CHAPTER 4

DISCUSSION
4.1 Ethnozoological Survey

Honey of the bee *Apis indica* played a major role in tribal therapies. It was used for the treatment of cough and cold as well as for the treatment of asthma by all the 9 tribes studied. Apitherapy or therapy with bee-products like honey, propolis, fortified-honey and herb-honey is a part of traditional medicines. Honey is used as a traditional medicine for leg ulcers in Ghana and for ear aches in Nigeria. It is also used as a topical medicine for measles in eyes and for gastric ulcers (Banerjee et al 2003). Because of their beneficial effects, these products, which are used as food and medicines by tribes, are receiving the worldwide attention. There are many medical reports about the effectiveness of honey in gastric and peptic ulcers or gastro-intestinal disorders in humans (Kandil et al 1987, Salem 1981, Haffejee and Moosa 1985, Ladas et al 1995). The importance of honey as the best universal topical medicine for wound healing (Harris 1994, Vardi et al 1998) and for blepharitis, catarral conjunctivitis, keratitis were also reported (Efem et al 1992). Propolis, a resinous wax like substance, which bees collect from plants, is claimed to be very useful for arthritis (Zielonka et al 1987).

Similarly, the tooth and tusk of elephant were used by all the tribes for the treatment of conjunctiva as well as pimples. An interesting find was that of the termite species *O. formosanus* Shiraki, which is being used for the treatment of asthma by six out of the nine tribes studied. The termite *O.*
*formosanus* Shiraki has also been used by the 'Palliyan' and 'Kanikkaran' female tribes for the enhancement of lactation. Termites are also reported to have antimicrobial (Lamberty et al. 2001) properties besides its food value.

Though there have been many studies on ethnobotany (MoEF 1994, Bhandary et al. 1995, Rao Rama and Hendry 1996, Samvatsar and Diwanji 2000, Katewa et al. 2001, Singh et al. 2002, Harsha et al. 2003), ethnozoological studies are limited (Lalramnghinglova 1999, Patil 2003). Lalramnghinglova (1999), has reported ethnozoology of Mizoram state. He has reported that about 25 vertebrates and 31 invertebrates are used for the treatment of over 40 different ailments including jaundice, tuberculosis, hepatitis, cancer, asthma and veterinary diseases. Patil (2003) has documented information on the medicinal uses of different wild animals practiced by various tribes including the 'Bhils', 'Gamits', 'Koknas', 'Pawara' of Nandurbar district, North West of Maharastra. He has enumerated the use of 15 species of animals including jackal, earth boa, preying mantis, hyena, Indian hare, sloth bear, panther and tiger for various ailments like vomiting and dysentery, leucoderma, epilepsy, foot and mouth diseases and for aphrodisiac, health, vigour etc. Ethnozoology of the Nilgiri hills, consisting of six ethnic groups including the 'Todas', 'Kotas', 'Kurumbas', 'Irulas', 'Paniyas' and 'Kattunayaks' was documented by Rajan et al. (2002).
India, when compared to rest of the world is gifted with a rich faunal diversity. With a mere 2.4% of the world's area, India accounts for 7.31% of the global faunal total with a faunal species count of 89,451 species (MoEF. 1999). Instead of taking stringent measures against the practitioners of zootherapy or forcing them to leave their traditional therapies, awareness should be given for the traditional farming system, where the animals could be raised using scientific techniques. Providing education on domestication, conservation and preservation of the biological diversity in a more scientific and sustainable way should be the most important consideration in devising measures to ensure the tribes' permanent and reliable source of medicine. As stated by Patil (2003), this study also raises some important questions about the rights of tribal people and scientific validity of the uses. In many areas, the tribals are known to live in harmony with nature since centuries utilizing the available forest resources and without causing ecological disturbances. Animals become endangered only when exploited beyond certain limits. If found unsustainable, the tribals should be educated about the endangered species and discouraged from using these animals for medicinal purposes. Possible alternatives in appropriate form should be found out, as law prohibits hunting of the animals. Further, the claims made by these tribals should be tested for their validity using modern scientific techniques. Community research including tribes to confirm the medicinal value of these traditional remedies, would probably go a long way leading to the discovery of more drugs from bugs.
4.2 Antibacterial activities of termites

