EFFECT OF GINGER (*Zingiber officinale*) ON NAPROXEN INDUCED LIVER DAMAGE IN WISTAR RATS

INTRODUCTION

Herbal Medicine is the study and or practice of the medicinal and therapeutic use of plants; herbalism (Erci, 2012). Herbal medicines have important roles in traditional medicines as traditional medicines practitioners often incorporate the use of herbal medicines in their practice. Herbal medicines are parts of plants commonly employed as raw materials for self-administered pharmaceutical remedies and as supplementary products in the general population (Aderibigbe, 2015).

The plant materials used include fruits, seeds, berries, roots, rhizomes, leaves, bark and flowers (Gupt et al., 202). The World Health Organization (WHO) approximates that about 80% of the world's population depends on traditional medicinal system for some aspect of primary health care (Van Lerberghe, 2008).This encourages developing countries to complement their health program with traditional herbal preparation provided they are proven to be non-toxic. Vast majorities of people use these herbal medicines as first line remedies and as their primary form of health care (Wachtel-Galor & Benzie, 2012).). Herbal prescriptions are employed in both developed and developing countries for the treatment of various diseases (WHO, 2009).

The rising use of traditional medicine has informed the WHO's decision to encourage its incorporation into the national health care systems of countries and to inspire the formulation of national policy and regulations as important indicators of the extent of incorporation of

such medicine within a national health care system (WHO, 2001).

Herbal medicines have important roles in traditional medicines as traditional medicines practitioners often incorporate the use of herbal medicines in their practice. Herbal medicines are parts of plants commonly employed as raw materials for self-administered pharmaceutical remedies and as supplementary products in the general population (Lee et al., 2022). The plant materials used include fruits, seeds, berries, roots, rhizomes, leaves, bark and flowers (Kankanamalage et al., 2024). The World Health Organization (WHO) approximates that about 80% of the world's population depends on traditional medicinal system for some aspect of primary health care (Akerele et al., 1985). Thus encourages developing countries to complement their health program with traditional herbal preparation provided they are proven to be non-toxic (WHO, 1985). Vast majorities of people use these herbal medicines as first line remedies and as their primary form of health care (Van Lerberghe, 2008). Herbal prescriptions are employed in both developed and developing countries for the treatment of various diseases (WHO, 2009).

The liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a centre for metabolism of nutrients and excretion of waste metabolites (Ozougwu et al*.,* 2017). Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system (Allen *et al.,* 2002). A total loss of liver function could lead to death within minutes, demonstrating the liver ‘s great importance (Ozougwu, 2017) in view of this, this study was undertaken to review the physiology of the liver with a view to keep it functioning at its optimum and maintaining good health so as to avoid liver damages such as fatty liver, liver fibrosis and cirrhosis.

The liver has numerous functions best grouped into secretion of bile, metabolism of bilirubin, vascular and hematologic functions, metabolism of nutrients, metabolic detoxification and storage of minerals and vitamins.

There are several enzymes in the liver, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyltranspeptidase (GGT).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the centrepiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases. The epidemiological risk of clinically apparent liver injury is low (1–8 cases per 100 000 patient years of NSAID use), but when it occurs, it can be serious and can cause diagnostic confusion (Bessone, 2010).

Naproxen is a popular over-the-counter nonsteroidal anti-inflammatory drug (NSAID) that is widely used for therapy of mild-to-moderate pain and arthritis. Naproxen has been associated with rare cases of clinically apparent drug induced liver injury.

Naproxen (naprox' en) belongs to the propionic acid class of NSAIDs similar to fenoprofen, ibuprofen, ketoprofen and oxaprozin (Shah et al., 2017). The antiinflammatory and analgesic properties of NSAIDs such as naproxen are mediated by inhibition of tissue cyclo-oxygenases (Cox-1 and -2), which results in a decrease in pro-inflammatory prostaglandins, important mediators in inflammatory and pain pathways. Naproxen has analgesic as well as antipyretic and antiinflammatory activity. It has a longer half-life than other commonly used NSAIDs, making a twice daily regimen feasible. Currently more than 10 million prescriptions for naproxen are filled yearly and these numbers do not capture the wide scale over-the-counter sales. Naproxen is indicated for mild-to-moderate pain from various causes including

