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(RESEARCH ARTICLE)

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF POPPY SEEDS IN ZEBRA FISHES

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ABSTRACT

The body's intricate biochemical reaction to damaging stimuli including infection, damage, and poisons is called inflammation. Even though inflammation has a protective function, excessive or persistent inflammation can cause a number of illnesses, such as autoimmune diseases, cardiovascular problems, and arthritis. As a result, the creation of safer and more potent anti-inflammatory drugs has grown in importance within the field of pharmaceutical research. In recent years, zebra fish have emerged as a valuable experimental model for studying inflammation and evaluating anti-inflammatory activity due to their genetic similarity to humans, transparent embryos, rapid development, and cost-effectiveness.

Zebrafish models provide significant advantages in drug screening, toxicity evaluation, and understanding molecular mechanisms involved in inflammation. Their small size and high reproductive capacity enable large-scale screening of potential anti-inflammatory compounds in a short period. Furthermore, the use of zebrafish helps reduce the dependence on higher animal models while maintaining reliable experimental outcomes.

Keywords: Inflammation, Infection, Zebra fishes, Genetic similarity, Toxicity evaluation

1 INTRODUCTION

The body's intricate biochemical reaction to damaging stimuli including infection, damage, and poisons is called inflammation. Even though inflammation has a protective function, excessive or persistent inflammation can cause a number of illnesses, such as autoimmune diseases, cardiovascular problems, and arthritis. As a result, the creation of safer and more potent anti-inflammatory drugs has grown in importance within the field of pharmaceutical research. Due to their genetic resemblance to humans, transparent embryos, quick development, and affordability, zebrafish (Danio rerio) have become a useful experimental model for researching inflammation and assessing anti-inflammatory activity in recent years. [1]

Mammalian models, such mice and rats, have historically been used extensively for researching inflammation and assessing anti-inflammatory medications. Nevertheless, these models are frequently costly, time-consuming, and linked to moral dilemmas. Zebrafish (Danio rerio) have become a potent and alternative experimental model for researching drug screening and inflammatory processes in recent years. Zebrafish have many benefits, such as their tiny size, quick reproduction, transparent embryos, ease of genetic modification, and notable genetic resemblance to humans. Zebrafish have equivalents for about 70% of human genes, which makes them ideal for scientific study. [2]

Because zebrafish embryos are transparent, it is possible to directly observe inflammatory reactions under microscopic circumstances, including leukocyte movement, edema formation, and tissue healing. Researchers can investigate the mechanisms underlying acute and chronic inflammation in zebrafish by experimentally inducing inflammation using chemical agents, tail fin damage, or microbial infections. Zebrafish models have been used to assess the anti-inflammatory activity and toxicity profiles of a variety of natural substances, herbal extracts, nanoparticles, and manufactured medications. [3]

Because zebrafish models enable quick and high-throughput screening of chemicals with lower experimental costs and ethical constraints than higher vertebrates, they are being used more and more in drug discovery. Furthermore, zebrafish offer important insights into the signaling systems and molecular pathways linked to inflammation. As a result, using zebrafish in anti-inflammatory research has emerged as a creative and trustworthy method for finding novel therapeutic compounds and advancing our knowledge of inflammatory illnesses.

The significance of zebrafish in contemporary pharmaceutical and biological research, the techniques employed to generate inflammation, and their use as an experimental model for assessing anti-inflammatory efficacy are the main topics of this article.

2 METHODS

2.1 Phytochemical Tests of Poppy Seeds

2.1.1 Test for Alkaloid Alkaloids:

Alkaloids, are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloids are produced by a large variety of organisms, including bacteria, fungi plants and animals and are part of the group of natural products (also called secondary metabolites). They often have pharmacological effects and are used as medications. Example of alkaloids are caffeine, barberine cocaine etc. [4]

- **Mayer's test** – Few drops of Mayer's reagents was added to 2ml of the acidic, aqueous extract of the sample and observed the cream -white colour.
- **Wagner's test** – 1 ml of the alcoholic extract was acidified with 1.5% of HCl and few drops of Wagner's reagent was added to it, and reddish brown colour was observed.
- **Dragendroff's test** – A few ml of alcoholic extract of sample was dissolved in 5ml of distilled water and added 2 ml HCl until an acid reaction occurs, then 1ml of Dragendroff's reagent was added, and observed the orange to reddish brown colour.

