, and both the single oil and the combined oil group reduced immobility, increased rearing and increased the overall ambulation/ exploration of the test arena compared to diazepam. These data are summarised in Table 6.

3.3. Social Interaction

All analyses for the SI test were carried out using non parametric statistics as the pairs of animals were scored as a unit resulting in an n of five per group. Mann-Whitney U tests were used for pair-wise comparison of odour and vehicle control groups to determine whether the control groups could be pooled. These analyses revealed the vehicle control spent significantly longer crawling over or under test partner than the odour control group [U= -2.24, p<0.05]. Considering there were no other statistically significant variables in any of the behaviours measured between the odour and vehicle controls, the two groups were pooled for any subsequent analyses. These data are summarised in Table 7.

Table 7.
Social interactive behaviour of odour and vehicle control groups in open-field

Behaviour	Odour Control	Vehicle Control
Contact latency	(8.5)9.9(16.6)	(5.7)9.9(28.9)
Separation frequency	(16.0)17.0(24.0)	(14.5)17.0(19.0)
Passive interaction frequency	(9.0)14.0(26.0)	(7.0)12.0(14.5)
Sniffing frequency	(21.0)22.0(29.0)	(20.0)21.0(26.0)
Following frequency	(4.0)7.0(21.0)	(6.0)9.0(10.5)
Crawl frequency	(1.0)2.0(2.0)	(2.0)2.0(2.0)
Fight frequency	(0.0)0.0(0.0)	(0.0)0.0(0.0)
Separation duration	(128.9)211.8(219.4)	(179.7)201.5(204.1)
Passive interaction duration	(13.9)30.8(50.9)	(16.8)23.5(29.9)
Sniffing duration	(28.2)37.9(38.2)	(46.8)57.3(69.5)
Following duration	(6.9)12.7(20.4)	(15.2)19.5(26.0)
Crawl duration	(1.7)1.9(3.6)	(2.7)3.6**(6.6)
Fight duration	(0.0)0.0(0.0)	(0.0)0.0(0.0)
% Separation	(23.3)29.1(29.3)	(24.5)27.4(30.0)
% Passive interaction	(16.4)24.1(25.2)	(10.9)19.4(25.0)
% Sniffing	(28.2)37.9(38.2)	(33.0)36.8(40.3)
% Following	(6.9)12.7(20.4)	(10.4)14.8(16.2)
% Crawling	(1.7)1.9(3.6)	(3.1)3.4(5.9)
% Fighting	(0.0)0.0(1.0)	(0.0)0.0(0.0)
% Active interaction	(46.6)51.5(54.5)	(48.4)53.2(61.2)

Data are represented as (25th percentile) *median* (75th percentile). Duration is measured in seconds (s). Sample size - n=3 odour control, n=5 for vehicle control. Levels of significance – two tailed Mann-Whitney U-test, **= p<0.05 compared to odour control.

To determine if the 2mg/kg dose of diazepam altered behaviour in the open-field (OF), Mann-Whitney U tests were used for pair-wise comparisons between the pooled control and diazepam groups. Compared to the control group, the diazepam group made significantly fewer sniffs [U=-2.94, p<0.01], followed [U=-2.50, p<0.05], crawled [U=-2.30, p<0.05] separated [U=-2.66, p<0.01] and made passive contact [U=-1.92, p<0.05] with partner significantly less often than the control group. Proportionate to the total frequency of behaviours measured, the diazepam group separated marginally more often [U=-1.76, p<0.1], and made

significantly fewer active interactions [$U=-2.34$, $p<0.05$] with test partner than the control group. On the whole, the analysis illustrates diazepam decreased the frequency of separations, sniffs, follows, crawls, passive and active interactions with test partner (Fig 7). These data are summarised in Table 8.

