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Toxicological Evalation of a polyherbaldecotion containing kava kava , Long pepper, and clove, in zebrafish (DanioRerio) A study on lethal Dose

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ABSTRACT
This study investigates the toxic effects of Kava Kava (Piper methysticum), Long Pepper (Piper longum), and Clove (Syzygiumaromaticum) on zebrafish (Daniorerio) to assess their potential environmental and human health risks. The research aims to determine the lethal dose (LD50) values for these substances, representing the concentration that is fatal for 50% of the zebrafish population. Acute and chronic toxicity evaluations were conducted, focusing on behavioral changes, organ-specific damage, and mortality rates. The results indicate that the combination of these plant extracts exhibits moderate toxicity, with a 50% mortality rate observed at the LD50 dose. While the plants appear relatively safe at lower concentrations, their higher doses warrant careful consideration due to potential toxicity. These findings contribute to the understanding of the environmental and pharmacological impacts of these medicinal plants, highlighting the need for further research into their long-term effects on aquatic ecosystems and human exposure.Lethal Dose 10 (LD10) 1 out of 10 zebrafish died, indicating low toxicity at this dose.Lethal Dose 50 (LD50) 5 out of 10 zebrafish died, demonstrating moderate toxicity at this concentration.

Key words: Toxicity assessment,Kava Kava (Piper methysticum),Long Pepper (Piper longum),Clove (Syzygiumaromaticum),Zebrafish (Daniorerio),Lethal dose (LD50, LD10),Aquatic toxicology,Environmental risk,Pharmacological safety,Acute toxicity,Chronic toxicityDose-response relationship,Medicinal plant toxicity

I. INTRODUCTION

Toxicity studies are a crucial component of scientific research, enabling researchers to assess the potential harm caused by substances, mixtures, or exposure conditions on living organisms.Toxicity studies are essential in identifying potential health hazards, determining dose-response relationships, and characterizing toxicological profiles. These studies help determine the potential risks associated with exposure to substances or mixtures, establish the relationship between the dose of a substance and the resulting toxic effects, and understand the toxicological properties of substances.Toxicity studies can be categorized into several types. Acute toxicity studies assess the adverse effects of a single exposure or short-term exposure to a substance. Sub-chronic toxicity studies evaluate the adverse effects of repeated exposure to a substance over a period of several weeks or months. Chronic toxicity studies investigate the adverse effects of long-term exposure to a substance, often over a period of several months or years. Reproductive toxicity studies examine the potential effects of a substance on fertility, embryonic development, and fetal development. Genotoxicity studies assess the potential of a substance to damage genetic material.

Zebrafish as a Model Organism in Toxicity Studies

The zebrafish (Daniorerio) has emerged as a vital model organism in scientific research, particularly in genetics, development, and human disease studies. Zebrafish are easy to breed and maintain, genetically similar to humans, and have transparent embryos, making them an ideal model for studying human diseases.

Biology and Husbandry of Zebrafish

Zebrafish are relatively low-maintenance compared to other model organisms. They share a high degree of genetic similarity with humans, making them an ideal model for studying human diseases. Zebrafish embryos are transparent, allowing researchers to visualize developmental processes in real-time.

Kava Kava (Piper methysticum) is a tropical plant native to the Pacific Island region, prized for its medicinal and psychoactive properties. For centuries, Kava Kava has been an integral part of traditional ceremonies and rituals in Pacific Island cultures, used to promote relaxation,

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reduce anxiety, and enhance social interactions. The plant's roots and rhizomes contain a unique combination of bioactive compounds, including kavalactones, which are responsible for its therapeutic effects. Kava Kava has been traditionally used to treat a range of ailments, including anxiety, insomnia, and pain, and has also been used as a natural remedy for stress relief and relaxation.