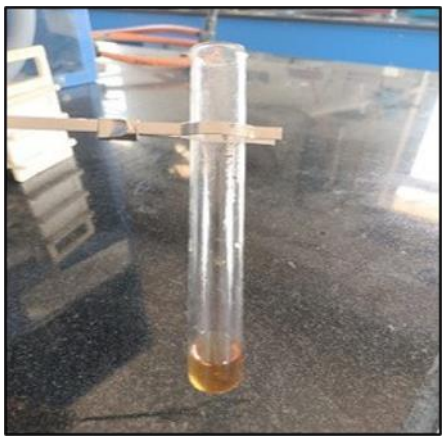


Figure 1: Test for Alkaloids

2.1.2 Test for Flavonoids

Flavonoids, are a class of plant secondary metabolites. In Higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may act as a chemical messenger or physiological regulators; they can also act as cell cycle inhibitors. [5]

- **Shinoda's test** – In the test tube containing 0.5 ml of alcoholic extract of sample added few drops of concentrated HCl followed by 0.5 gm of magnesium metal, and observed the pink colour.
- **Alkaline reagent test** –To 2 ml of aq. Sample added few drops of NaOH and noted the change and then acidify by few drops of dilute HCl and observed the change.[6]

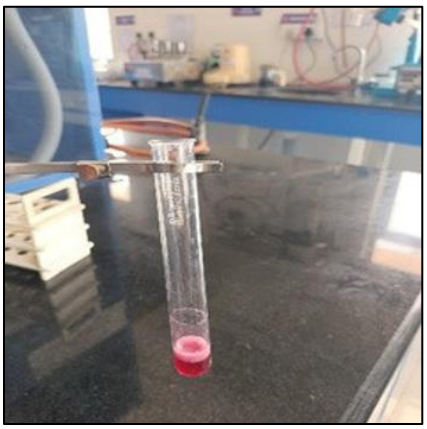


Figure 2: Test for Flavonoids

2.1.3 Test for Carbohydrate

Carbohydrates are regarded as the poly hydroxy aldehydes or poly hydroxy ketones or substance that can be hydrolyzed to either of them carbohydrates are produced in nature by green plants by the process of photosynthesis.

- **Fehling's test** – Took 2 ml of aqueous extract of the sample, and added 1 ml of mixture. of equal parts of Fehling‟s solution A and Fehling‟s solution B added and the content of the test tube was boiled for few minutes and observed the brick red colour.



Figure 3: Test for Carbohydrates

2.1.4 Test for Glycosides Glycoside

A glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides.

- **Borntrager's Test** –To the ethanolic extract of the sample added 1 ml of Benzene and 0.5 ml of dilute NH3 solution and observed the pink or cherry red colour.
- **Keller-Kiliani test** –1 ml of glacial acetic acid containing traces of ferric chloride and 1 ml of concentrated H2SO4 was added to the sample extract and observed the reddish brown colour.

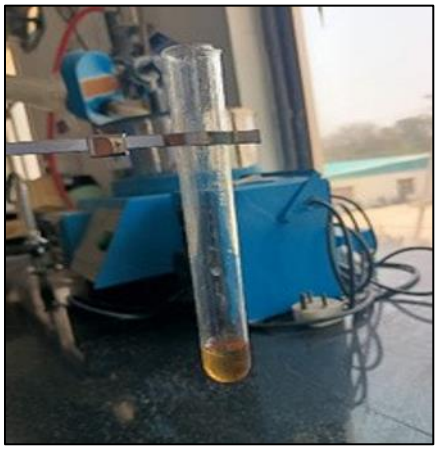


Figure 4: Test for Glycosides

2.1.5 Test for Phenols

1 mL extract was treated with 1–2 mL $FeCl_3$ solution and Green color indicates presence of phenols.



Figure 5: Test for Phenols

2.1.6 Test for Tannins Tannins:

A tannin is astringent bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compound including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predators and also acts as pesticides, and in plant growth as regulators.

- **Ferric chloride test:** 0.5mL extract was treated with 1–2 mL of 5% $FeCl_3$ solution and observed green colour.