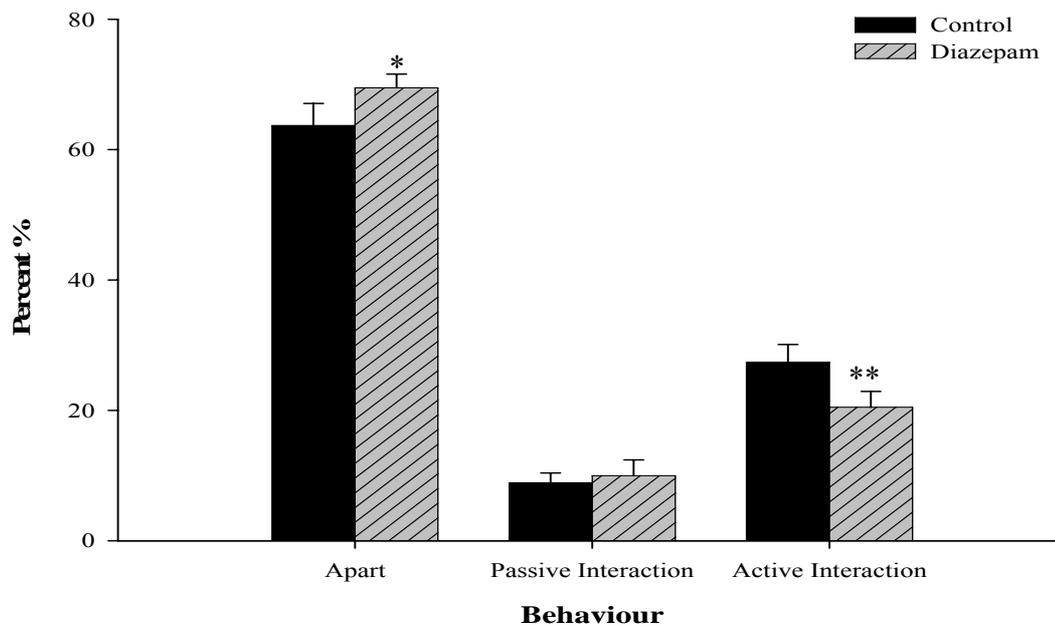


Figure 7. Social interactive behaviours of rats in control and diazepam groups in open-field (OF). Rats were placed in centre of arena and left to interact for 5 minutes. Data are represented as mean percentage of frequency \pm S.E.M. Level of significance - $p<0.1$, $*p<0.01$.

Kruskal-Wallis tests were conducted to determine whether there were any significant differences in the behavioural measures between the combined/pooled Control group and the Essential Oil groups. Where an effect was found ($p<0.1$), two-tailed Mann-Whitney U-tests were used for pair-wise comparisons.

Firstly, a series of Kruskal-Wallis tests were conducted to investigate the behavioural effects of bergamot and the combination of bergamot and geranium compared to the control rats. The Kruskal-Wallis tests revealed significant differences among these groups for; contact latency [$W(2, N=18)=6.28$, $p<0.05$], the number of separations [$W(2, N=18)=4.28$, $p<0.1$], sniffs [$W(2, N=18)=8.95$, $p<0.05$], follows [$W(2, N=18)=3.94$, $p<0.1$], and time spent apart [$W(2, N=18)=8.87$, $p<0.5$] and sniffing duration [$W(2, N=18)=9.41$, $p<0.01$].

Secondly, a series of Kruskal-Wallis tests were conducted to investigate the behavioural effects of geranium and a combination of bergamot and geranium

compared to control rats. The Kruskal-Wallis tests revealed significant differences among these groups for; contact latency [$W(2, N=18)=4.96, p<0.1$], the number of sniffs [$W(2, N=18)=5.19, p<0.1$], and the time spent apart [$W(2, N=18)=9.55, p<0.01$] and sniffing duration [$W(2, N=18)=9.10, p<0.05$].

Further analysis with Mann-Whitney U-tests revealed, bergamot group experienced a significantly shorter contact latency [$U=-2.05, p<0.05$], made marginally more sniffs [$U=-1.70, p<0.1$] and follows [$U=-1.54, p<0.1$]; and spent significantly less time sniffing [$U=-2.34, p<0.05$] and apart [$U=-2.05, p<0.05$] from test partner compared to the control group. The geranium group, spent significantly less time apart [$U=-2.34, p<0.05$] and sniffing [$U=-2.20, p<0.05$] test partner than the control group. Further more, Mann-Whitney U-tests revealed, the combined group experienced a significantly shorter contact latency [$U=-2.05, p<0.05$], made significantly more separations [$U=-2.21, p<0.05$] and sniffs [$U=-2.69, p<0.01$], and followed partner marginally more often [$U=-1.32, p<0.1$] than the control group. The combined group also spent significantly less time apart [$U=-2.64, p<0.01$] and sniffed partner for significantly longer [$U=-2.64, p<0.01$] than the control group.