trauma, tendonitis, headache, dysmenorrhea, and various forms of arthritis including osteoarthritis, rheumatoid arthritis, gout and ankylosing spondylitis. Generic and over-the-counter formulations are available as tablets, capsules and oral suspensions in multiple doses (125, 250, 225, 375, 500, 550 mg) under multiple commercial names including: Aleve, Anaprox, Naprosyn, Naxen, Naxodol, Neo-Prox, Nu-Naprox, Nycopren, Proxen, Synflex. Over-the-counter combinations with antihistamines are also available. The typical dose is 250 to 500 mg taken orally twice daily. As with other NSAIDs, naproxen is generally well tolerated, but side effects can include headache, dizziness, somnolence, dyspepsia, nausea, abdominal discomfort, heartburn, peripheral edema and hypersensitivity reactions. Rare but serious adverse events from NSAIDs include gastrointestinal ulceration and bleeding, increased risk for cardiovascular disease, renal dysfunction, exacerbation of asthma and hypersensitivity reactions including anaphylaxis, exfoliative dermatitis and Stevens Johnson syndrome. Serum aminotransferase levels can be elevated in as many as 4% of patients receiving prolonged courses of naproxen, particularly with high doses. Clinically apparent naproxen induced liver injury is very rare (~1-3 per 100,000 users), but convincing cases have been reported that resemble acute hepatitis and arise within 1 to 6 weeks of starting naproxen (Cases 1 and 2). The time to onset can be as long as 12 weeks, but convincing instances of liver injury arising after long term use have not been described. The pattern of serum enzyme elevations has ranged from hepatocellular to cholestatic injury. Immunoallergic features and autoantibodies are not common. In most instances, recovery is rapid once naproxen is stopped. Rare instances of acute liver failure attributed to naproxen have been published, but the role of naproxen in these cases was not very convincingly shown. Reviews of hepatotoxicity often

mention that naproxen is the least likely NSAID to cause serious liver injury (Sukasem et al., 2018)

Ginger (*ZingiberofficinaleRosc.*) belongs to the family *Zingiberaceae*. It originated in South-East Asia and then used in many countries as a spice and condiment to add flavor to food (Park et al., 2002). Besides this, the rhizome of ginger has also been used in traditional herbal medicine. The health-promoting perspective of ginger is attributed to its rich phytochemistry (Jolad et al. *2004;* Shukla et al., 2007*)* grouped fresh ginger into two wide range categories, i.e. volatiles and non-volatiles. Volatiles include sesquiterpene and monoterpenoid hydrocarbons providing the distinct aroma and taste of ginger. On the contrary, non-volatile pungent compounds include gingerols, shogaols, paradols, and zingerone (Jolad et al., 2004).

MATERIALS

Materials used for this experiment include:

Animal Cages, Weighing balance, Beaker, Measuring Cylinder, Animal Feeds, Conical Flask, Permanent Marker, Syringes (2mL and 5mL) and needle, Distilled water and Drinkers

Drugs and Chemicals

All Chemical drugs were obtained commercially and were of analytical grade. Naproxen was purchased from a pharmaceutical store.

Experimental Animals and Care

Twenty-five Wistar rats were used for this research; the animals were purchased from and housed in the Animal Care Unit Bingham University, Karu. The animals were kept in cages under normal environmental temperatures and were fed with a standard pellet diet and water and libitum. The rats were allowed to acclimatize to the laboratory environment for two weeks before the experiment commenced. This

research was carried out in Bingham University in accordance with the rules governing the care and use of laboratory animals as accepted internationally.

EXPERIMENTAL DESIGN

Liver damage was induced by oral administration of naproxen at a dose of 20mg/kg body weight, the treatment lasted for a period of four weeks and the group was treated with ginger supplements. The Wistar rats were divided into five groups of rats as follows;

Group I: This will served as the control group, the animals were fed with animal feed and water for two weeks.

Group II: Rats in this group were administered naproxen 20mg/kg body weight and ginger supplement 20g/kg of animal feed for two weeks.

Group III: Rats in this group were administered naproxen 20mg/kg body weight and ginger supplement 40g/kg of animal feed for two weeks.

Group IV: Rats in this group were administered naproxen 20mg/kg body weight and ginger supplement 60g/kg of animal feed for a period of two weeks.

Group V: This served as the negative control group. The animals were fed with animal feed and administered only naproxen 20mg/kg body weight for a period of two weeks.