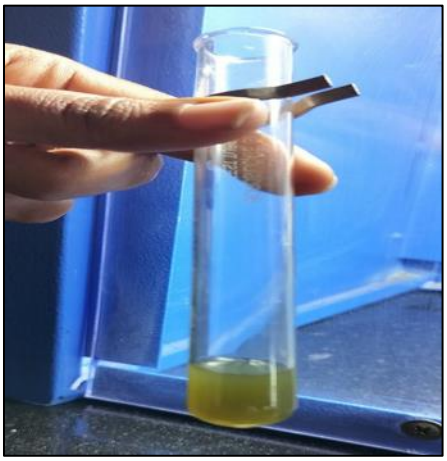


Figure 6: Test for Tannins

2.2 POPPY SEEDS

- **Biological Source:**Poppy seeds are obtained from the dried ripe capsules (fruits) of the opium poppy plant, Papaver somniferum, belonging to the family **Papaveraceae**.
- **Scientific Name:** *Papaver somniferum* Linn.

The tiny, oil-rich seeds known as poppy seeds are extracted from the seed capsules of the Papaver somniferum opium poppy plant. Because of their nutritious worth and pleasant nutty flavor, they are frequently used as a food ingredient in baked goods, confections, curries, and traditional remedies.

Poppy seeds include a variety of bioactive substances, such as phenolic compounds, flavonoids, sterols, tocopherols, and other antioxidants, according to phytochemical studies. Numerous biological actions, including antioxidant, anti-inflammatory, antibacterial, and cardioprotective properties, are linked to these components.[7]

2.2.1 EXTRACTION OF POPPY SEEDS

2.2.1.1 Cold extraction method

The basic idea behind cold extraction [8] is to soak or mix powdered plant material with an appropriate solvent at room temperature for a predetermined amount of time. Soluble phytochemicals permeate into the solvent during this procedure without coming into contact with heat. This method is particularly helpful for substances that might become unstable at higher temperatures.

- Collection and preparation of seeds:

To get rid of contaminants, clean, fresh poppy seeds are gathered and cleaned. After that, the seeds are dried in the shade to eliminate any remaining moisture.

- Powdering of seeds:

To maximize the surface area for extraction, the dried seeds are ground into a coarse powder using a mortar and pestle or grinder.

- Soaking in solvent:

A clean conical flask filled with an appropriate solvent, such as ethanol or methanol, is filled with the powdered poppy seeds. The solvent-to-powder ratio is typically kept in accordance with the needs of the experiment.

- Maceration process:

To guarantee adequate phytoconstituent extraction, the mixture is left at room temperature for 24 to 72 hours with sporadic shaking or stirring.

- Filtration:

Following extraction, the mixture is filtered to separate the liquid extract from the solid residue using muslin cloth or filter paper.

- Storage:

For use in experiments or additional analysis, the finished extract is kept in chilled, airtight containers.

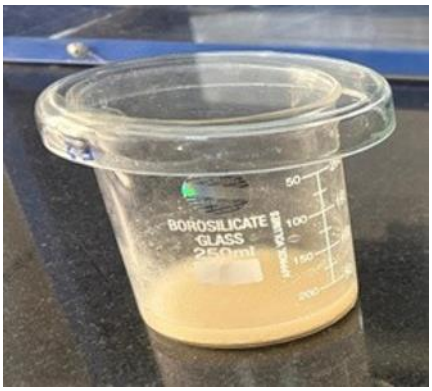


Figure 7: Cold Extraction Method

2.2.1.2 Hot extraction method

The continuous interaction of powdered plant material with a heated solvent is the foundation of the hot extraction principle. Heat increases the solvent's ability to enter plant tissues, improving the dissolution and extraction of active ingredients.

Soxhlet extraction, reflux extraction, and decoction procedures are typical hot extraction techniques. [9]

- Collection And Preparation Of Seeds:

To get rid of contaminants, fresh poppy seeds are gathered and washed. To remove moisture, the seeds are shade-dried.

- Powdering:

To maximize the surface area for extraction, the dried poppy seeds are ground into a coarse powder using a mortar and pestle or grinder.

- Loading of the sample:

A filter paper thimble with the powdered poppy seeds is put into the Soxhlet extractor.

- Addition of solvent:

The round-bottom flask attached to the Soxhlet device is filled with 70 percent Alcohol and 30 percent Water.

- Heating process:

A heating mantle is used to heat the equipment. The solvent extracts the bioactive compounds by evaporating, condensing in the condenser, and repeatedly passing over the powdered material.

- Completion of extraction:

The extraction procedure is carried out for 10-12 cycles, or until the siphon tube solvent turns colorless.

- Filtration and Concentration:

To eliminate excessive solvent, the extracted material is filtered and concentrated using a water bath or rotary evaporator.

