COMPARISON OF THE ANXIOLYTIC EFFECTS OF DIAZEPAM AND KAVA ON BEHAVIOUR IN RATS

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Abstract

Due to the scarcity of research on kava in animal populations, the current study aimed to cover weaknesses in previous investigations in this area by comparing the anxiolytic effects of chronic kava and diazepam treatment on male and female rats. 90 mg/kg and 180 mg/kg of kava, and 5 mg/kg and 10 mg/kg of diazepam were administered through food daily to 40 male and 40 female PVG/c rats over a period of four weeks. During treatment the rats were tested on three animal models of anxiety; the open field, light dark box, and elevated plus maze. Few significant dose effects occurred for either kava or diazepam. In the elevated plus maze both the 90 mg/kg and 180 mg/kg kava dose groups spent more time in the open arms compared to the control group, suggesting that both doses had an anxiolytic effect, but this was the only dose effect found for kava. A sex X dose interaction for diazepam occurred for males in the open field, as males in the 10 mg/kg dose group spent more time in the corner squares than the 5 mg/kg dose group. Another interaction effect occurred for female rats in the open field, as female controls walked more often than those in the 10 mg/kg dose group. In the light dark box female rats in the 5 mg/kg dose group and the control group entered the light side more times than those in the 10 mg/kg dose group, and the control and 5 mg/kg dose group entered the light side more often than those in the 10 mg/kg overall. These results did not allow meaningful comparisons between kava and diazepam to be made, but suggested that a chronic 10 mg/kg/day dose of diazepam may not be as effective as a lower dose. Future research comparing the two substances is recommended.

Introduction

Anxiety disorders are some of the most commonly diagnosed mental illnesses, and include generalized anxiety disorder (GAD); panic attacks, agoraphobia, social phobia, specific phobias, and post-traumatic stress disorder (PTSD). In America the 12 month prevalence of anxiety disorders is estimated at 22.2%, and lifetime prevalence is estimated at 31.6% (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). Their study found social and specific phobias were the most common at 13-13.8% lifetime prevalence, and that GAD had a lifetime prevalence of 6.2%. With such a wide prevalence of anxiety related disorders, many treatment avenues have been explored. Benzodiazepines are the currently preferred class of anxiolytic prescribed, but antidepressants such as selective serotonin reuptake inhibitors (SSRIs), and tricyclic antidepressants (TCAs) are also frequently used, as depression and anxiety related disorders often occur together (Kessler et al., 2012; World Health Organisation [WHO], 2016).

Current anxiety treatments

Benzodiazepines have a sedative effect which occurs through their action on the GABA-A receptors in the brain. Some benzodiazepines commonly prescribed to treat anxiety disorders include diazepam, lorazepam, and alprazolam. SSRIs work on the serotonin receptors in the brain to elevate mood, while TCAs work on serotonin receptors as well as norepinephrine receptors. Commonly prescribed SSRIs for both anxiety and depression are fluoxetine, paroxetine, and citalopram; well-known TCAs are imipramine, amitriptyline, and nortriptyline. An issue with TCAs is that they can affect other areas of the brain such as the cholinergic, histaminic, and adrenergic receptor sites, unlike SSRIs which affect only the serotonin receptors (Ferguson 2001). Side effects from TCAs can include dizziness, dry mouth, and tachycardia, and although SSRIs can cause similar side effects as well as sexual dysfunction and sleep disturbances, these are less severe compared to the side effects of TCAs (Anderson, 1998, Ferguson, 2001). Benzodiazepines can have side effects such as memory

impairment, drowsiness, ataxia, or increased aggression or hostility; and can also be toxic when used in combination with alcohol or other drugs such as SSRIs (Longo and Johnson, 2000). The benefits of benzodiazepines appear much earlier compared to antidepressants, which means that their "greatest asset is also their greatest liability" (Longo & Johnson, 2000), as they can quickly become habit forming. Physical dependence on these drugs can cause unpleasant and sometimes severe withdrawal effects once the drug treatment has stopped. Such withdrawal effects can include tremors, insomnia, increased anxiety, and in some cases even seizures and psychosis, with such effects being found in both animals and humans (Chouinard, 2004; File & Andrews, 1991; Mellor & Jain, 1982). A meta-analysis of studies comparing SSRIs and TCAs to benzodiazepines for the treatment of anxiety found that benzodiazepines were more effective for the treatment of GAD and panic disorder, and that their side effects were more tolerable, as fewer people on benzodiazepines ceased treatment due to side effects compared to TCAs (Offidani, Guidi, Tomba, & Fava, 2013). This led the authors to question the increase of antidepressants being prescribed for anxiety disorders, and suggested that benzodiazepines should remain the frontline treatment for anxiety, with the caveat their "role and usefulness … need to be reappraised", considering they still have some serious side effects and dependency issues (Offidani et al., 2013). It is therefore apparent that both benzodiazepines and antidepressants such as SSRIs and TCAs can be effective for anxiety treatment, but have some major drawbacks. While benzodiazepines may currently be the preferred treatment option for anxiety, it would be beneficial to explore alternatives with fewer, less severe side effects, that are also more readily available for people who cannot access a doctor for diagnosis or treatment. Consequently, the current study intends to compare the commonly prescribed benzodiazepine diazepam with an alternative to prescription drugs, the kava plant. Natural, plant-based treatments for anxiety are gaining popularity, with more and more studies exploring their efficacy and mechanisms of action on the brain.

Natural alternatives to prescription drugs

Natural alternatives to benzodiazepines such as lemon balm, kava, chamomile, and St John's wort, are derived from plants and have demonstrated anxiolytic effects in both humans and animals, and have also been used as traditional medicines for a range of illnesses for centuries (Lakhan & Vieira, 2010; Sarris, McIntyre & Camfield 2013). In a 2012 survey of 235 college students in America, 36.3% responded that they used some kind of natural product to treat anxiety because it was easy to obtain, and 23.1% responded that the cheaper cost compared to prescription drugs was a reason for their use (Birkett, 2012). Many of these natural alternatives are available in some form at local pharmacies, or are easily obtainable from online websites, making them more accessible than prescription drugs.

In a review of plant-based medicines for treating anxiety, Sarris et al. (2013) stated that kava was the only plant "sufficiently studied" for anxiety, compared to other plants such as chamomile, lemon balm, and St John's wort. Some issues with studies conducted on these other plant-based treatments for anxiety are that they lack replicated results over several studies, and that sample sizes have been very small (Cases, Ibarra, Feuillere, Roller, & Sukkar, 2011; Sarris et al., 2013). Kava was therefore chosen for the current study to be compared to diazepam, as they are both reasonably well studied and well known. By comparing the two anxiolytic substances, more evidence supporting the benefits of natural alternative may emerge and therefore encourage people with anxiety disorders to consider their use.