SAMPLE COLLECTION ANALYSIS

All the animals were sacrificed at the end of the experiment. The rats were anaesthetized at the time of sacrifice by being placed in a sealed cotton wool soaked chloroform inhalation jar. Blood sample was collected directly from the heart via cardiac puncture from each Wistar rat and the sample obtained was used to determined:

i. Serum total protein

ii. Direct bilirubin

iii. L-Lactate dehydrogenase (LDH)

iv. Alkaline phosphatase (ALP)

v. Aspartate aminotransferase (AST)

vi. Alanine aminotransferase (ALT)

Albumin

DATA ANALYSIS

Data obtained were analyzed using One Way Analysis of Variance (ANOVA) a statistical package SPSSversion 23 was used and the results obtained were presented as mean ± standard error of mean (SEM) and an LSD post hoc test of multiple comparison was used and p values (P< 0.05) were considered statistically significant.

RESULT

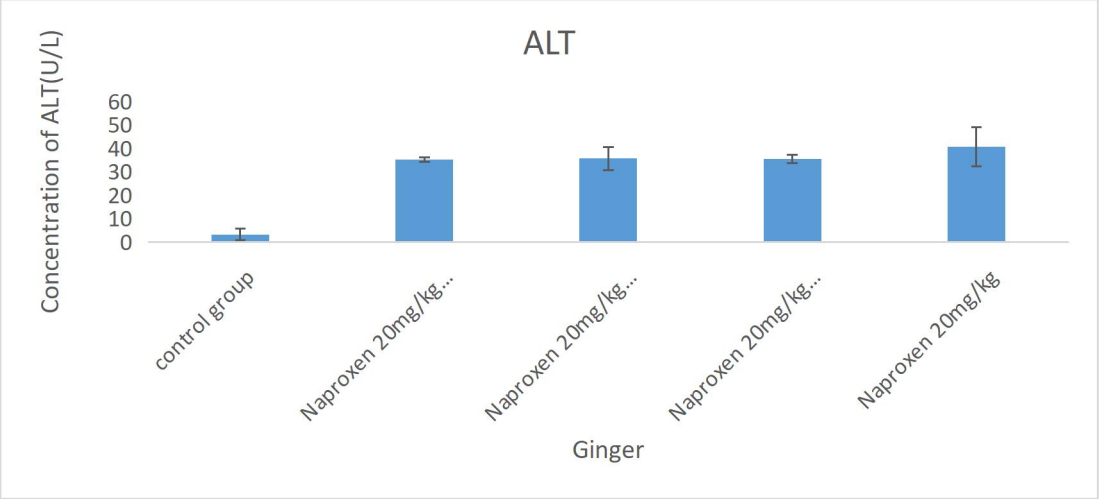

Figure 4.1 Effects of ginger and naproxen on serum ALT concentration in wistar rats. Values are represented as mean ± standard error of mean.
* stands for statistical significance p<0.05

4.2 SERUM AST

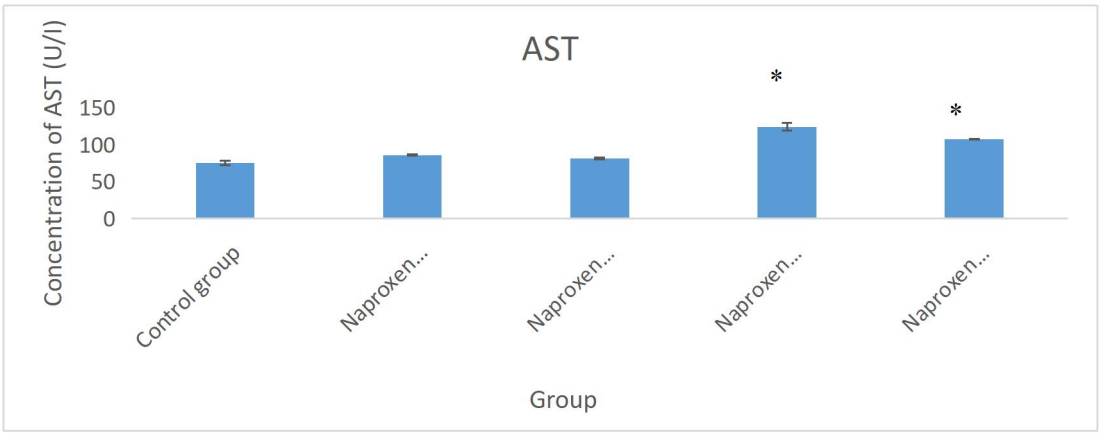

Figure 4.2 Effects of ginger and naproxen on serum AST concentration in Wistar rats. Values are represented as mean ± standard error of mean.
* stands for statistical significant $p<0.05$