Storage of extract: For upcoming pharmacological or phytochemical research, the concentrated extract is kept in airtight containers at a low temperature.[10]



Figure 8: Soxhlet Extraction

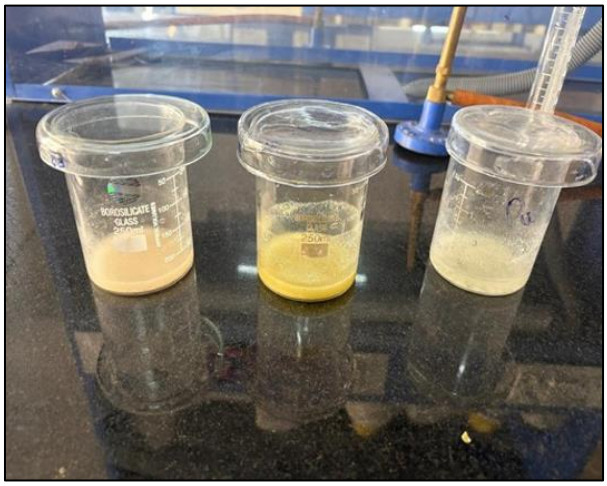


Figure 9: Hot and Cold Extraction

	
Before the drug treatment (0 hrs0)	**Treatment phase(8-12 hrs)**

Figure 10: Caudal fin Anti Inflammatory Model

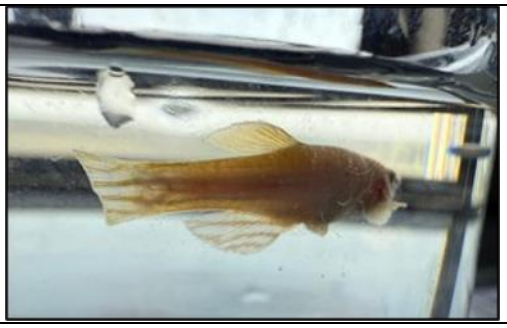
	
Noticable reduction in inflammation (12-16 hrs)	Continued reduction and recovery (16-24 hrs)