Overview of kava

Kava is a natural anxiolytic that has demonstrated benefits in both humans and animals (Sarris et al, 2009; Sarris, LaPorte, & Schweitzer, 2011). The name "kava" refers to both the plant native to western Pacific islands as well as the traditional and ceremonial drink made from finely ground powder of kava mixed with water. In Pacific nations such as Fiji, kava is more frequently used for recreational purposes, rather than as an anxiolytic as it produces a "tranquil state of intoxication"

(Norton & Ruze, 1994) when consumed in its traditional form, directly sourced from plants. In Western countries kava is readily available in many forms, either from pharmacies or over the internet. Ground kava root can be purchased online from producers in countries such as Fiji and Vanuatu, while capsules and other supplements with ground kava root can be found in many pharmacies throughout New Zealand (Tomlinson, 2007). The kava herbal supplements found in pharmacies and online retailers are generally of a lower dose than the drink prepared by Pacific Island peoples, possibly reflecting its use as an anxiolytic rather than a recreational or ceremonial substance. The kava sold for treatment of anxiety generally produces a sedating and calming effect, without the intoxicating effects found in the product used for recreational consumption (Tomlinson, 2007; Sarris et al, 2011). Studies on the anxiolytic effects of kava have been conducted on both human and animal samples, and unlike several other natural remedies, kava's potential mechanisms of action have also been found and explored (Smith et al, 2001; Sarris et al., 2009).

Kavalactones are understood to be the active elements in kava, and the six main kavalactones that have been discovered are yangonin, desmethoxyyangonin, kavain, diyhdrokavain, methysticin, and dihydromethysticin. It is still not fully understood where in the brain the kavalactones exert their influence, although suggestions include effects on the GABA-A receptor, sodium and calcium ion channels, and norepinephrine and dopamine receptors (Lakhan & Vieira, 2010; Sarris et al., 2011). It has been suggested that the concentrations of these six kavalactones can vary between kava plants and strains and can therefore result in different effects after consumption. For example, in the Sarris et al. (2009) study discussed below, it was suggested that the nausea experienced by one participant after ingesting kava may have been due to high levels of the kavalactone dihydromethysticin. The kavalactones are concentrated in the roots and root stems of the kava plant which is the reason these parts of the plant are used when preparing kava to drink.

Human studies

One of the most important studies on kava in humans to date was the Kava Anxiety Depression Spectrum Study (or KADSS) conducted by Sarris et al. (2009). Their study was interested in observing the effects of kava administered five times daily over a three week period on 37 adults who were screened for an anxiety or depressive disorder. The authors used an aqueous extract of kava that was pressed, dried and made into a tablet form as well as a placebo tablet identical in appearance to the kava tablet. The kavalactone concentration for each tablet was 50 mg/kg, meaning participants obtained 250 mg/kg per day after consuming all five tablets. Half the participants received the kava dose for 1 week then switched to placebo, while the other half received placebo for one week then switched to kava. The study found that scores on the Hamilton Anxiety Scale (HAMA) and Beck Anxiety Scale (BAI) were reduced in the kava group compared to the placebo group, with reduced scores on these scales indicating reduced anxiety (Hamilton, 1959; Beck, Epstein, Brown, & Steer, 1988). This result was taken from a weighted mean of the anxiety responses from both the kava-placebo and placebo-kava groups. Furthermore, scores on the Montgomery-Asberg Depression Rating Scale (MADRS) were also reduced compared to the placebo group, with reduced scores indicating reduced depressive symptoms (Montgomery & Asberg, 1979). The authors therefore concluded that an aqueous extract of kava was useful in participants with generalised anxiety, and may be useful for those with depressive disorders. The authors stated that the kava dose used and duration of the study did not cause any significant negative side effects, although there was one participant withdrawal due to mild nausea after taking the kava for one day and the issue disappeared completely after one day of receiving no kava. One participant also complained of a stomach ache the first day of taking kava which ceased after the second day. Furthermore, the authors noted no rebound or withdrawal symptoms once the kava-placebo group stopped their kava dose and switched to placebo, which they contrasted with the effects that can occur when benzodiazepine treatment is suddenly stopped. As this study highlighted, some side

effects of kava such as nausea and stomach ache can occur but they are less frequent and much less severe than the side effects that occur with diazepam, as was found in a review of multiple studies on kava conducted by Sarris et al. (2011). A unique and relatively rare side effect of prolonged kava use in humans is that it can cause skin to become dry and scaly, although this effect appears to be limited to people in countries such as Fiji who consume kava for more recreational purposes, and in larger doses (Norton & Ruze, 1994; Sarris et al., 2011). While side effects do not appear to be a problem with kava use, a significant issue with kava is that there have been concerns of potential hepatotoxicity in humans, particularly in Western countries.

In 2002, the American Food and Drug Administration issued a warning about the potential hepatotoxic effects of kava, leading to Canada, England, and some European countries banning kava supplements (FDA Consumer Advisory, 2002). The Sarris et al. (2011) review of kava stated that at the time of publication over 100 cases of hepatotoxicity that were potentially linked to kava had been reported. The authors proposed that this hepatotoxicity could be related to a deficiency in the enzyme CYP2D6 in Caucasian people which is implicated in the ability to metabolize drugs. Interestingly, cases of hepatotoxicity related to kava have not been reported in Pacific Island countries, and Sarris et al. (2011) also cited a study that showed Pacific-Asian populations do not appear to suffer from the CYP2D6 deficiency, meaning it could be a problem limited only to Caucasian populations. Other explanations for potential hepatotoxic effects related to kava were discussed in meta-analyses by Singh (2005), Lakhan and Vieira (2010), and Sarris et al. (2013) who all suggested that any liver damage related to kava consumption was most likely from poor quality product, from using the toxic aerial parts of the plant, or from the use of solvents in the extraction process. An aqueous extract of kava such as that used in the KADSS study appears to be the best option for kava extraction for use in treating anxiety (Sarris et al., 2009; Sarris et al., 2013). The authors of the aforementioned studies also suggested that people already experiencing liver damage or difficulties should avoid kava in case they receive a poor quality version which may exacerbate their condition. The issues of

hepatotoxicity in kava therefore seem to be strongly related to its quality, which is something that can be ensured through proper regulation of the product.

Quality of kava is important in determining its anxiolytic effects because the aerial parts of the kava plant can contain toxic substances and should not be consumed, meaning that people buying kava should take care to ensure it has been sourced from the safe parts of the plant. This can be difficult as there is little regulation for kava and other plant-based medicines, particularly ones shipped to Western countries from Pacific nations (Davis, 1999). The risk of purchasing toxic or impure kava can be mitigated by buying from reputable sellers and learning about which parts of the plants the kava should be sourced from. A final health concern for kava is that like diazepam, it can have a toxic effect when used in combination with alcohol or other drugs, which can lead to hospitalisation (Davis, 1999; Tomlinson, 2007).

Because kava has been used by Pacific peoples for so long with little ill effect, animal studies observing its properties have been relatively scarce and many more studies have been conducted on human populations, meaning that there is an untapped area of kava study in animal populations. Furthermore, most of the animal studies were conducted over 10 years ago, when information on kava's effects and properties were not as well established. The current research is therefore interested in filling the gaps left from previous animal studies with the aim to promote kava as a suitable alternative to benzodiazepines, and to encourage further research in the area of comparing kava and diazepam.

Animal studies

The two significant animal studies demonstrating kava's anxiolytic effect on rodents were conducted by Rex, Morgenstern, and Fink in 2002, and Garrett, Basmadjian, Khan, Schaneberg, and Seale in 2003. Both studies compared acute kava with acute diazepam by using one or more behavioural tests of anxiety on either rats or mice.

