ACTIVITY OF THE SEMI-PURIFIED FLAVONOIDS FROM DANDELION (*Taraxacum officinale*) AND KANGKONG (*Ipomea aquatica*) ETHANOLIC LEAF EXTRACTS AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY MALE WISTAR RATS (*Rattus norvegicus*)

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ABSTRACT

The aim of this study is to determine the hepatoprotective effect of the semi-purified flavonoids from Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) ethanolic leaf extracts against acetaminophen-induced male Wistar rats (*Rattus norvegicus*) which weigh 120-140 grams. The test subjects were divided into 5 groups namely: Treatment 1 treated with Semi- Purified Flavonoids from Dandelion (*Taraxacum officinale*) Ethanolic leaf extract, Treatment 2 treated with Semi-Purified Flavonoids from Kangkong (*Ipomea aquatica*) Ethanolic Leaf Extract, Treatment 3 treated with Mixture of the Semi-purified Flavonoids from Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) Ethanolic leaf extract, Treatment 4 treated with Silymarin (Silybum marianum) and Treatment 5 treated with Normal saline solution (NSS). The test subjects were subjected to the induction of 250mg/kg acetaminophen via oral gavage for 7 days. The leaves of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) were macerated with 70% ethanol and evaporated with sufficient amount of time. The extract was filtered, then evaporated to dryness, and weighed. The flavonoids were obtained. The test subjects were given treatments for 10 days. Results of the study revealed that there is no significant difference between the Semi-Purified Flavonoids from mixed Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) and Silymarin (*Silybum marinum*).

Key words: *hepatoprotective, kangkong, dandelion, sylimarin*

INTRODUCTION

Liver is one of the most complex organs of the gastrointestinal tract. It performs a fundamental role in the regulation of diverse physiological processes, and its activity is related to different vital functions, such as metabolism, detoxification of endogenous and exogenous substances, storage and secretion. Because of all of these functions, hepatic diseases continue to be among the principal threats to public health leading to a worldwide problem (Vargas-Mendoza et al., 2014). Dandelion (*Taraxacum officinale*) is distributed widely as a member of the daisy family and has been used since the 10th century as a medicinal plant grown in temperate zones. Kangkong (*Ipomea aquatica*) normally flourishes in water and moist soil found in most Southeast Asian countries. The different

constituents of these plants, most especially the flavonoids are the focus of the study.

As per Philippine Council for Health Research and Development, there are hundreds of millions of people with hepatitis, including Filipinos. The World Health Organization said that Hepatitis B and C infections lead to chronic liver disease, which is the most common cause of cirrhosis and liver cancer. Many effective drugs to combat viral Hepatitis B and C are only slightly more accessible now due to prohibitive cost in the Philippines. Most medicinal plants occur naturally in a large number of countries. WHO has estimated that perhaps 80% of the more than 4000 million inhabitants of the world rely chiefly on traditional medicines for their primary health care needs, and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts or their active principles (Farnsworth & Akerele, 1985).

The fact that hepatic problems are responsible for the number of deaths recorded worldwide, the number of available commercial drugs for liver diseases are very limited. The World Health Organization has stressed the need to develop drugs from plant origin, which will be: inexpensive; accessible to the rural people in the developing countries; and will show less/no side effects. Therefore, the researchers had decided to make an alternative prevention in having hepatotoxicity using the leaves-extract from Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*).

Research Questions

The aim of this study was to determine the hepatoprotective effect of the semi-purified flavonoids from Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) ethanolic leaf extracts against acetaminophen-induced male Wistar rats (*Rattus norvegicus*). Specifically, it aimed to answer the following questions:

1. What is the hepatoprotective activity of the concentrations of the following?
    a. Dandelion (*Taraxacum officinale*) ethanolic leaf-extract
    b. Kangkong (*Ipomea aquatica*) ethanolic leaf-extract
    c. Mixed Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) ethanolic leaf-extracts.
    d. Silymarin (Silybum marianum)
2. Is there a significant difference in hepatoprotective activity when grouped according to plant extracts/ treatment administered?
3. Is there a significant difference in hepatoprotective activity when grouped according to days after acetaminophen induction?
    a. Kangkong (*Ipomea aquatica*) vs Dandelion (*Taraxacum officinale*)
    b. Kangkong (*Ipomea aquatica*) vs Silymarin (*Silybum marianum)*

Hypotheses

- There is no significant difference in the activity of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*).
- There is no significant difference in the activity of Dandelion (*Taraxacum officinale*) versus Silymarin (Silybum marianum)
- There is no significant difference in the activity of Kangkong (*Ipomea aquatica*) and Silymarin (Silybum marianum).
- There is no significant difference in the activity mixed leaf extracts and Silymarin (Silybum marianum).

Significance of the Study

This study is significant to the community through: providing a safe and accessible source of therapy for people especially to those at remote places where healthcare services are difficult to avail; and increasing awareness to an alternative and natural remedy for preventing liver disease. This study will provide the baseline for the development of the Medical Technology field of research by providing information for future reference.

Literature Review

Kangkong (*Ipomoea aquatica*)

Kangkong (Ipomoea aquatica), which belongs to the family Convolvulaceae is supposed to be originated in China and is usually consumed as a green leafy vegetable.

This plant is widely distributed all around the World, especially India, Malaysia, Indonesia, China, Hong Kong and some parts of USA. This plant is grown as an aquatic plant which grows abundantly in marshy areas. Various parts of the Kangkong (*Ipomea aquatica*) plant are used medicinally in Southeastern Asia and reported to be useful for the treatment of liver diseases.