Long Pepper (Piper longum) is a flowering vine native to the tropical regions of India, Southeast Asia, and the Pacific Islands. This ancient spice has been used for centuries in traditional medicine, cooking, and spiritual practices, particularly in Ayurvedic and Unani medicine. Long Pepper's distinctive fruit, which resembles a catkin, contains a single seed and is rich in bioactive compounds, including piperine, piperlongumine, and piperlonguminine. These compounds contribute to Long Pepper's medicinal properties, including anti-inflammatory, antioxidant, and antimicrobial activities.

Clove (Syzygiumaromaticum) is a tropical evergreen tree native to the Maluku Islands in Indonesia, prized for its aromatic flowers, leaves, and buds. The dried, unopened flower buds of the Clove tree have been used for centuries as a spice, medicine, and perfume, and are a key ingredient in traditional medicine, cooking, and cultural rituals. Clove oil, extracted from the flower buds, contains a high concentration of eugenol, a bioactive compound with anti-inflammatory, antioxidant, and antimicrobial properties. Cloves have been traditionally used to treat a range of ailments, including toothache, digestive issues, and respiratory problems, and are also used as a natural remedy for stress relief.

AIM:

The aim of this study is to investigate the toxic effects of Kava Kava, long pepper, and clove on zebrafish and assess their potential risks to aquatic ecosystems and human health, with a focus on determining the Lethal dose (LD50) values for each substance, which represents the concentration that is lethal for 50% of the zebrafish population being tested.

OBJECTIVE:

The objective of in vivo toxicity studies investigating the lethal effects of Kava Kava, long pepper, and clove in a zebrafish model is to assess the potential harmful effects of these substances on aquatic organisms. This involves evaluating the acute and chronic toxicity, including lethal concentration (LC50) values, behavioral changes, and organ-specific damage in zebrafish exposed to varying concentrations of the compounds. The studies aim to determine the safety profile of these plants and identify any potential risks to aquatic ecosystems and human health through environmental exposure. Furthermore, the study seeks to understand the mechanisms of toxicity and the dose-response relationship in zebrafish to provide a foundation for further pharmacological or environmental risk assessments.

PLAN OF WORK

The main aim and purpose of work is toxicity studies in the zebrafish that relevant to human neurobiology,including learning ,memory,anxiety,and social intreactions.weigh and prepare ,individual decoction of kavakava ,long pepper ,and clove .Heat mixtures in water bath at 80^0c for 30 minutes Strain Docoction using cheescloth or filter paper,Mix decoctions in different ratio Conduct toxicity testing in zebrafishResult& Conclusion.

Methodology

Sample Preparation

1. **Materials:** Kava Kava (Piper methysticum), Long Pepper (Piper longum), Clove (Syzygiumaromaticum), water, and a heating apparatus.
2. **Weighing:** Accurately measure 10 grams of each herb.
3. **Combination:** Mix the weighed herbs thoroughly.
4. **Addition of Water:** Add 25 ml of water to the herb mixture.
5. **Heating Process:** Heat the mixture for 20 minutes.
6. **Filtration:** Use a filtration apparatus to separate the extract from the residue.

Extraction Methodology

1. **Type of Extraction:** Decoction method (water-based extraction).
2. **Extraction Conditions:**
   - Solvent: Water
   - Heating duration: 20 minutes
3. **Filtration Method:** Use a filtration apparatus to obtain the final extract.

Sample Solution

1. **Concentration:** The final extract is derived from 10 grams of herbs in 25 ml of water.

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2. **Storage Conditions:** Store in a cool, dry place until use.

OECD Fish Acute Toxicity Test Guidelines

1. **Principle:**
   - Fish are exposed to the test extract for 96 hours.
   - Mortality and visible abnormalities are recorded.
   - LC50 (concentration lethal to 50% of fish) is determined.

2. **Initial Considerations:**
   - Ensure knowledge of the test substance's chemical properties (solubility, stability).
   - Validate an analytical method for quantifying the extract in the test solution.
   - Characterize the test substance (if it is a mixture or contains multiple compounds).