In the Rex et al. (2002) study, 50 male rats were used and there were three dose groups for kava, one for diazepam, and one control group. The authors stated that only one dose of 15 mg/kg of diazepam was used in their study because a preliminary dose-finding study did not find an anxiolytic effect for doses lower than 15 mg/kg, and that doses higher than 20 mg/kg produced only a sedating, not anxiolytic effect. The kava used was an ethanol extract of kava root, and was administered orally to the rats in doses of 120, 180, and 240 mg/kg of kavalactones (called kavapyrones in the study). The rats were then tested on the elevated plus maze, a regularly used assay of anxiety related behaviour in animals. The three doses of kava in the study generally produced an inverted U-curve, with the middle dose of kava having the highest anxiolytic effect based on increased entries into, and time spent in the open arms of the plus maze. The lowest and highest doses did not have as great an effect as the middle dose, but they still were generally at least twice as high as the control group. The authors concluded that the kava used in their study acutely produced "anxiolytic behaviour comparable with the effects of diazepam", as the 180 mg/kg dose of kava had almost the same effect on rat behaviour in the elevated plus maze as the 15 mg/kg dose of diazepam.

In the Garrett et al. (2003) study, kava and diazepam were compared in acute doses using male mice in the elevated plus maze and the mirrored chamber avoidance assay. Kava root was ground into powder then formed into oil, and finally evaporated using ether into a resin, which along with the diazepam was administered intraperitoneally. Several doses of kava, ranging from 32 – 316 mg/kg kavalactones, and several doses of diazepam that ranged from 0.0316 – 5 mg/kg were used, although the authors did not state how many mice, or how many specific dose groups they used. They found kava had an anxiolytic effect compared to the control in both animal models, but not to the same extent as diazepam. In the mirrored chamber avoidance assay, an anxiolytic effect was demonstrated through an increased time spent in, and decreased latency to enter the mirrored chamber. Kava had the greatest anxiolytic effect in this behavioural model at a dose of 178 mg/kg, with responses returning to control level at the 316 mg/kg dose. In the elevated plus maze the optimal dose was

found at 133 mg/kg, with responses returning to control level at 215 mg/kg. This resembled the results found in Rex et al. (2002) as higher doses of kava in that study did not produce a stronger anxiolytic reaction. Unlike the Rex et al. (2002) study however, kava was not considered comparable to diazepam for reducing anxiety. Another difference between the two studies was that Garrett et al. (2003) explored the mechanisms of action of kava, and used high-performance liquid chromatography (HPLC) analysis to determine the distribution of kavalactones. This meant that in the results the authors were able to suggest which levels of certain kavalactones had the greatest effects. The highest individual kavalactone concentrations for the optimal doses of both the mirrored chamber avoidance assay and elevated plus maze were diyhdrokavain and kavain, although concentrations of these kavalactones were higher in the mirrored chamber avoidance assay dose. This may reflect the fact that the optimal kava dose for the mirrored chamber avoidance assay was 178 mg/kg, and the optimal dose for the elevated plus was 133 mg/kg, so individual kavalactone concentrations may differ as dose increases.

Both studies comparing kava and diazepam on rodents found an acute anxiolytic effect of kava, although only Rex et al. (2002) concluded that kava may be of a similar potency to diazepam. These studies form a good basis for further animal research comparing diazepam and kava, as there are a number of improvements that can be made.

The current study

The current study was intended to compare chronic treatment with kava and diazepam in both male and female rats, using three animal models of anxiety.

The anxiolytic effects of kava and diazepam were determined through the use of three animal models of anxiety; the open field, light-dark box, and elevated plus maze. All three models are considered to show face validity, meaning that the anxiety responses demonstrated by rodents correspond to anxiety behaviour in humans. Construct validity has also been found for all three tests, meaning that the tests actually explore anxious behaviours. Using three different animal models of anxiety is

important, as Garrett et al. (2003) used the elevated plus maze and mirrored chamber avoidance assay, and Rex et al. (2002) used only the elevated plus maze to test for anxiety. It will be possible to see if results generalise across behavioural tests if more are used, which is why the open field and light dark box were chosen along with the elevated plus maze.

The open field test is a square shaped platform with raised walls on all four sides that has markings on the floor dividing it into evenly shaped squares. After an anxiolytic substance has been administered, there are various behaviours in the open field considered to demonstrate reduced anxiety. These include time spent in the middle squares of the field, because rodents naturally prefer to be close to the edge or corner as it offers more protection; walking around which can demonstrate an interest in their surroundings; and rearing on their hind legs which again suggest the rat is not afraid of its surroundings and is motivated to explore (Walsh & Cummins, 1976; Carola, D'Olimpio, Brunamonti, Mangia, & Renzi, 2002).

The light-dark box comprises two compartments, a covered dark side, and a larger, open side with an open top. Reduced animal anxiety in this apparatus is shown when more entries from the dark side to the light side are made, as rodents naturally prefer to be in a darkened, enclosed environment (Bourin & Hascoet, 2003). Anxiety is also considered to be reduced the faster the animal first emerges from the dark side to the light.

The elevated plus maze (or just plus maze) is a raised cross shaped apparatus with two opposing arms having dark, high raised edges, and the other two opposing arms having clear see-through edges. Similar to the light-dark box and open field, this tests a rodent's natural aversion to light and open spaces. Therefore an anxiolytic effect on behaviour would be shown through increased entries into the open arms of the plus maze, more time spent there, as well as more walking and rearing (Pellow, Chopin, File, &Briley, 1985; Walf & Frye, 2007). Across all three models of anxiety, decreases in grooming, immobility and defecation are also considered to show reduced anxiety (Moody, Merali, & Crawley, 1988; Pellow et al., 1985).

The use of both male and female rats is important as behaviour can differ between the two sexes when exposed to different drugs or different stressful stimuli, and sex differences exist in anxiety symptoms and prevalence between male and female humans (Palanza, 2001). In an assessment of the use of male and female rodents in animal studies, Hughes (2007) found that over 80% of studies used only male rodents. The survey also found that while the number of studies featuring both sexes was not as common, over half of those studies reported drug effects that were sex dependent. Furthermore, Johnston and File (1991) found female rats made more open arm entries on the elevated plus maze than male rats without receiving any drug treatment, indicating that they may be more naturally inclined to explore, and be less fearful than male rats. Another study on sex differences between male and female rats found that female rat behaviour in the elevated plus maze appeared to be driven by activity, while male rat behaviour was driven by anxiety (Fernandes, Gonzalez, Wilson, & File, 1999). This indicates that results for male-only rat studies may not be generalizable to female rats, as their behaviours may be influenced by different factors. It is therefore clear that there are differences between female and male rats both in behaviour and their responses to drugs, which provides support for the use of both sexes in the current study.

There were also two dose groups for kava and diazepam each, and a control group, so as to determine if there is an optimal dose for reducing anxiety. The kava and diazepam was administered to the selected rats through their food, and not through injection or drinking water as was the case in the Rex et al. (2002) and Garrett et al. (2003) studies. This is because doses are easier to measure into food than water and because the process of handling and injecting rats or mice can be a source of stress and consequently increases their anxiety. An example of this was demonstrated by Lapin (1995), who found that mice injected intraperitoneally with a saline solution and then placed in an elevated plus maze spent more time in the closed arms and had fewer entries into both open and closed arms compared to mice that were not injected. This indicated the process of injecting the mice was a source of stress and caused an anxiogenic effect. This therefore provides a precedent for using

food to administer the drug doses, so as to avoid the stress of rats repeatedly being picked up and injected. Once data on all rats had been gathered, it was used to explore if and how the different doses of kava and diazepam had impacted rat behaviour in the three animal models of anxiety. Because kava has fewer negative side effects than diazepam, no withdrawal effects, and can be purchased over-the-counter, it is already plausible to suggest that, in some cases, it may be a preferable option for treating anxiety compared to diazepam or other benzodiazepines. Therefore, in the current study, results were expected to further support this suggestion, and thus add to the knowledge base of kava studies in animals through the use of different approaches from previous animal research.