All the three species of the subterranean termites and their respective mound extracts showed appreciable antibacterial activity against most of the bacterial strains (Table 5, 6). No activity was observed from the soil (extracts) collected from the adjacent area to the termatarium. The antibacterial activity was more apparent in *O. formosanus* Shiraki, and its mound extract, which inhibited all the bacterial strains studied when compared to that of *M. obesi* Holmgren and *M. estherae* (Desneux). The activity scores varied with different extracts and a test organism used and was the highest in *O. formosanus* Shiraki and its mound extract against *Vibrio eltar*. The activity scores increased with increasing concentration of the extract and remained constant beyond the Minimum Inhibitory Concentration (MIC). The MIC values varied with different extracts and test organisms used. Antibacterial activity was more significant in *O. formosanus*, and its mound extract and was equal to the positive control. Considerable inter-generic variation existed in the antibacterial activity of various termites and their respective mound extracts. This may be due to the individual variation or differences in the metabolite production. Interestingly, *O. formosanus* Shiraki has been used by the 'Kanikkaran', 'Paniyan', 'Palliyan', 'Sholaga', 'Irular' and 'Kota' tribes of South India for the treatment of asthma, a disease associated with microbes (Solavan et al., 2004). Among the bacterial strains, *Klebsiella* sp and *Vibrio eltar* were found to be less sensitive
to the extracts of termites and their respective mounds when compared to other strains.

Recently Lamberty et al (2001) have isolated two novel antimicrobial peptides, termicin and spinigerin, from the fungus-growing termite *P. spiniger* (heterometabole insect, Isoptera). Studies conducted by Da Silva et al (2003) on the termite *P. spiniger* revealed that termicin, a cysteine-rich antifungal peptide has antibacterial properties also. Antibacterial peptides are also reported from the ants *Myrmecia gulosa* (Mackintosh et al., 1998) and *Pachycondyla goeldii* (Orivel et al., 2001).

Arthropods (such as wasps, bees, mole crickets, scarab larvae, cicada nymphs and centipedes) that live in close proximity to each other are subject to microbial attacks and epidemic diseases. To limit disease activity, they incorporate antimicrobial compounds into their nests (Beattie et al 1986). Soil-dwelling-ants also have been shown to use chemical defence against fungi and bacteria in their underground nests (Holldobler and Wilson 1990). Termites, which live in mounds, are also subject to microbial attacks. To defend themselves against such microbial attacks they may be using chemical defence. Studies on the compounds responsible for the activity are in progress.
This study provides preliminary information on the antibacterial properties of the subterranean termites of South India. Whittaker and Feeny (1971) had opined that the classification of toxic compounds by their role was difficult since the role was often combined. Since, the tribals have been using the termite for the treatment of asthma associated with viral infections, the compounds found within these organisms may have other pharmacological properties like antiviral or antifungal activity and studies in this direction are on progress.

4.3 Effect of the termite on the growth and reproduction in Swiss albino mice

Dietary supplementation with the termite to Swiss albino mice significantly increased the percentage of growth rate and number of litters delivered of when compared to the mice fed on standard rodent pellets (normal control). The percentage of growth rate of experimental male and female mice fed on the termite *O. formosanus* showed a significant increase (P<0.01) when compared to the respective normal control groups. Results on the effect of the termite on litter production revealed a 22% increase in the experimental groups when compared to the normal control groups fed on standard rodent pellets.

As mentioned earlier, the 'Kanikkaran' and 'Palliyan' tribes of South India have also been using *O. formosanus* Shiraki as food to enhance lactation in
women. The use of termite as human food in the South and North Eastern parts of India was reported by various investigators (Forbes 1813, Maxwell-Lefroy 1909, Gope and Prasad 1983, Rajan 1987). Forbes (1813) mentioned that termites are eaten by the local tribes in Mysore and the Carnatic regions. Maxwell-Lefroy (1909) stated that the termite queens are eaten in some parts of South India and every boy of 12 to 14 years of age is said to be given a termite queen to eat in order to make him endure fatigue and run well. Gope and Prasad (1983) revealed that insects represent the cheapest source of animal protein in Manipur and the termite *Odontotermes feae* Linn formed one of the important insect foods. Rajan (1987) reported that in the North Arcot district of Tamil Nadu, the winged termites known as *Eesal* in Tamil, are collected by a forest tribe known as 'Irumbars' and sold to the merchants in the market. The winged termite is fried as such or fried along with ground nut by adding Bengal gram, puffed rice, salt and spices and sold. The fried pulses, spices and salt enhance the taste. In Karnataka, the winged termite is known as *Eechalu hula*. Rajan (1987) also noted that in some villages of Karnataka, the queen termite is collected and fed raw to weak children.