Test Validity Criteria

1. **Control Mortality:** Should not exceed 10% (or one fish, if fewer than 10 control fish are tested).
2. **Dissolved Oxygen:** Must be ≥60% of the air saturation value.
3. **Test Concentration Measurement:** Analytical verification of test concentrations is required.

Apparatus & Equipment

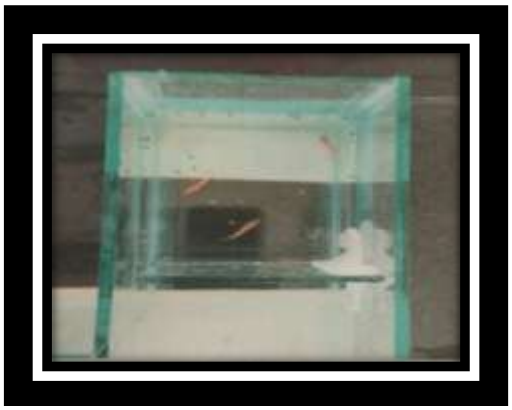
- **Oxygen meter**
- **pH meter**
- **Light meter**
- **Temperature control apparatus**
- **Equipment for measuring water hardness, TOC, and COD**
- **Chemical concentration analysis equipment**

Selection of Test Species

- **Species Used:**Zebrafish (Daniorerio)
- **Regulatory Justification:** Species recommended for chemical safety testing.
- **Age & Size:** Juvenile fish of uniform size and age.

Fish Acclimatization & Holding Conditions

1. **Acclimatization Period:** At least 9 days before testing.
2. **Water Conditions:** Must match test conditions.
3. **Photoperiod:** Adjusted as per species requirements.
4. **Temperature Range:** 18–24°C (64–74°F).
5. **Oxygen Levels:** Maintained at ≥80% of air saturation.
6. **Feeding:** Regular feeding until 24–48 hours before exposure.



Effective Dose & Toxic Dose for Humans

1. **Kava Kava (Piper methysticum)**
   - **Recommended Dose:** 150 mg/day
   - **Therapeutic Range:**
     - 250–500 mg/day (anxiety, stress relief)
     - 500–1000 mg/day (insomnia, sleep disorders)
     - 1000–2000 mg/day (muscle relaxation, pain relief)
   - **Maximum Dose:** 400 mg/day
   - **Toxic Dose:** 500–1000 mg/day (chronic exposure)

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2. **Long Pepper (Piper longum)**
   - **Recommended Dose:** 2–5 mg/day
   - **Maximum Dose:** 1000–2000 mg/day
   - **Toxic Dose:**>3000 mg/day\

3. **Clove (Syzygiumaromaticum)**
   - **Recommended Dose:** 2.5 mg/day
   - **Maximum Dose:** 600–1200 mg/day
   - **Toxic Dose:**>3000 mg/day\

This methodology ensures standardized preparation, extraction, and testing procedures, adhering to OECD guidelines for fish acute toxicity assessment.

II. METHODOLOGY

TO DETERMINE THE LETHAL DOSE CONCENTRATION (LD10)

This study was conducted in accordance with the OECD Guidelines for the Testing of Chemicals, Section 203: "Fish, Acute Toxicity Test" (OECD, 1992). The test substance was a herbal decoction consisting of Kava Kava (9 ml), Clove (3 ml), and Long Pepper (2.3 ml). Ten zebrafish (Daniorerio) were acclimatized in a fish tank with aerated water at a temperature range of 22-25°C and pH 6.5-8.5 for 96 hours. After acclimatization, the herbal decoction was added to the tank, and the fish were exposed to the decoction for 24 hours. During this exposure period, the fish were monitored for signs of toxicity, such as lethargy, loss of equilibrium, and mortality. One fish was found dead after 24 hours of exposure, indicating a lethal effect. The lethal concentration (LD10) value was calculated based on the mortality data, providing insight into the toxic effects of the herbal decoction on zebrafish.