4.3 SERUM ALP

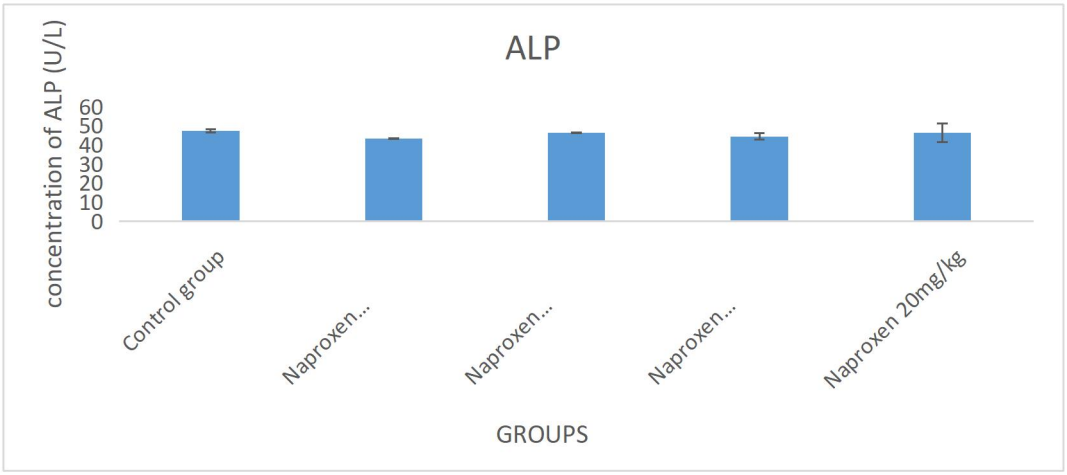

Figure 4.3 Effects of ginger and naproxen on serum ALP concentration in wistar rats. Values are represented as mean ± standard error of mean.
* stands for statistical significant $p<0.05$

4.4 SERUM LDH

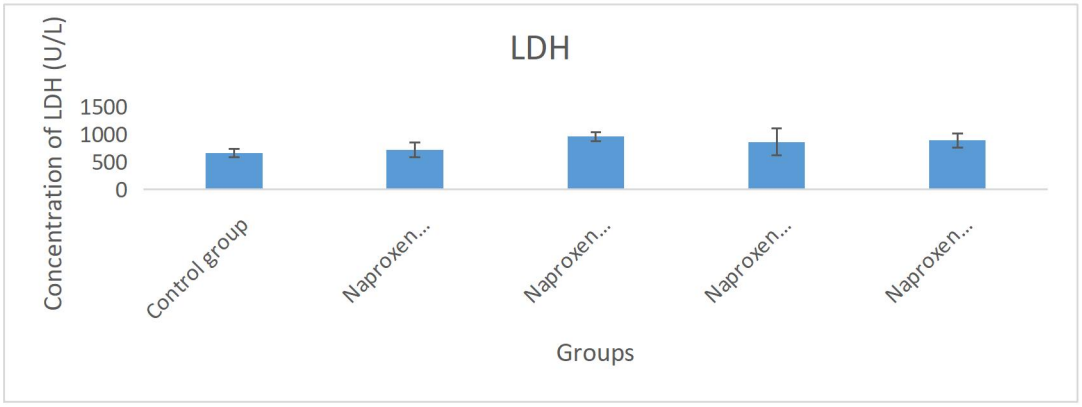

Figure 4.4 Effects of ginger and naproxen on serum LDH concentration in Wistar rats. Values are represented as mean ± standard error of mean.