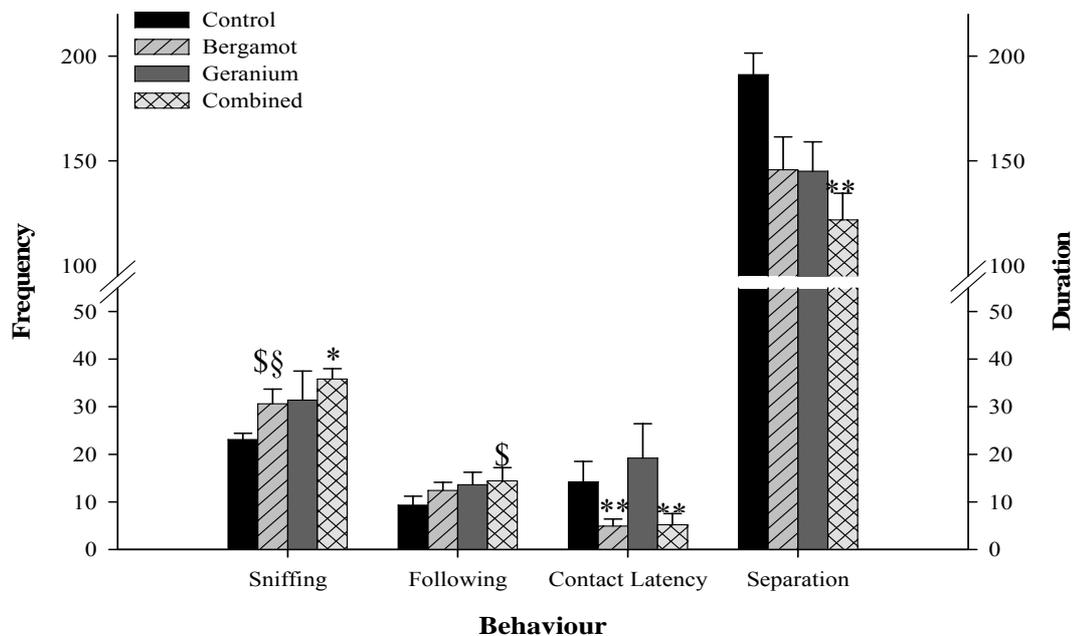


Figure 8. Frequency of social interaction of rats in OF for control & essential oil groups over a 5 minute test session. Data are represented as mean \pm S.E.M. Level of significance- \$ $p<0.1$, ** $p<0.05$, * $p<0.01$.