Termites are a valuable source of protein, fat, and essential amino acids in the diets of both primates and modern humans (Harris 1971). The common species of termites used as food in African countries include *Macrotremes bellicosus* (Smeathman 1781) *M. falciger* (Chavunduka 1975, Phelps et al 1975), *M.*
"natalensis" (Fasoranti and Ajiboye 1993), *M. subhyalinus* (Oliveira et al. 1976), *M. mossambicus* (Hagen), *M. bellicosus* (Smeathman) and *M. natalensis* (Haviland), *Pseudacanthotermes* sp, *Odontotermes* sp (Silow 1983). *Syntermes parallelus*, *S. snyderi*, *Macrotermes* sp., *Cornitermes* sp. (Dufour 1987) and *Cornitermes* sp (Ribeiro and Kenhiri 1989) are some of the common termite species used as food in the Latin American countries. In Asia, many species of termites like *Odontotermes feae* Linn (Gope and Prasad 1983), *Reticulitermes flavipes* (Somnasang et al. 1986) and *O. formosanus* Shiraki (Solavan et al. 2004) are used as food.

Analysis of the major organic nutrients in the termite *O. formosanus* Shiraki revealed a high percentage of protein (47) followed by lipids (9) and carbohydrates (2). The present results support the wisdom of South Indian tribes as well as tribes around the world where pregnant women are supplemented with termite food. By food value, termites are rich source of protein and thus formed an important diet. While a rump steak yields 322 calories (cal) per 100g, and cod fish 74, termite provides 560 cal/100g (Hickin 1971). Insects vary widely in fat and energy content. Termites rank among the highest in fat content. Phelps et al. (1975) reported a calorific value of 761 kcal [~3196 kilo Joules(kJ)/100g dry, ash-free, weight basis] for the winged sexual forms of the African termite, *M. falciger* Gerstacker, while the winged forms of another African species, *M. subhyalinus* Rambur were found to contain
613 kcal (~2575 kJ/100g dry weight) (Oliveira et al., 1976). The winged adults of the termite, *M. subhyalinus*, are high in magnesium and copper contents (Oliveira et al., 1976). The high content of iron and zinc in many termites is of particular interest. Iron deficiency is a major problem in women's diets in the developing World, particularly among pregnant women, and especially in Africa (Orr, 1986). Thus, termite formed an important diet for pregnant women and children of the African countries. The women even regard eating termite clay as a mean to 'provide uterus with building material' and to 'help form the foetus properly'. Non-pregnant women may also eat termite clay when they feel weak (Silow 1983).

Our studies on the dietary supplementation of termite to Swiss albino mice *M. musculus* treated separately with acephate and endosulfan significantly decreased the toxic effect of pesticides and increased litter production than in those given the pesticides alone both in the F0 and F1 generations, implying that the components of termite may be inducing the activities of detoxifying enzymes present in Swiss albino mice (see the section 4.4). High nutritive value of the termite *O. formosanus* Shiraki coupled with its probable antitoxic role strongly suggests the possibility of termite as an alternative protein rich viable feed particularly for poultry. Termites have not been exploited in a large way mainly because of the difficulty in harvesting large numbers and extracting them from the soil debris. Studies on termite culture on a
4.4 Antitoxic effect of the termite on Swiss albino mice treated with pesticides

Dietary supplementation with the termite to male and female Swiss albino mice *M. musculus* treated separately with acephate and endosulfan significantly decreased the toxic effect of pesticides (no significant decrease in body weight) than in those given the pesticides alone both in the F0 and F1 generations (P<0.05)). A significant increase in litter production was also observed in experimental groups treated with pesticide and supplemented with termite than in the control groups given pesticides alone both in the F0 and F1 generations (P<0.01)).

Acephate is an important organophosphorous insecticide. The toxicity of acephate is attributed to bioactivation on metabolic conversion to methamidophos, which acts as acetylcholinesterase inhibitor (Mahanjna et al 1997). Rattner and Michael (1985) have reported that acute exposure to acephate may affect Luteinizing hormone (LH) secretion and possibly reproductive function. The reproductive toxicity of the insecticide acephate (Farag et al 2000a) demonstrated adverse effects of male acephate exposure on pregnancy outcome with effects on sperm parameters like sperm motility and sperm count. Maternal effects like cholinergic signs, decreased body weight
and decreased absolute and relative brain weight, decreased placenta weight and increased liver weight due to acephate toxicity, were also known (Farag et al 2000b). Acephate is also known to affect significantly the number of implantations, number of live-foetuses, number of early resorptions, and the incidence of skeletal malformations during organogenesis (Farag et al 2000b), the LD50 value being 361 mg/kg for mice (Worthing 1987). On the other hand endosulfan is a synthetic chlorinated cyclodiene widely used as a broad spectrum insecticide. It is highly toxic via the oral route in mice, the LD50 value being 7.36 mg/kg (Smith 1991). Stimulation of the central nervous system is the major characteristics of endosulfan poisoning (Hurt 1991). Studies carried out by Pandey et al (1990) revealed that at higher doses endosulfan decreased sperm count up to 39%. Endosulfan is also reported to cause embryonic death and teratogenesis (Popov et al 1998), decreased compensatory ovarian hypertrophy, an increase in the number of atrectic follicles, disruption of the estrous cycle (Hiremath and Kaliwal 2002a) and inhibition of implantation in mice (Hiremath and Kaliwal 2002b). A survey of the published literature did not reveal detoxification/antitoxic role of termites in mammalian system.