Methods

Subjects

One hundred PVG/c rats, 50 male and 50 female, were used for the study. This ensured enough rats of each sex were available for each test condition (control, kava or diazepam) to obtain sufficient power for the determination of statistically significant interactions between all conditions, including sex. The rats were housed with the same sex three or four to a cage, with free access to water.

Kava and diazepam treatment

The rats were changed from their usual pellet diet to a mash diet a week before kava and diazepam were added to the food, to familiarise the rats with the change, and to determine how much was being eaten so appropriate amounts of mash were given during testing. The mash was made by grinding the usual rat pellets into powder form and mixing it with water, which was then placed into small plastic or ceramic bowls and placed into each cage. The rats in each cage had free access to the bowls of mash, and more mash was given to the cages housing four rats, so that each rat had enough to eat. The diazepam was in the form of 2mg tablets which were then ground to a fine powder using a mortar and pestle. The kava was in the form of capsules containing 90mg of kavalactones per capsule as specified on the container they were purchased in, which were simply opened and added to the food as required. It was observed that an average 151 gram male PVG/c rat ate around 7.7 grams of dry food a day, while an average female ate around 5.5 grams, therefore it was determined that each dose of either kava or diazepam would be added to 1 kg of dry food and then mixed with water to form the mash. Each 1 kg batch of mash was then distributed as evenly as possible between the small bowls used in each cage (around 40 bowls for each dose). As the kava capsules contained 90 mg kavalactones, 10 capsules were added to the dry food for the 90 mg/kg dose group, and 20 capsules were added to the dry food for the 180 mg/kg dose group. For the 2 mg diazepam tablets, 50 were added to the dry food for the 5mg/kg dose group, while 100 tablets were added to the dry food

for the 10 mg/kg dose group. It was not possible to determine the exact quantities of kavalactones in the kava used for the study due to the lack of access to methods such as HPLC analysis used in the Garrett et al. (2003) study. Rats were given the required dosage of drug mixed in with mash each day at approximately the same time, and this was given to them after behavioural testing had finished for the day.

For 20 rats (10 male, 10 female), kava was added to their daily quantity of food to enable a daily intake of 90 mg/kg of kavalactones, and for the other 20 rats in the kava group (10 male, 10 female) kava extract was added to their daily food to enable a daily intake of 180 mg/kg of kavalactones. These doses were chosen based on the Rex et al. (2002) that suggested doses in this range were anxiolytic for males.

Another 20 rats (10 male, 10 female) received diazepam daily in the same method described above, to enable a daily intake of 5 mg/kg. The remaining 20 rats in the diazepam group (10 male, 10 female), received daily doses of diazepam through their food to achieve a daily intake of 10 mg/kg. These doses have been shown to be anxiolytic in male rats (Wada and Fukuda, 1991).

The 20 rats (10 male, 10 female) in the control group did not receive any kava or diazepam in their daily food quantities, but apart from this were treated the same as the kava and diazepam groups.

The number of rats used and all procedures outlined above were approved by the University of Canterbury Animal Ethics Committee on 15 July 2015.

Behavioural testing procedure

After 7-9 days of kava, diazepam, or no treatment, the rats were randomly split into several cohorts and had their first behavioural test in the open field which took six days to complete for all dose groups. During the testing period both test groups and dose groups continued to receive the aforementioned daily doses of either kava or diazepam. Three days after the open field test was completed by all rats, they then underwent the second behavioural test, the light-dark box test. The

rats completed the second test in the same order as the open field test, whilst also continuing to receive their daily doses of kava or diazepam, and this took five days to complete. Four days after all rats were tested in the light-dark box they underwent the final behavioural test, the elevated plus maze, in the same order as the previous two tests were completed. After each rat was tested the apparatus used was wiped using a 70% ethanol solution and then dried. The observer scoring all three behavioural models was not blind to the dosage groups, as feeding and scoring was done by the same person.

Open field test

Each rat was placed in the centre of the wooden 60 x 60 x 25 cm open field, which had a black floor with white lines painted over it to divide the floor into 16 squares. The apparatus was situated on top of a table 70 cm high, and was illuminated from above with dim fluorescent lighting. For 5 min, by means of a momentary time-sampling procedure, every 3 s (signaled by a faint tone delivered to the observer through an ear piece) it was noted (for a total of 100 observations) which square of the apparatus the rat was occupying (all of the body excluding the tail). It was also recorded which of the following responses it was engaged in: standing up on its hind legs (rearing); grooming its body (grooming); or remaining completely inactive (immobile). All observations were made via CCTV with the camera positioned above the apparatus, which was connected to a television screen situated roughly 2 metres away, allowing the observer to watch the rat's behaviour while staying out of sight. The number of fecal boluses left in the apparatus was also counted. The location measures enabled subsequent determination of the rats' occupancy of the four corners (corner occupancy) and centre four squares (centre occupancy) of the apparatus. This location data also enabled the distance travelled (ambulation) to be estimated by counting the number of times a rat was seen occupying a different square from that occupied at the onset of the immediately preceding 3-s signal.

Light-dark box test

Each rat was placed in the dark 30 x 20 x30 compartment of the wooden box for 30 seconds, with a removable slide covering the 10 x 10 opening in the centre and separating the two compartments. The light side was covered by a hinged clear Perspex lid, while the dark side was covered by a hinged wooden lid that did not allow light through. The box was placed on a 70 cm high table, with dim fluorescent lighting illuminating it from above. The slide was then withdrawn, and the rat allowed free access to both compartments for 5 minutes. Latency to enter the light side after the slide was withdrawn was measured using a stopwatch. Every 3 seconds it was noted which side the rat was in as well as the total number of entries of the light side, and fecal boluses were counted after the test finished. All observations were made via CCTV with the camera positioned above the apparatus and connected to a screen in the same set up as the open field.

Elevated plus maze

Each rat was placed on the 15 x 15 cm central platform of the elevated plus maze, which was raised 1 metre above the ground, facing one of the enclosed arms. The arms of the plus maze measured 50 x 10 x25 cm each, and were connected to the central platform. The two opposing closed arms had side and end walls of black painted wood, while the open arm sides and ends were made from clear Perspex. The high clear walls were intended to prevent rats from falling or jumping from the open arms, and do not appear to make the open arms of the plus maze any less aversive for the rats (Martinez, Cardenas, Lamprea, & Morato, 2002). For 5 minutes, the number of entries (involving all four feet) into the enclosed and open arms was counted. In addition, the same time-sampling procedure used in open field and light-dark box testing was used to record which arm the rat was occupying every 3 s. All observations were made via CCTV with the camera positioned above the apparatus and connected to a screen in the same way as the previous two behavioural tests. It was

subsequently possible to calculate the rat's number of entries into each arm, percentage of open-arm entries, and percentage of times it was observed occupying the open arms. It was also recorded every 3 sec if the rat was rearing or walking, and in which arm, and the number of fecal boluses left in the maze was also tallied.