Table 8.
Social interactive behaviour of test groups in open-field

Behaviour	Pooled Control	Diazepam (2mg/kg)	Bergamot	Geranium	Combined
Contact latency	(7.9)9.9(16.2)	(6.5)8.5(81.6)	(2.9)3.6**(7.5)	(5.8)13.8£(35.4)	(2.1)2.5**(9.6)
Separation frequency	(16.0)17.0(19.5)	(7.5)8.0*(12.5)	(16.0)23.0°°(25.5)	(11.5)19.0°°(28.0)	(20.0)21.0**°(25.0)
Passive interaction frequency	(7.5)13.0(14.8)	(4.0)7.0**(10.5)	(8.0)12.0£(24.5)	(11.0)15.0°°(15.0)	(14.0)15.0°°°(24.5)
Sniffing frequency	(21.0)21.5(27.0)	(5.5)10.0*(14.0)	(24.0)32.0\$§°(36.5)	(19.0)28.0°(45.5)	(32.5)38.0**°(38.0)
Following frequency	(5.5)8.0(10.8)	(0.5)2.0**(5.0)	(9.0)12.0°(16.0)	(8.0)15.0°°(18.5)	(9.0)12.0\$°(21.0)
Crawl frequency	(2.0)2.0(2.0)	(1.0)1.0**(1.5)	(1.0)2.0(3.5)	(0.5)2.0(3.5)	(0.0)2.0(5.0)
Fight frequency	(0.0)0.0(0.0)	(0.0)0.0(0.0)	(0.0)0.0(0.5)	(0.0)0.0(0.0)	(0.0)0.0(1.0)
Separation duration	(175.6)201.6(210.5)	(194.9)211.6(220.7)	(116.7)129.2**(183.1)	(121.7)154.2**(163.6)	(98.5)115.2*(148.3)
Passive interaction duration	(16.0)24.3(33.7)	(15.6)24.8(47.2)	(19.8)29.3(56.3)	(20.6)24.4(59.5)	(28.8)47.4(54.1)
Sniffing duration	(47.6)55.2(65.1)	(37.2)48.8(54.2)	(64.5)75.9**(105.1)	(63.2)86.5**(109.1)	(77.4)94.7*(128.8)
Following duration	(14.4)18.0(26.3)	(1.5)6.5(27.2)	(15.6)27.2(42.3)	(22.8)31.0(36.3)	(16.5)19.5(41.5)
Crawl duration	(1.5)2.7(3.7)	(0.9)1.0(4.2)	(1.8)2.9(4.9)	(0.6)2.0(3.5)	(0.0)3.3(11.6)
Fight duration	(0.0)0.0(0.0)	(0.0)0.0(0.0)	(0.0)0.0(7.6)	(0.0)0.0(0.0)	(0.0)0.0(4.6)
% Separation	(24.0)28.3(29.4)	(26.7)32.4\$(39.6)	(24.1)26.0°(27.6)	(20.9)24.7°(26.9)	(19.2)22.8°(31.1)
% Passive interaction	(12.7)21.7(25.1)	(17.1)25.0(27.6)	(11.4)15.8(25.7)	(13.7)18.2(22.5)	(15.7)20.0(23.0)
% Sniffing	(32.6)37.4(39.1)	(26.3)30.8(37.2)	(35.5)36.8(39.4)	(36.3)38.2(41.2)	(35.7)38.6(41.1)
% Following	(9.4)13.7(16.2)	(1.8)6.5(15.1)	(10.4)18.2(19.3)	(12.8)18.8(19.7)	(11.1)13.4(19.7)
% Crawling	(2.2)3.3(3.6)	(2.6)3.6(7.1)	(1.1)2.2(5.1)	(1.1)3.0(3.5)	(0.0)1.9(5.2)
% Fighting	(0.0)0.0(0.0)	(0.0)0.0(0.0)	(0.0)0.0(0.9)	(0.0)0.0(0.0)	(0.0)0.0(1.1)
% Active interaction	(47.8)52.9(58.9)	(41.0)44.4**(47.3)	(47.4)60.0°°(62.9)	(52.4)59.2°°(62.6)	(51.9)55.6°(61.0)

Data are represented as (25th percentile)*median*(75th percentile). Duration is measured in seconds (s). Sample size - n=5 for diazepam, bergamot, geranium & combined groups, n=8 pooled control. Levels of significance – two tailed Mann-Whitney U-test, \$= p<0.1, **= p<0.05, *= p<0.01 compared control, §= p<0.1 compared to combined group, £= p<0.1, °°= p<0.05, °= p<0.01 compared to diazepam.

Mann-Whitney U-tests between the EO groups revealed, the bergamot group made marginally fewer sniffs [$U=-1.83$, $p<0.1$], and the contact latency between test partners of the geranium group was marginally longer [$U=-1.78$, $p<0.1$] than the combined group. On the whole; bergamot decreased contact latency, increase sniffing and following and decreased separation, geranium increased sniffing and decreased separation, and the combination of bergamot and geranium decreased contact latency and separation, and increased sniffing and following of test partners (Fig 8). These data are summarised in Table 8.

Discussion

The overall aim of the thesis was to evaluate the effects of bergamot and geranium essential oils in three different tests of anxiety. The anxiolytic diazepam was used as a positive control.

Diazepam increased the time spent in and the number of entries to the open arm of the EPM, along with increased risk assessment behaviour. In the OF diazepam produced an increase in immobility time, and a reduction in ambulation and time spent in Zone2. Exploratory behaviour was also decreased by diazepam, with rats making fewer rears and spending less time sniffing the arena. In addition grooming frequency was increased by diazepam. In the SI test, diazepam decreased passive and active interactions between rat pairs. Behaviourally, diazepam caused a decrease in sniffing and following of partner as well as decrease in the frequency of separations in the SI test. The increase exploration and risk assessment in the open arms is indicative of an anxiolytic effect of diazepam on rats in the EPM. The decrease in locomotion and exploration in the OF might be indicative of a sedating effect of diazepam on rat behaviour. However when take in context to the increased grooming behaviour and immobility, the decreases in locomotion and exploration indicates diazepam had an anxiogenic effect. The decreased frequency of separation together with the decrease in active interaction between test partners would suggest diazepam had a sedating effect on rat behaviour. However if one considers the decreased separation frequency is relative to the decreases in passive interaction, diazepam had an anxiogenic effect on social interactive behaviours.