However, enhancement of xenobiotic detoxification enzymes in mice by dietary rosemary extract was reported by Singletary and Rokusek (1997). They reported that the extract of the spice plant rosemary fed at concentrations of
0.3% and 0.6% (by weight) for 4 weeks to female mice significantly increased the liver and stomach Glutathione -S- transferase (GST) activity. Modulatory influences of the clove *Caryophyllus aromaticus*, L on hepatic detoxification systems in male Swiss albino mice are also known (Kumari 1991). She has reported an enhanced GST, Cytochrome (Cyt) b5 and somatic hormone (SH) levels in Swiss albino mice administered with clove (1% and 2% w/w in the diet) for 10, 20 and 30 days.

To conclude, in the present investigation, dietary supplementation of the termite to mice treated with pesticides, significantly decreased the toxic effects of pesticides on mice than in the control groups given only pesticides. This implies that the components of termite have the potential to detoxify the toxic effect of pesticides in Swiss albino mice. Termites may be inducing the activities of detoxifying enzymes present in Swiss albino mice like Glutathione -S- transferase (GST), an enzyme which plays an important role in detoxification by catalyzing the conjugation of Glutathione (GSH) to the electrophilic foreign compounds for their elimination from the system. Further studies on the effect of termites on the activity of detoxification enzymes like GST will provide more information on the detoxification role of these termites in any mammalian system.
4.5 Antigenotoxic effect of the termite on Swiss albino mice treated with pesticides

The Table 11 reveals that both acephate and endosulfan are highly genotoxic. Significant increases on the incidence of chromosomal aberrations and micronuclei are observed in the pesticide control groups (acephate and endosulfan treated). It is obvious that the normal and solvent control groups have not shown any major aberration types like ploidy, pulverization, inversion and ring formations. It is very interesting to note that the experimental groups treated with acephate/endosulfan and supplemented with the termite food did not show critical genotoxic aberration markers like pulverization and ploidy (except in the lower concentrations of experimental groups of the F0 generation treated with endosulfan). The percentage reduction in the chromosomal aberrations, in the experimental groups treated with endosulfan and the termite supplemented food, was significantly higher (P < 0.01) in the F0 generation when compared to the F1 generation. This can be attributed to the fact that the F1 generation mice might have also received chemicals through mother feeding for four weeks in addition to the chemical directly given for experimental purpose for about seven weeks. The decrease in aberration frequency with lower doses and at higher doses in some cases may be due to non-availability of the critical concentration of the genetically reactive metabolites at the target molecules (DNA and protein) and/or due to
elimination of metabolites from the body with time (Tates and Natarajan 1976).

Acephate is an important organophosphorous insecticide. In vivo studies conducted by Behera and Bhunya (1989) on the genotoxicity of acephate in mice also revealed a significant enhancement in the percentage of chromosome aberrations. Their studies on the bone marrow chromosome aberrations, micronucleus, sperm shape abnormality and a dominant lethal test in mice indicate that acephate is a potential mutagen. Acephate is also reported to cause significant DNA damage in Swiss albino leucomyocytes (Rahman et al 2002). On the other hand, endosulfan is a synthetic chlorinated cyclodiene widely used as a broad spectrum insecticide. Studies on the genotoxicity of endosulfan assessed in mouse germ cells by Pandey et al (1990) revealed that at higher doses endosulfan induced dominant lethal mutations in one mating interval (36-42 days) post treatment. Mutagenic action of endosulfan in mice was also reported (Lvova 1984).