Statistical analyses

Behavioural responses across all three tests were subjected to separate 2-way (sex X dose) ANOVAs, and Fisher PLSD post hoc ($p < 0.05$) tests were performed for significant main effects and interactions.

Results

Overall, relatively few statistically significant dose effects of either diazepam or kava occurred. The results are presented in tables showing the means and standard error of the means, with significant effects indicated by asterisks, and significant interactions displayed in graphs.

Open Field

Diazepam

For the diazepam group in the open field test, a significant effect was found for the sex x dose interaction on the frequency of corner square occupation [$F(2,54) = 5.27$, $P<0.01$]. Figure 1 shows the significant difference between male rats in the 5 mg/kg dose group and male rats in the 10 mg/kg group, as the male 10 mg/kg dose group were observed occupying the corner squares more frequently than the male 5 mg/kg dose group. This meant that the dose level for corner occupancy was only significant when sex was included. Walking in the open field was also significantly affected by the sex x dose interaction [$F(2,54) = 3.09$, $P<0.05$]. Figure 2 shows there was a significant difference between female control rats and female rats in the 10mg/kg dose group, with the female control group observed walking more frequently than the female 10 mg/kg dose group. Therefore the dose level for walking in the open field was significant only for females. For faecal boluses in the open field, a significant effect was found for sex (Table 1), with male rats eliminating significantly more faecal boluses than female rats. No significant drug or sex effects occurred for ambulation, centre square occupancy, rearing, grooming, or immobility in the open field for the diazepam group (Table 1).

Table 1: Mean (± SEM) values of each measure recorded in the open field for each dose of diazepam, and each sex, and results of ANOVAs.

Response	Diazepam				Sex		
	Control	5 mg/kg	10 mg/kg	F(2,54)	Males	Females	F(1,54)
Open Field:							
Ambulation	54.70 (2.18)	54.10 (2.01)	53.50 (1.80)	0.09	52.00 (1.63)	56.20 (1.52)	3.38
Centre Occupancy	7.00 (0.52)	8.50 (1.11)	8.40 (0.80)	1.01	8.67 (0.80)	7.27 (0.54)	1.01
Corner Occupancy[a]	53.60 (2.69)	54.20 (2.10)	53.60 (1.98)	0.03	52.37 (1.61)	55.23 (2.01)	1.38
Walking[a]	40.05 (2.35)	41.05 (1.70)	36.45 (0.79)	2.15	37.57 (1.08)	40.80 (1.70)	2.99
Rearing	38.25 (2.23)	36.55 (1.94)	40.80 (1.56)	1.30	40.43 (1.65)	36.63 (1.46)	3.08
Grooming	4.65 (1.17)	4.00 (0.81)	3.05 (0.80)	2.71	3.00 (0.53)	4.80 (0.93)	2.71
Immobile	17.00 (2.44)	18.45 (1.80)	19.15 (1.56)	0.31	18.97 (1.78)	17.43 (1.38)	0.46
Faeces	2.4(0.53)	2.6(0.53)	2.95(0.51)	0.33	3.47(0.37)	1.83(0.41)	**8.41****

*p <0.05; **p <0.01; ***p <0.001.

aDrug x sex interaction significant (see text)

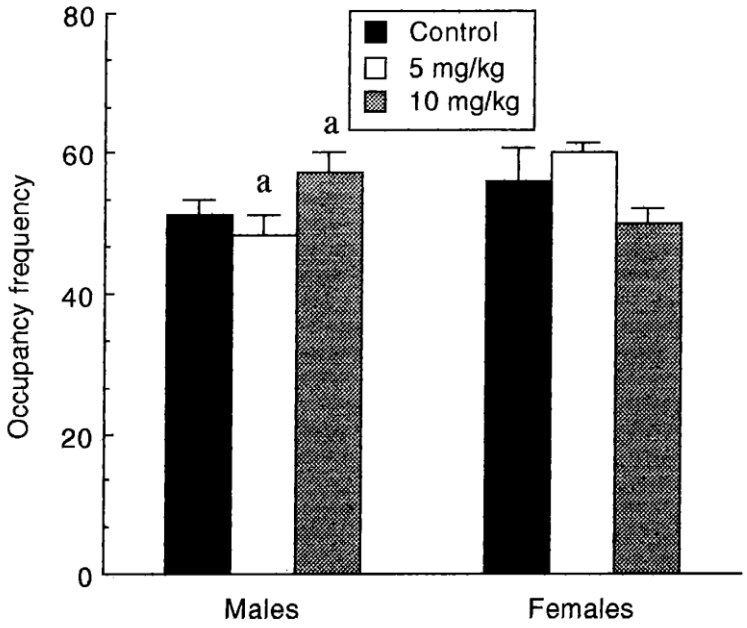

***Figure 1**: Mean (± SEM) frequencies of corner occupancy in the open field following exposure to 0 (control), and approximately 5 and 10 mg/kg/day of diazepam for males and females separately.* *[a]Difference between groups with superscripts in common significant for males ($p < 0.05$).*

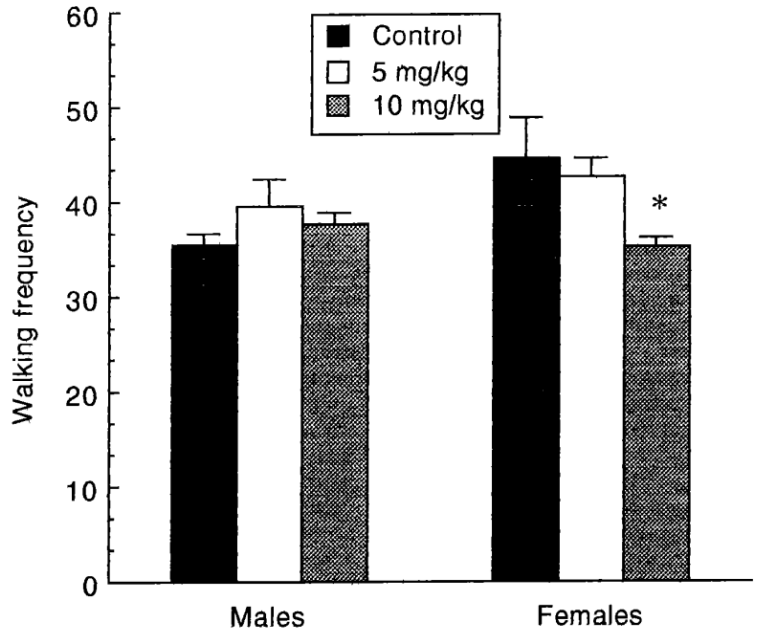

***Figure 2**: Mean (± SEM) frequencies of walking in the open field following exposure to 0 (control), and approximately 5 and 10 mg/kg/day of diazepam for males and females separately. *Significantly different from control ($p < 0.05$).*

Kava

For the kava group in the open field, a significant effect was found for sex on walking. Table 2 shows the significant difference between male and female rats, as female rats were observed walking more often in the open field than males regardless of dose level. Another significant effect of sex was found for rearing in the open field. Table 2 demonstrates that male rats overall were observed rearing more often than female rats, regardless of dose group. For grooming in the open field, sex was once again found to have a main effect. Table 2 demonstrates that female rats displayed more grooming behaviours than male rats overall, regardless of dose group. The final significant result found for the open field data was a main effect of sex on faecal boluses, with male rats eliminating more faecal boluses than female rats overall, irrespective of dose. There were no significant drug or sex effects for any other measure.