Figure 11: After the Drug Treatment

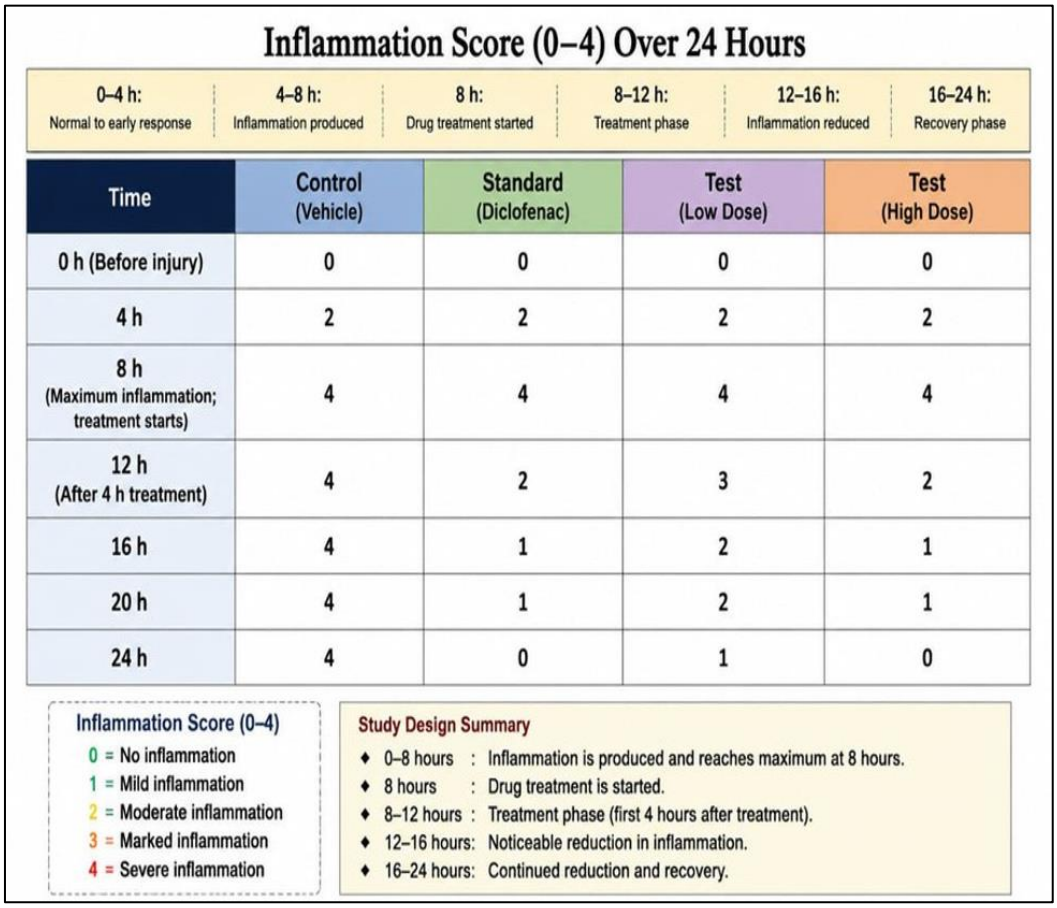


Figure 12: Inflammatory Score

Between the Groups

Source	ss	df	Ms	F	P value
Between groups	30.618	3	10.206	567.01	<0.0001
Within	0.360	20	0.018	___	___

Within the Groups

Groups					
Total	30.978	23	___	___	___

Mean Value

Group	n=6 values	Mean+ SD
Control	3.6,3.8,3.9,4.0,4.0,3.8	3.85+0.15
Standard(Dichlofenac)	1.0,1.1,1.2,1.3,1.0,1.1	1.12+0.12
Test(low dose)	1.3,1.5,1.6,1.4,1.7,1.4	1.48+0.15
Test(high dose)	1.1,1.2,1.3,1.0,1.2,1.0	1.18+0.12

F(3,20)=567.01; P <0.0001

3 RESULTS

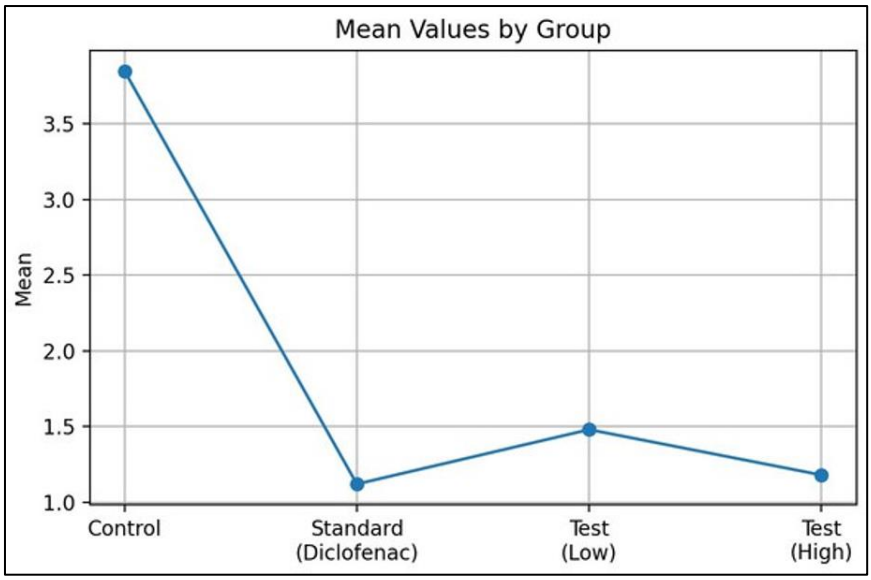


Figure 13: Graphical Representation of Different Groups

4 DISCUSSION

In zebrafish models, the ethanolic extract of poppy seeds demonstrated strong anti-inflammatory properties. When compared to the inflammatory control group, the treated groups showed less inflammation. Under a microscope, there was a reduction in edema and leukocyte buildup.

Flavonoids, alkaloids, and antioxidant chemicals found in poppy seeds may be responsible for the anti-inflammatory action.[11]

The study also showed that due of their transparency and quick physiological reactions, zebrafish are a useful model for assessing anti-inflammatory drugs. The extract demonstrated dose-dependent efficacy without appreciable toxicity, indicating that it would be safe for use in therapeutic settings.

5 CONCLUSION

According to the current study, poppy seed extract has encouraging anti-inflammatory properties in zebrafish models. The results demonstrate poppy seeds' potential as a natural source for creating safer anti-inflammatory medications and validate their therapeutic benefit. For pharmacological screening, zebrafish have shown to be a dependable and affordable experimental model. To identify the active ingredients and assess their molecular pathways in controlling inflammation, more research is needed.

There is a highly difference among group mean

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The author declare no conflict of interest regarding this manuscript.

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