A 24 hour exposure to bergamot odour produced an increase in close arm, centre square and the total number of entries made in the EPM. Following exposure to bergamot, rats showed fewer episodes of immobility, increased the time spent sniffing and rearing, and the time ambulating/exploring the OF. In the SI test, rats exposed to the odour of bergamot for 24 hours produced shorter contact latency, decreased the time spent sniffing partner, but increased the frequency of sniffing and follows between test partners. Furthermore, bergamot decreased the time test partners spent apart. The increase in total entries suggests that bergamot had a stimulating effect in the EPM. The increase in locomotion and

exploratory behaviour suggests bergamot had an anxiolytic effect in OF. In the SI test, the shorter contact latency and increased passive and active interaction between the rats is indicative of the anxiolytic effect of bergamot.

A similar exposure to the odour of geranium produced an increase in close arm, centre square and total entries in the EPM. Geranium produced a decrease in the frequency and duration of immobility, increased the frequency of grooming and increased the time spent rearing in the OF. In the SI test, geranium caused a decrease in the time test partners spent apart and an increase in the time spent sniffing partner. The increase in total entries also indicates the stimulating effect of geranium in the EPM. The increase of grooming frequency could indicate that geranium had an anxiogenic effect in the OF. However, when compared to a decrease in immobility and the increase in time spent rearing, it appears that geranium had an anxiolytic effect in the OF. In the SI test, the decrease in time the rats spent apart from partner and the increase in active interaction, i.e. partner sniffing suggests geranium had an anxiolytic effect.

Exposure to combination of bergamot and geranium produced an increase in centre square and total entries in the EPM. The oil combination increase the number of entries into Zone2 and Zone3 and the time spent in Zone2 in the OF. Furthermore, the combination produced an in decrease in immobility frequency and duration and an increase in the frequency and duration of rears. The combination of bergamot and geranium also produced an increase in the frequency of line crossing and the time spent ambulating around the arena. In the SI test the essential oil combination produced shorter contact latency between test partners. Furthermore, the frequency of sniffing and following of partner was increased by the combination of oils. The duration of time spent following partner was also increased. In addition, test partners separated more often but the time spent apart was decreased by the combination of bergamot and geranium. In the EPM the bergamot and geranium combination had a stimulating effect as demonstrated by an increase in total entries. The increased locomotion and exploration of Zone2 as well as the increase in frequency and duration of rears suggests the combination of EOs had an anxiolytic effect in the OF. In the SI test, shorter contact latency between test partners coupled with an increase in active and passive interaction indicates the anxiolytic effect of the combination of bergamot and geranium.

With regards to diazepam, the results of this study are consistent with other research which indicate diazepam can have an anxiogenic effect on rat behaviour in the OF and SI tests (Prut & Belzung, 2003). What is surprising however is considering the analogous nature of the EPM, OF and SI models; one would have expected diazepam to have had a similar effect in all three tests. A plausible explanation for the differing results could be that the different models of anxiety are tapping into different levels or types of anxiety, i.e. stress, fear or worry. It also suggests that when studying anxiolytics you need to look at their effects in more than one test.

In contrast to the present study, previous studies reported the essential oils, lemon verbena (Vale, Matos, de Lima, & Viana, 1999), woundwort (Rabbani, Sajjadi, & Zarei, 2003) and bitter orange (Carvalho-Freitas & Costa, 2002) demonstrated an anxiolytic effect on mice in the EPM by increasing the amount of entries and time spent in the open arms. These differences are hardly surprising considering different oils and species are being compared. More importantly however, is the fact the present study used inhalation as the mode of essential oil administration, not ingestion (oral) or the intra-peritoneal (injection) mode as was used in these studies. The inhalation of lavender has also demonstrated anxiolytic effect on gerbils in the EPM by increasing risk assessment behaviour (Bradley, Starkey, Brown, & Lea, 2007) but once again species and oil variation could account for this difference. On the other hand, the effects of EOs in the OF and SI in this study are supported by previous research, which report EOs demonstrate anxiolytic properties in the OF (Ambavade, Mhetre, Tate, & Bodhankar, 2006) and SI tests (Leite et al., 2008) by increasing locomotion and exploration, active and passive interaction, respectively.