Table 2: Mean (± SEM) values of each measure recorded in the open field for each dose of kava, each sex, and results of ANOVAs.

Response	Kava				Sex		
	Control	90 mg/kg	180 mg/kg	F(2,54)	Males	Females	F(1,54)
Open Field:							
Ambulation	54.70(2.18)	53.25(1.99)	55.2(1.38)	0.28	53.60(1.39)	55.17(1.65)	0.50
Centre Occupancy	7.0(0.52)	8.50(0.45)	7.85(1.03)	0.75	8.50(0.68)	7.07(0.71)	2.05
Corner Occupancy	53.6(2.69)	53.85(2.48)	53.55(1.75)	0.01	51.23(1.44)	56.1(2.17)	3.27
Walking	40.05(2.35)	35.45(1.55)	40.3(1.70)	2.31	36.27(0.08)	40.93(2.06)	**5.06***
Rearing	38.25(2.23)	36.95(2.44)	39.20(1.88)	0.28	40.83(2.01)	35.43(1.35)	**4.72***
Grooming	4.65(1.17)	4.95(0.97)	3.7(0.90)	0.43	3.10(0.55)	5.77(0.99)	**5.36***
Immobile	17.0(2.44)	21.9(1.81)	16.8(1.80)	2.14	19.8(1.78)	17.33(1.59)	1.17
Faeces	2.40(0.53)	2.40(0.50)	2.25(0.57)	0.03	3.27(0.40)	1.43(0.40)	**10.71****

*p <0.05; **p <0.01; ***p <0.001.

Light Dark Box

Diazepam

For the diazepam group in the light dark box, significant effects occurred for dose, sex (Table 3), and the dose x sex interaction [$F(2,54) = 3.32$, $P<0.05$] on entries into the light side. Rats in the control and 5mg/kg dose groups made more entries into the light side than those in the 10 mg/kg group, regardless of sex (Table 3). Figure 3 shows the significant interaction, as female rats in the 5mg/kg dose group made significantly more entries into the light side than females in the 10 mg/kg dose group. Furthermore, the female control group also made significantly more entries into the light side than the female 10 mg/kg group. Female rats overall made more entries into the light side than male rats, regardless of dose. Another significant result was found for sex on faecal boluses, as female rats overall eliminated fewer faecal boluses than males. No significant results were found for latency or observations in the light side (Table 3).

Table 3: Mean (± SEM) values of each measure recorded in the light dark box for each dose of diazepam, each sex, and results of ANOVAs.

Response	Diazepam				Sex		
	Control	5 mg/kg	10 mg/kg	F(2,54)	Males	Females	F(1,54)
Light Dark Box:							
Latency (In Seconds)	16.60 (5.70)	14.45 (3.48)	15.30 (3.38)	0.07	13.00 (2.55)	17.97 (4.19)	1.00
Entries into Light[a]	6.50 (0.39)	7.00 (0.46)	5.65 (0.37)	**3.22***	5.87 (0.30)	6.90 (0.37)	**5.53***
Observations in Light	45.95 (9.61)	36.85 (2.87)	33.00 (2.74)	1.20	38.83 (6.68)	38.37 (2.15)	<0.01
Faeces	0.95(0.32)	1.35(0.45)	1.05(0.34)	0.42	1.97(0.35)	0.27(0.15)	**21.15*****

*p <0.05; ***p <0.001.

[a]Drug x sex interaction significant (see text)

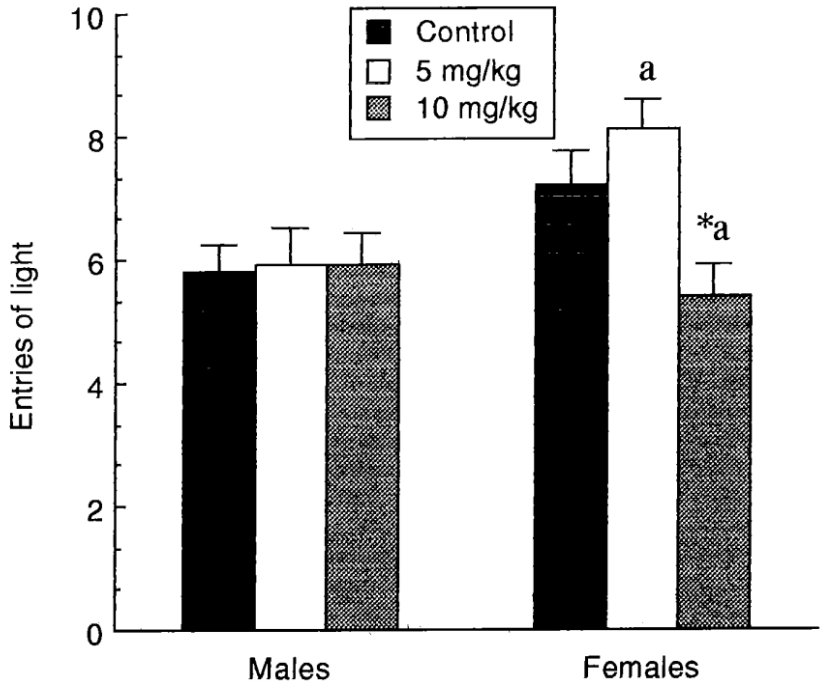

Figure 3*: Mean (± SEM) entries into the light side of the light dark box following exposure to 0 (control), and approximately 5 and 10 mg/kg/day of diazepam for males and females separately.* ***[a]****Difference between groups with superscripts in common significant for females.* *******Significantly different from control for females.*

Kava

For the kava group in the light dark box, the only significant effect was again for sex on number of faecal boluses. Female rats overall eliminated fewer faecal boluses in the light dark box than male rats (Table 4). No significant drug or sex effect occurred for any other measure.

Table 4: Mean (± SEM) values of each measure recorded in the light dark box for each dose of kava, each sex, and results of ANOVAs.

Response	Kava				Sex		
	Control	90 mg/kg	180 mg/kg	F(2,54)	Males	Females	F(1,54)
Light Dark Box:							
Latency (In Seconds)	16.70(5.70)	13.75(2.38)	15.45(4.02)	0.12	13.33(2.42)	17.27(4.21)	0.66
Entries into Light	6.50(0.39)	6.80(0.48)	6.35(0.42)	0.29	6.47(0.39)	6.63(0.31)	0.11
Observations in Light	45.95(9.61)	40.7(1.86)	39.9(2.46)	0.31	43.5(6.39)	40.87(2.01)	0.15
Faeces	0.95(0.32)	1.10(0.46)	0.90(0.46)	0.07	1.77(0.43)	0.20(0.10)	**12.03*** **

***p <0.001

Elevated Plus Maze

Diazepam

For the diazepam group in the elevated plus maze, a main effect for sex occurred for entries into the closed arms of the maze (Table 5). Female rats entered the closed arms more frequently than male rats overall. Table 5 also shows a significant sex effect for walking in the closed arms of the maze, with females walking more than males overall, regardless of dose. Finally, a significant effect for sex was again found for faecal boluses, as female rats eliminated no faecal boluses at all in the plus maze compared to the male rats overall. No significant drug or sex effects occurred for percentage of open arm entries, percentage of open arm occupation, or closed arm rearing.