* stands for statistical significance p<0.05

4.5 SERUM ALBUMIN

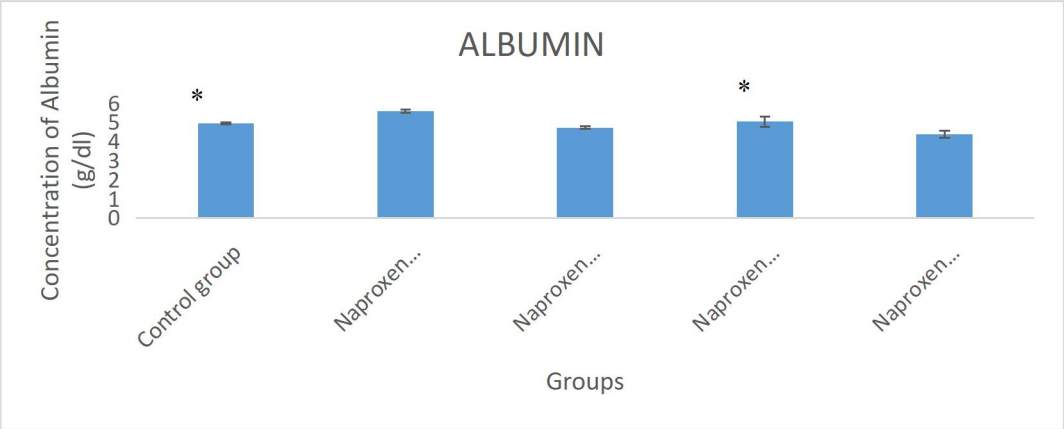

Figure 4.5 Effects of ginger and naproxen on serum albumin concentration in wistar rats.
Values are represented as mean ± standard error of mean.
* stands for statistical significance p<0.05

4.6 SERUM PROTEIN

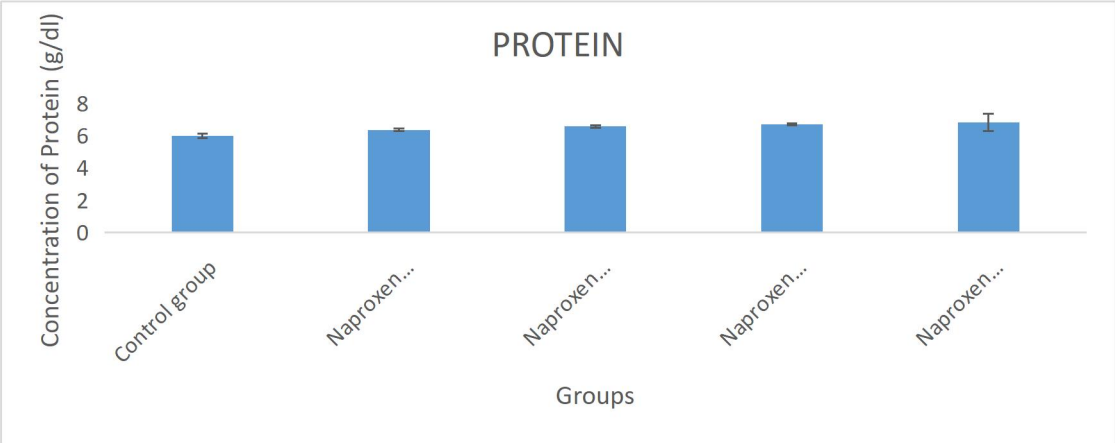

Figure 4.6 Effects of ginger and naproxen on total protein concentration in wistar rats.
Values are represented as mean ± standard error of mean.
* stands for statistical significance p<0.05

4.7 SERUM BILIRUBIN

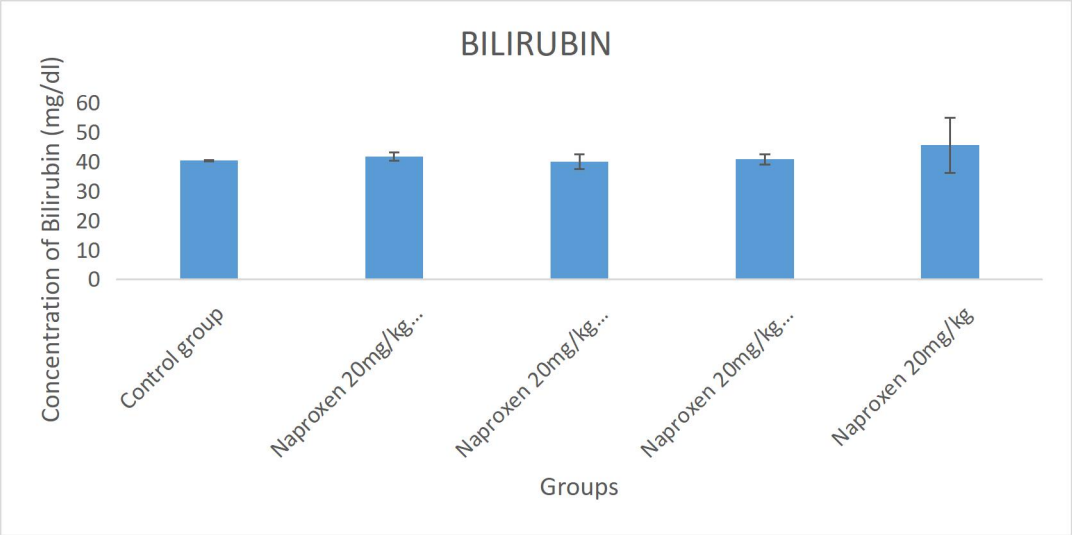

Figure 4.7 Effects of ginger and naproxen on serum bilirubin concentration in wistar rats.
Values are represented as mean ± standard error of mean.
* stands for statistical significance $p<0.05$