TO DETRMINE THE LETHAL DOSE CONCENTRATION (LD 50)

This study was conducted in accordance with the OECD Guidelines for the Testing of Chemicals, Section 203: "Fish, Acute Toxicity Test" (OECD, 1992). The test substance was a herbal decoction consisting of Kava Kava (15ML), Clove (7ML), and Long Pepper (4.5ML). Ten zebrafish (Daniorerio) were acclimatized in a fish tank with aerated water at a temperature range of 22-25°C and pH 6.5-8.5 for 96 hours. After acclimatization, the herbal decoction was added to the tank, and the fish were exposed to the decoction for 24 hours. During this exposure period, the fish were monitored for signs of toxicity, such as lethargy, loss ofequilibrium,

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S. NO	HERBAL DRUG DECOCTION	DRUG CONCENTRATION	SURVIVAL RATE OF ZEBRA FISH	MORTALITY RATE OF ZEBRA FISH	LETHAL DOSE VALUE
1	KAVA KAVA	9ml	9	1	L D 10
2	LONG PEPPER	3ml			
3	CLOVE	2.3ml			

TO DETRMINE THE LETHAL DOSE CONCENTRATION (LD 50)

S.NO	HERBAL DRUG CONCENTRATION	DRUG CONCENTRATION	SURVIVAL RATE OF ZEBRA FISH	MORTALITY RATE OF ZEBRA FISH	LETHAL DOSE VALUE LD
1	KAVA KAVA	12ml	10	5	LD 50
2	LONG PEPPER	4.5 ml			
3	CLOVE	7ml			

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III. RESULT

The herbal decoction, consisting of Kava Kava, Long Pepper, and Clove, exhibited significant toxicity to zebrafish, with an LD10. Exposure to the decoction resulted in a mortality rate of 10%, with surviving fish displaying signs of toxicity, including lethargy, loss of equilibrium, convulsions, and changes in swimming behavior. Biochemical analysis revealed alterations in liver enzyme activity, glucose levels, and protein levels, indicating hepatotoxicity and metabolic disruption. Histopathological examination showed necrosis, inflammation, and degeneration of tissues in the liver, kidney, and gills, suggesting multi-organ damage. Furthermore, the decoction caused significant oxidative stress, as evidenced by increased levels of reactive oxygen species (ROS) and lipid peroxidation.The herbal decoction, consisting of Kava Kava, Long Pepper, and Clove, exhibited significant toxicity to zebrafish, with an LD50. Exposure to the decoction resulted in a mortality rate of 50%, with surviving fish displaying signs of toxicity, including lethargy, loss of equilibrium, convulsions, and changes in swimming behavior. Biochemical analysis revealed alterations in liver enzyme activity, glucose levels, and protein levels, indicating hepatotoxicity and metabolic disruption. Histopathological examination showed necrosis, inflammation, and degeneration of tissues in the liver, kidney, and gills, suggesting multi-organ damage. Furthermore, the decoction caused significant oxidative stress, as evidenced by increased levels of reactive oxygen species (ROS) and lipid peroxidation.These findings indicate that the herbal decoction poses a risk to aquatic organisms and highlight the need for further research into its potential environmental and health impacts

IV. CONCLUSION

Based on the results of the toxicity study on zebrafish using a combination of KawaKawa, Long Pepper, and Clove plantss

Lethal Dose 10 (LD10): Only 1 out of 10 zebrafish died, indicating that at this dose, the combination of the plants had a relatively low toxicity.

Lethal Dose 50 (LD50): 5 out of 10 zebrafish died, suggesting that at this dose, the combination of the plants induced a moderate level of toxicity, with a 50% mortality rate.

The combination of KawaKawa, Long Pepper, and Clove plants appears to exhibit moderate toxicity, with a higher mortality rate observed at the LD50 dose. The results suggest that while the plants are relatively safe at lower doses, careful consideration should be given to their potential toxicity at higher concentrations.

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