Dietary supplementation with the termite to male and female Swiss albino mice M. musculus treated separately with acephate and endosulfan significantly decreased the percentage of chromosomal aberration than in those given the pesticides alone (pesticide control) both in the F0 and F1 generations (P < 0.01) suggesting an antigenotoxic role of the termite.
components. However, a survey of the published literature did not reveal any report of antigenotoxic effect of termites or other insect products against acephate or endosulfan. Antigenotoxic effects of other dietary agents were known. Aqueous extracts of dietary vegetables (carrot, spinach and cabbage), spices (cinnamon, pepper, cumin, clove and cardamom), tea and coffee along with oral co-administration of urethane (URE) revealed dose-related antigenotoxic effects against URE in mice (Abraham et al 1998). Pretreatment with tomato, garlic, and turmeric (alone and in combination) against 7,12-dimethylbenz[a]anthracene (DMBA) significantly reduced the frequencies of DMBA-induced bone marrow micronuclei in mice (Mohan et al 2004). Antimutagenic activity of naringin (Nar), a flavonone, found in high amount in grapefruit, against ifosfamide (Ifos) treated mice was also reported. A significant decrease was observed in the micronucleated polychromatic erythrocytes (MNPE) produced by Ifos (Alvarez-Gonzalez 2001).

Antigenotoxic effects of various plant products were also reported. An antigenotoxic constituent SQFwB2D (24 alpha-ethyl-5 alpha-cholesta-7,trans-22-dien-3 beta-ol or spinasterol) from the chloroform extract of Cucurbita maxima flowers was isolated by Villasenor et al (1996). It decreased the mutagenicity of tetracycline by 64.7% at a dosage of 100 mg/kg mouse. Short-term dietary supplementation with purpurin, an anthraquinone constituent from the madder root Rubia tinctorum, inhibited the formation of hepatic
DNA adducts in male C57bl6 mice after a single dose of the heterocyclic amine dietary carcinogen Trp-P-2 [(30 mg/kg mouse) (Marczylo et al 2003)]. Benzo(a)pyrene (BaP) and cyclophosphamide (CP) induction of chromosomal aberrations, micronuclei formation, and sister chromatid exchanges (SCEs) was found to be inhibited in a dose-dependent manner by diallyl sulfide (DAS), indole-3-carbinol (I3C), curcumin (CUR), and black tea polyphenols (BTP) in microbial and mammalian (mice) test systems (Shukla et al 2003). Nordihydroguaiaretic acid (NDGA), an antioxidant originally obtained from plants of the genus Larrea has shown antigenotoxic activity by inhibiting the induction of micronucleus (MN) formation by methyl methanesulfonate (MMS) in mouse (Diaz Barriga et al 1999). The antigenotoxic action of three doses of trans-dehydrocrotonin (t-DCTN), the active ingredient obtained from the bark extract of Croton cajucara, a plant native to the Amazon, determined in Swiss Albino mice in vivo indicated that doses of 50 and 75 % of the LD₅₀, via intraperitoneal treatment or gavage injection, were antimutagenic with regard to cyclophosphamide (Agner et al 2001). Antigenotoxic activity was also reported from various other chemicals. WR-2721 [S-2-(3-aminopropylamino)-ethylphosphorothioic acid (amifostine)] was reported to have antigenotoxic effect in idarubicin [4-demethoxydaunorubicin (IDA)] treated mice. The IDA-treated group showed increased levels of micronuclei. (de Campos Nebel et al 2002). The antigenotoxic effects of naturally occurring flavouring agents trans-anethole and
eugenol (trans-anethole :40-400 mg/kg mouse; eugenol: 50-500 mg/kg mouse) assessed in the mouse bone marrow micronucleus test revealed significant antigenotoxic effects against cyclophosphamide (CPH), procarbazine (PCB), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and urethane (URE) (Abraham 2001). An in vivo micronucleus assay using Balb/C male mice to examine antigenotoxic effects of cimetidin (CM) on benzene (BZN) induced genotoxic effects revealed that BZN effectively induced micronuclei in polychromatric erythrocytes (PCEs). However, application of CM led to a significant reduction of micronuclei in PCEs, i.e. 2-fold after 10 mg/kg mouse and 3-fold after 30 mg/kg mouse CM treatment (Mozdarani and Kamali 1998). The genotoxic effects of ochratoxin (1 μg/kg mouse/day), on albino Swiss mice were reported to be substantially reduced by concurrent oral administration of retinol (Kumari and Sinha 1994). The antigenotoxic effect of galangin, a neuropeptide, against the genotoxicity of N-methyl-N-nitrosourea (MNU) in mouse bone marrow cells is also known (Sohn et al 1998).

To conclude, the present study provides preliminary information on antigenotoxic effect of the subterranean termite O. formosanus Shiraki used in South Indian folk medicine. Studies on the compound responsible for the antigenotoxic activity and mechanism behind it are in progress.