Like diazepam, there appears to be a conflict between the effect of the EOs observed in the EPM and the effects of the EOs in the OF and SI tests. Research suggest that poor correlations (Tonissaar, Philips, Eller, & Harro, 2004) and inconsistent drug effects (Green, 1991) across different animal models of anxiety and depression indicates that there are other mechanisms at work than just generalised anxiety, i.e. different animal models could be tapping different aspects of anxiety. One explanation for these behavioural inconsistencies could be the difference between trait (a motive or acquired behavioural disposition that predisposes an individual to perceive a wide range of objectively non-dangerous

circumstances as threatening) and state anxiety (characterized by subjective, consciously perceived feelings of apprehension and tension, accompanied by or associated with activation or arousal of the autonomic nervous system) (Spielberg, as cited in Wrisberg, 1994). It has been suggested that lack of test/re-test stability illustrates that the elevated plus-maze is a better measure of state anxiety because novel exploration of the open arms is not stable but reduces with successive testing (Andreatini & Bacellar, 2000). Contrastingly, it has been suggested the open-field test is a better test of trait anxiety because of the lack of consistency between effect of different benzodiazepines (Prut & Belzung, 2003). As discussed earlier, effect variation across different models of animal anxiety is justified if one assumes the different tests are measuring different forms of anxiety. However, an alternative explanation could be that the EOs are operating via a different pathway to diazepam, i.e. not via GABA receptors, because they share similar behavioural profiles to SSRIs or amphetamines in the EPM (Prut & Belzung, 2003). As such it might be more appropriate to assess the anxiolytic effectiveness of EOs in comparison to SSRI or amphetamines rather than benzodiazepines.

A plethora of research has focused on single essential oils or their individual component (Sakamoto, Minoura, Usui, Ishizuka, & Kanba, 2005; Torii, 1997), few have examined the potentiating or additive effects of the combined components on the effectiveness of the individual essential oils which is a common practice amongst aromatherapists (Atanassova-Shopova & Roussinov, 1970; Komori et al., 2006; Lemon, 2004). The present study demonstrates the addition of bergamot had a potentiating effect on the anxiolytic properties of geranium in the OF because it caused an increase in the number of entries into Zone2 and Zone3, and the number of line crossings. Furthermore the addition of bergamot to geranium increased the time spent in Zone2. The combination of EOs also had an additive influence on the anxiolytic properties of bergamot in the SI test by increasing active interactive behaviours, i.e. the sniffing and following of test partners. Similarly, the combination of EOs had potentiating effect on geranium because the contact latency between the test partners was decreased. As such, this study indicates that further research is necessary to determine the importance of the unique composition of EOs on their overall effectiveness, and whether the potentiating effect of the combined oils observed in this study

transcends to different combinations and if there is an optimal number of oils that may be combined at any one time.

In this study the different effects of diazepam and the EOs, in the EPM, OF and SI tests clearly highlight the need for further research to determine whether the animal models of anxiety are indeed testing the same level/type of anxiety. Although diazepam is considered the gold standard for the evaluation of anxiolytic effects in animal models of anxiety and depression, differences between the effect of diazepam and the EOs in this study suggest further research is needed to determine if the anxiolytic profiles of EOs are best compared to SSRIs or amphetamines rather than diazepam. As outlined earlier, lack of conformity in the research methodologies has produced conflicting conclusions as to the anxiolytic and anti-depressant properties of EOs. Considering the normal method of EO administration in humans is via absorption through the skin or inhalation, it would seem that studies utilizing i.p. or oral methods of administration are inappropriate. Furthermore, in some studies animals have been placed in an enclosed chamber or restraint tube where the flow or volume of EO the animals are exposed to is precisely controlled (Shaw, Annett, Doherty, & Leslle, 2007). However, considering the delivery of EO in humans is either through a vaporiser or steam/oil bath this form of delivery should also be considered inappropriate when studying the effect of EO. It is therefore suggested that these points be taken into consideration when conducting further research into the effects of EOs on human or animal behaviour.