Table 5: Mean (± SEM) values of each measure recorded in the elevated plus maze for each dose of diazepam, each sex, and results of ANOVAs.

Response	Diazepam				Sex		
	Control	5 mg/kg	10 mg/kg	F(2,54)	Males	Females	F(1,54)
Plus Maze:							
% Open-Arm Entries	50.11 (1.89)	52.88 (1.69)	53.26 (1.69)	0.39	51.42 (1.64)	52.75 (1.21)	0.43
% Open-Arm Occupation	51.65 (3.43)	54.33 (2.94)	57.09 (2.26)	0.41	51.62 (2.93)	57.08 (1.56)	2.75
Closed-Arm Entries	6.8 (0.45)	7.25(0.37)	7.15(0.49)	0.35	6.17(0.32)	7.97(0.31)	**15.37*****
Closed-Arm Rearing	14.85 (1.18)	15.40 (1.06)	14.65 (0.94)	0.14	15.03 (0.95)	14.90 (0.77)	0.01
Closed-Arm Walking	14.4(0.81)	13.15(0.70)	12.5(0.81)	1.75	12.23(0.60)	14.47(0.62)	**7.03***
Faeces	0.2(0.20)	0.8(0.32)	0.35(0.35)	1.24	0.9(0.33)		**7.73****

*p <0.05; **p <0.01; ***p <0.001.

Kava

For the kava group in the elevated plus maze, there were significant main effects of both dose and sex for the percentage of open arm observations (Table 6). The control group occupied the open arms a significantly lower percentage of times compared to both the 90 mg/kg dose group, and the 180 mg/kg dose group, regardless of sex. However there was no significant difference between the 90 mg/kg and 180 mg/kg groups. Female rats overall showed a higher percentage of open arm occupation than male rats, regardless of dose group. No significant drug or sex effects occurred for any other measure.

Table 6: Mean (± SEM) values of each measure recorded in the elevated plus maze for each dose of kava, each sex, and results of ANOVAs.

Response	Kava				Sex		
	Control	90 mg/kg	180 mg/kg	F(2,54)	Males	Females	F(1,54)
Plus Maze:							
% Open-Arm Entries	50.11(1.89)	51.93(1.72)	55.43(1.42)	2.51	51.56(1.53)	53.42(1.29)	0.89
% Open-Arm Occupation	51.65(3.43)	58.8(2.12)	60.39(1.76)	**3.71***	53.18(2.53)	60.71(1.43)	**7.27****
Closed-Arm Entries	6.8(0.45)	7.6(0.34)	7.35(0.39)	1.14	6.83(0.33)	7.67(0.30)	3.54
Closed-Arm Rearing	14.85(1.18)	16.05(1.13)	13.7(0.92)	1.19	15.87(0.47)	13.87(0.77)	2.59
Closed-Arm Walking	14.4(0.81)	14.3(0.73)	13.7(0.86)	0.21	14.3(0.66)	13.97(0.64)	0.12
Faeces	0.20(0.20)		0.15(0.15)	0.52	0.23(0.16)		1.96

*p <0.05; **p <0.01.

Discussion

This study aimed to compare the anxiolytic effects of kava and diazepam on three different animal models of anxiety, with the goal of encouraging further research into natural alternatives to benzodiazepines, and to improve on the existing literature describing kava's effects on animals. Because there were so few significant results obtained however, it was not possible to draw strong conclusions about the effects of either kava or diazepam. Nonetheless, the few significant effects and their practical implications will be addressed, as well as weaknesses in methodology, and directions for future research.

Dose and dose X sex interaction effects

Kava

The finding that both the 90 mg/kg and 180 mg/kg dose groups for kava were observed more frequently in the open arms of the elevated plus maze compared to the control group regardless of sex, provides some evidence of kava's efficacy as an anxiolytic. However because this was the only significant dose effect for kava across all three models of anxiety, it is difficult to conclude that kava does indeed have an anxiolytic effect at those doses. Future research is therefore necessary to determine any further effects for kava, as well as any potential dose X sex interaction effects, as none occurred in the current study. Garret et al. (2003) and Rex et al. (2002) both found more consistent acute dose effects for kava in the elevated plus maze, and were able to compare it favourably to diazepam. Rex et al. (2002) found the greatest anxiolytic effect occurred for the 180 mg/kg dose of kava, and an anxiolytic effect also occurred at that dose in the current study, which gives some justification for that dose to be used again in further studies. However, as will be discussed in the limitations, Rex et al. (2002) used an ethanol extract of kava root which may have been more potent than the capsules used in the current study and thus led to that study producing more significant results. In the current study, only one dose effect for kava occurred in the elevated plus maze, and no

dose effects for diazepam occurred in the plus maze, so comparisons could not be drawn between the effectiveness of the two substances.

Diazepam

In the open field and light dark box test, a consistent result was that the 5 mg/kg dose of diazepam reduced anxiety more than the 10mg/kg dose, depending on the sex of the rats. Males in the 5 mg/kg dose group were in the corner squares of the open field less often than those in the 10 mg/kg dose, and females in the 5 mg/kg dose group walked around the open field more often than those in the 10 mg/kg dose group. Furthermore, in the light dark box females in the 5 mg/kg dose group and control group entered the light side more than those in the 10 mg/kg dose. These behaviours indicated decreased anxiety, and therefore suggested that a 5mg/kg daily dose of diazepam may be more effective at alleviating symptoms of anxiety than a higher dose. However, because these were interaction effects, it also appears that sex may determine the particular behaviour that diazepam affects. The finding that the 5mg/kg dose group also entered the light side of the light dark box more frequently than the 10mg/kg regardless of sex adds further support for the notion that 5 mg/kg of diazepam may be the preferable dose for rats, and it also suggests that this may be true for both male and females. There is also the possibility that the 10 mg/kg dose of diazepam was too high and therefore increased anxiety in the rats, as the female control group in the light dark box entered the light side more frequently than the 10 mg/kg dose group. This indicated that the female control group was less anxious than the female 10 mg/kg dose group, which therefore suggests the 10 mg/kg dose had increased the anxiety of female rats. This finding contrasts Rex et al. (2002) as they found that their acute dose of 15 mg/kg of diazepam had an anxiolytic effect, which indicates that in the current study it may have been a chronic dose of 10 mg/kg of diazepam that led to an anxiogenic effect. Garrett et al. (2003) found acute anxiolytic effects for diazepam in doses ranging from 0.0316 – 5 mg/kg of diazepam, so the current study appears to be closer in results to their study as the 5 mg/kg chronic dose of diazepam produced a stronger anxiolytic effect than the 10 mg/kg dose. Unlike Rex

et al. (2002) and Garrett et al. (2003), the current study was also able to determine that some dose effects for diazepam were sex dependent, and affected male and female rats differently. However, considering the number of animal models used in the study, and number of behaviours observed, the few dose results found for diazepam mean that only tentative conclusions can be drawn.

Sex effects

By far the most consistent sex difference was for faecal boluses, as male rats overall eliminated more faecal boluses than female rats across all three models of anxiety. This result indicates that for female rats, faecal boluses may not be a strong measure of anxiety, or that the three behavioural tests used did not provoke the anxiety response of defecation. Broadhurst (1957) also found female rats defecated significantly less than male rats in an open field test, which suggests that female rats in general may not eliminate faeces as often as males.