DISCUSSION

The result also shows a significant increase ($P<0.05$) in serum Aspartate Transaminase (U/I) (AST) in all the groups administered naproxen and those supplemented with 40g/kg of ginger (81.83±1.37) and 60g/kg of ginger (124.52±4.98) compared to the normal control group (75.87±3.09). AST activity is widely distributed across human tissues with the highest activity found in heart, liver, skeletal muscle, kidney and brain. An elevated AST activity may reflect tissue damage (plasma membrane disruption or apoptosis), plasma membrane bleb formation, increased tissue expression and macroenzymes (complexes of AST with plasma proteins). Serum AST activity is increased in patients with acute myocardial infarction in proportion with the extent of myocardial necrosis (Giuseppe et al., 2021) Analysis for serum Lactate Dehydrogenase (U/L) (LDH) shows that there was a significant increase ($P<0.05$) in serum lactate dehydrogenase in all groups administered naproxen however, treatment with various supplements of ginger could not reverse the elevated LDH when compared to the normal control group. Lactate

dehydrogenase is an enzyme that is present in almost all body tissues. Conditions that can cause increased LDH in the blood may include liver disease, anemia, heart attack, bone fractures, muscle trauma, cancers, and infections such as encephalitis, meningitis, encephalitis, and HIV (Drent et al., 1996). the increased LDH maybe a clear indication of damage to some organs caused by naproxen. Also, there was an increase in total protein level (g/dl) in all groups treated with the various fractions of ginger and the naproxen group (6.85±0.52) as compared to the normal control group (6.02 ± 0.14) with no statistical significance ($P<0.05$).

The result obtained for Albumin(g/dl) shows that the naproxen group (4.39±0.19) and the group treated with 40g/kg of ginger (4.72±0.06) significantly ($P<0.05$) decreased serum albumin as compared to the control group (4.94±0.05), while the group treated with 20g/kg of ginger (5.59±0.07) significantly increased ($P<0.05$) compared to the control group (4.94±0.05). Whereas the group treated with 60g/kg ginger supplement showed a statistical significant increased in serum albumin (5.03±0.28)when compared to the control group (4.94±0.05).

The analysis for total bilirubin (uMol/L) shows a significant increase ($P<0.05$) in the naproxen group (45.59± 9.37) and all the groups treated with the various fractions of ginger as compared to the normal control group (40.39± 0.24). The group treated with 40g/kg of ginger (39.98± 2.53) significantly decreased($P<0.05$) as compared to the normal control group (40.39± 0.24).

This study shows that administration of naproxen for 14 days in Wistar rats causes an increase in the serum concentrations of AST, ALT, LDH and protein which are biomarkers for liver damage. The most prominent result of liver damage is the release of the intracellular enzymes AST, ALP and ALT from the liver into the blood. The serum concentrations of AST, ALT, LDH and protein can serve as indicators of the

state of the liver. Higher levels of AST, ALT, LDH and protein are indicators of liver damage. Therefore, the increase in serum concentration of AST, ALT, LDH and protein after naproxen administration reveals the liver damage caused by naproxen in Wistar rats. The close relationship between naproxen and the liver is due to the fact that naproxen is metabolized by the cytochrome P450 enzymes in the liver. Naproxen generates free radicals resulting in the generation of reactive oxygen species (ROS). ROS generation induces oxidative stress and is associated with cell death. Naproxen has been associated with liver injury; the mechanism is thought to be immunological idiosyncracy (Sriuttha et al., 2018).

Ginger has anti-oxidative and anti-inflaflammatory effects. Previous studies have shown that ginger and its active compunds can exhibit anti-diabetes, anti-cancer and anti-inflammatory properties (Thomson et al., 2002). Ginger is an antioxidant that is known to play a role in boosting the immune system. The varying levels of serum Albumin and Bilirubin indicate the liver damage caused by naproxen toxicity.

5.2 CONCLUSION

In conclusion, this study revealed that naproxen administration to Wistar rats induces liver damage due to increase in the biomarkers of liver injury AST, ALP and ALT and the administration of ginger supplement at the graded doses of 20, 40 and 60g/kg of animal feed relieves the cytotoxicity of naproxen.

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