Research also indicates the psychological and physiological effectiveness of EOs may be dose dependant (Umezu, 2000). Animal studies have indicated the length of exposure to odourised EOs impacts on effectiveness of the oil to influence behaviour; stimulating essential oils appear more dose dependant than sedating oils (Shaw, Annett, Doherty, & Leslle, 2007). These findings are supported by MRI studies which illustrate that prolonged exposure to an odour results in the habituation of the olfactory receptors and the olfactory cortex (Poellinger et al., 2001). In this study the test animals were exposed to 4 drops of EO every 2 hours over a 24 hour period. Research has examined effects of acute and chronic exposure to EOs (Bradley, Starkey, Brown, & Lea, 2007), however no consensus has been established as to the optimal dose of EO per room size or an optimal exposure time or regime. Further research should also be conducted to

determine the effect of an intermittent period of exposure, i.e. a period of exposure that is broken intermittently by periods of non-exposure, because this schedule of exposure more accurately mirrors the process of exposure in the natural environment.

Test conditions may also alter rat's behaviour in many tests of anxiety. For example, File and Seth (2003) attest that high illumination of a novel arena (open-field) is sufficient to generate high levels of anxiety in rats. Consequently, rats display less social interaction under high illumination conditions (File & Seth, 1978). High illumination is also demonstrated to increase anxiety related behaviours in the elevated plus maze (Griebel, Moreau, Jenck, Martin, & Misslin, 1993; Hale, Bouwknecht, Spiga, Shekhar, & Lowry, 2006). There seems to be no consistency as to whether animals should be tested during the light or dark phase of their circadian cycle. Rats are nocturnal and therefore more active at night, it is possible that the lack of responsiveness to EOs reported in research could be attributed to a natural reduction of activity during the light phase of rats circadian cycle. Further studies could identify whether the time of testing impacts on the behavioural responses of rats exposed to EOs. Many animal models rely on the anxiety generated by high levels of illumination; it would be interesting to see if the same level of lighting generate the same level of anxiety when the animal are tested during the light or dark phase of their circadian cycle.

4.1. Limitations

As pointed out by Calatayud, Belzung and Aubert (2004), inter-individual testing variability could also be an influencing factor to lack of consensus as to the effect of EOs. In this study, prolonged care may have resulted in the rats habituating to handling which could have influenced their behaviour in the tests, i.e. the levels of anxiety observed in the EPM, OF and SI tests. Arakawa (2007) proposes that risk assessment behaviour emerges in juvenile rats (around 40 days), peaks during adolescent stage of development (65 days) and drops off as rats reach maturity (130 days) and remains stable throughout adulthood.

In this study sample size was limited to 10 rats per test group. In the SI test the combined assessment of test partners effectively halved the sample size. Research has demonstrated that larger samples show greater stability and power, smaller standard deviations and provide a more accurate projection about

population trends (MacCallum & Widaman, 1999). Consequently, as pointed out by Moher, Dulberg and Wells (1994), if a trial with negative results has insufficient power; a clinically important but statistically non-significant effect is usually ignored or taken to mean the treatment made of difference. With this in mind a larger sample per group would have provided more power to the study and produce a more robust indication of the anxiolytic effect of EOs in the tests of anxiety.

4.2. Conclusion

Considering the prevalence and debilitating effects anxiety and depression have on ones ability to function, it not surprising that that drugs like diazepam are prescribed because of the swiftness and efficacy with which they operate. Sadly psychotropic drugs are associated with serious negative side effects and the risk of addiction. For this reason, pharmaceuticals like diazepam are only recommended for short term use. With this in mind it would seem appropriate to find a treatment solution for anxiety and depression that can be used more extensively without the associated contraindicative risk to health.

Essential oils have been offered as a plausible alternative to conventional treatment approaches for anxiety and depression; however there is insufficient empirical evidence to support their efficacy. Unfortunately, most of the essential oil studies have relied on animal research to investigate the effects EOs may have on human behaviour, which is hardly surprising considering the hedonic valence associated with aromas. However, there is little consensus about the appropriate testing methods and which of the myriad of psychotropic drug associated with the treatment of anxiety or depression are more appropriate as a positive control. Furthermore, many of the more familiar essential oils have been investigated however they are not necessarily the most appropriate oils for the treatment of human depression and or anxiety. In addition, research has focused on the effects of single oils rather than the combination of several oils which is generally the practice in aromatherapy.

The present study established that bergamot and geranium, essential oils more commonly used for the treatment of anxiety and depression in aromatherapy had a positive anxiolytic effect on rat behaviour when inhaled. Furthermore the

study also demonstrated that the combining of the oils had a potentiating effect on the anxiolytic properties of the single oils.

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