Female rats also appeared to move around more in the three behavioural tests than male rats. Females walked in the open field, entered the light side of the light dark box, entered the closed arms of the elevated plus maze, and walked in the closed arms of the plus maze more than male rats, regardless of dose. These results may lend support to the finding from Fernandes et al. (1999) that female rat behaviour was driven by activity, while male rat behaviour was driven by anxiety. This may also explain the fact that male rats overall eliminated more faecal boluses than female rats, because they were driven more by anxiety than females.

Overall the fact that significant sex effects occurred in the current study across various behaviours and models of anxiety encourages the use of both rat sexes in future studies.

Limitations and future research

An issue in methodology that may have led to the lack of significant results in the study was the method of administration of both kava and diazepam. While mixing the drugs into the rat's food was far less invasive than repeatedly injecting them, it could not be guaranteed that all rats were receiving

the same daily doses. The rats had a tendency to flick their food over the cage while they were eating, and it is possible that one rat in the cage was eating more or less compared to the others and therefore getting more or less drug. If the rats had been individually housed rather than group housed it would have been easier to determine how much each rat was eating. Additionally, weighing the rats daily may have provided a better estimate of how much they were eating, although this could have caused added stress for the rats. Despite this, using food as the method of administration also ensured that rats were less stressed prior to testing as they would not have been through the ordeal of being injected (Lapin 1995).

Another issue that may have affected results is that it was not possible to test all the rats on one day due to their large numbers. Because the rats continued to receive their daily dose of kava and diazepam while testing occurred, the last rats to be tested on each behavioural model could have had slightly more drug in their bodies, depending on how often they ate. This contrasted with the two previous studies by Rex et al. (2002) and Garrett et al. (2003), because they were able to complete all testing in one day due to the fact that their study was focused on acute effects, and because they had fewer rats to test. A solution to this may be to have more than one person testing the rats, so that more can be tested in a day which would then reduce the amount of time it takes to complete testing, and thus ensure that doses are more even.

Another benefit of using more than one person during testing is that it would allow blind testing to be conducted. In the current study only one person prepared the mash and tested the rats, meaning that blind testing did not occur. A potential issue from this is observer bias, which could have influenced perceptions of the rats' behaviour in the anxiety models (Tuyttens et al., 2014). Therefore the inclusion of an additional person or people would allow the food preparation and testing to be completed by separate people and allow the person testing to be blind to the dose group of each rat. This would also allow for more testing to be completed in one day, as preparing the food and testing the rats in one day was a very time consuming process.

The form of kava used may not have been as effective, even though it contained 90mg kavalactones. A more direct source of kava such as the resin made from ground kava root as was used in the Garrett et al. (2003) study may have provided a more potent dose of kavalactones. Rex et al. (2002) and Garrett et al. (2003) both used kava sourced directly from the root, unlike the current study which used capsules purchased from a pharmacy. This meant that it was not possible to know the original source of the kava, and it is possible the pharmacy kava was not as effective as the kava used in the previously mentioned studies. However, the capsules from the pharmacy were also more representative of a typical product that people might purchase for treating anxiety-related disorders (Tomlinson, 2007).

One advantage Garrett el al. (2003) had over the present study was the use of HPLC analysis for identifying individual kavalactone concentrations. Because access to such an analysis was not available, the current study was unable to determine individual kavalactone concentrations and therefore comparisons between kavalactone levels in the current study and the Garrett et al. (2003) study were not possible. This however would be an interesting idea for future research, so that more could be understood about how different kavalactone concentrations might affect the anxiolytic properties of kava, as it is possible the kava used in the current study was lower in certain kavalactone concentrations than the kava used in Garrett et al. (2003).

Future studies may also benefit from comparing a different benzodiazepine to kava, or even an SSRI, as it appears that currently the literature has only compared kava to diazepam for treating anxiety. Diazepam is a frequently prescribed drug, but it is not the only one used for the treatment of anxiety disorders, meaning that if kava does not compare favourably to diazepam, it may show better results compared to a different anti-anxiety medication.

Due to the large number of rats used in the current study, they were bred and born over a period of two months, meaning that when dose treatment began, 20 rats each were 69, 83, 99, 111, and 134

days old respectively. As there can be age differences in anxiety, the older rats in the study may have shown increased anxiety in the elevated plus maze compared to younger rats (Imhof, Coelho, Schmitt, Morato, & Carobrez, 1993). However, the differently aged rats were randomly mixed between the dose groups, so it was not possible to determine if any age effects had occurred. Garret et al. (2003) used mice that were 98 days old and found anxiolytic effects occurred for kava, while Rex et al. (2002) did not specify the age of the rats used. Therefore future studies may benefit from the use of younger rats, or rats up to 98 days old, as increases in anxiety occur as both mice and rats age (Frick, Burlingame, Arters, & Berger-Sweeney, 1999; Imhof et al., 1993). Alternatively, if mixed age groups are used, it may be beneficial to determine which age is in which dose group so as to recognise differences between younger and older rats.

Conclusion

Despite the relative lack of significant drug effects, and thus an inability to draw stronger conclusions about kava's anxiolytic properties compared to diazepam, the current study was not entirely devoid of any implications. Awareness of kavalactone doses for individual rats and using kava derived from the root of the plant might enable better ways of assessing the anxiolytic potential of the drug. It would also be useful to compare effects of kava with those of other drugs used to treat anxiety (e.g., SSRIs) in order to determine whether or not such an alternative form of treatment is comparable or even superior to current treatment practices.

The main value of the current research has been in the use of both male and female rats and a chronic dosing regimen. Although the experimental outcomes were not outstanding, this thesis has served to highlight deficiencies to be rectified as well as suggesting some potentially fruitful lines of future enquiry.

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Appendix A

UNIVERSITY OF CANTERBURY

ANIMAL ETHICS COMMITTEE

Secretary, Lynda Griffioen
Email: animal-ethics@canterbury.ac.nz

Ref: 2015/12R

15 July 2015

Nicole Flynn
Department of Psychology
UNIVERSITY OF CANTERBURY

Dear Nicole

I am pleased to inform you that the Animal Ethics Committee (AEC) has approved your application entitled: "A comparison of the anxiolytic effects on behaviour of chronic administration of kava and diazepam".

Approval has been granted:
(a) for the use of 100 *Rattus norvegicus*
(b) for your research project to be undertaken from 15 July 2015 to 31 December 2015. If you require an extension of this period please contact the AEC Secretary.

As part of AEC's new Code of Ethical Conduct all applicants receiving approval to work on animals are required to provide a final report at the completion of their project. The purpose is to provide the AEC with a record of your use of animals and what was achieved by your research project. We are very much interested in your findings and to learn what you have achieved. Following the completion date indicated above you are asked to provide this report using the new Final Report form which is available at the AEC web site (https://intranet.canterbury.ac.nz/research/ethics.shtml).

On an annual basis the University is legally required to provide to MPI statistical data on all animal manipulations undertaken in a calendar year. To assist us in collating this information you are also required to complete and return to the AEC Secretary the attached MPI Animal Manipulation Statistical form 30 days after the completion of this project, or once every three years, whichever comes first. If no animals have been manipulated in your project please provide a "Nil" return. Please also find enclosed a copy of the Animal Welfare (Records and Statistics) Regulations 1999 for your information, together with a list of Animal Type Codes and brief guideline notes for your assistance.

Yours sincerely

James Briskie

Associate Professor Jim Briskie
Chair
University of Canterbury Animal Ethics Committee