The chemical constituents of this plant have revealed the presence of carotenes such as β-carotene, cryptoxanthin, lutein, lutein epoxide, violaxanthin and neoxanthin, flavonoids such as mycertin, quercetin, luteolin, and apigenin and some alkaloids. Research has shown that kangkong (*Ipomea aquatica*) was effective in the prevention of hepatic damage in rats. The results show that the protective effect of kangkong (*Ipomea aquatica*) in liver damage might contribute to its modulation on detoxification enzymes and its antioxidant and free radical scavenger effects. Moreover, it confirms the use of kangkong (*Ipomea aquatica*) traditionally for the treatment of liver disorders (S. Alkiyumi, & A. Abdullah, 2012).

Dandelion (*Taraxacum officinale*)

Dandelion (*Taraxacum officinale*) is a common meadow herb of the Asteraceae or sunflower family. Dandelion (*Taraxacum officinale*) is a rich source of vitamins A, B complex, C, and D, as well as minerals such as iron, potassium, and zinc (Hu & Kitts, 2003). Dandelion (*Taraxacum officinale*) has been used in folklore medicine andTraditional Chinese medicine in the treatment of inflammation and several women's diseases such as breast and uterine cancers (Choi et al., 2010). Dandelion leaves produce a diuretic effect while the roots act as an antiviral agent, appetite stimulant, digestive aid, and may help promote gastrointestinal health (Al-Malki, Kamel, Golayel, Elnaga & Al-Beshriand, 2013). It also has anti-angiogenic, anti-inflammatory and anti-noceptive properties (Jeon et al., 2008). Particular attention has been given also to its diuretic, choleretic, anti-inflammatory, anti-oxidative, anti-carcinogenic, analgesic, anti-hyperglycemic, anti-coagulatory and prebiotic effects (Schutz, Carle & Schieber, 2006). The perennial weed has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments such as dyspepsia, heartburn, spleen and liver complaints, hepatitis and anorexia. However, its use has mainly been based on empirical findings. This contribution provides a comprehensive review of the pharmacologically relevant compounds of *Taraxacum officinale* characterized so far and of the studies supporting its use as a medicinal plant. The extensive literature survey reveals the fact that *Taraxacum officinale* or dandelion to be safe and the available evidence on the mechanisms of action appears promising (Devaraj, 2016). The different chemical constituents of the plant include alkaloids, saponins, tannins and cardiac glycosides (Berezi, Monago, & Adelagun, 2013).

Silymarin (*Silybum marianum*)

Silymarin (*Silybum marianum*) is an extract of Silymarin (*Silybum marianum*) herb and a pharmaceutical plant. The therapeutic use of the plant dates back 2000 years, and in ancient Greek sources, it is stated as a liver protective agent. In recent times, several studies have investigated the impact of Silymarin (*Silybum marianum*) on various liver disorders and in most cases, it has many beneficial effects and no significant side effects mentioned. Herbal medicine has always been a cost-effective and less expensive remedy. Silymarin (*Silybum marianum*) is an herbal medicine with high therapeutic effects (Baghbahadorani & Miraj, 2017).

The mechanisms of action of Silymarin (*Silybum marianum*) involve different biochemical events, such as the stimulation of the synthetic rate of ribosomal RNA (rRNA) species through stimulation of polymerase I and rRNA transcription, protecting the cell membrane from radical-induced damage and blockage of the uptake of toxins such as alpha-amanitin. Studies in patients with liver disease have shown that Silymarin (*Silybum marianum*) increases superoxide

dismutase (SOD) activity of lymphocytes and erythrocytes, as well as the expression of SOD in lymphocytes. Silymarin (*Silybum marianum*) has also been shown to increase patient serum levels of glutathione and glutathione peroxidase. Silymarin (Silybum marianum) 420 mg/day was also shown to improve indices of liver function [AST, ALT, gamma-glutamyl transferase and bilirubin] in patients with liver disease of various etiology, including those exposed to toxic levels of toluene or xylene (Wellington & Jarvis, 2001).

The Silymarin (Silybum marianum) exerts membrane-stabilizing and antioxidant activity, it promotes hepatocyte regeneration; furthermore, it reduces the inflammatory reaction and inhibits the fibrogenesis in the liver. Based on the results of studies usingmethods of molecular biology, Silymarin (*Silybum marianum*) can significantly reduce tumor cell proliferation, angiogenesis as well as insulin resistance. Furthermore, it exerts an anti-atherosclerotic effect and suppresses tumor necrosis factor-alpha-induced protein production and mRNA expression due to adhesion molecules. The chemopreventive effect of Silymarin (*Silybum marianum*) on HCC has been established in several studies using in vitro and in vivo methods; it can exert a beneficial effect on the balance of cell survival and apoptosis by interfering cytokines. In addition to this, anti-inflammatory activity and the inhibitory effect of Silymarin (*Silybum marianum*) on the development of metastases have also been detected. In some neoplastic diseases Silymarin (*Silybum marianum*) can be administered as adjuvant therapy as well (Feher & Lengyel, 2012).

Hepatoprotection

The liver is a vital organ in the body that is primarily responsible for the metabolism of endogenous and exogenous agents. It plays an important role in drug elimination and detoxification. Liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anemia and medications (Mroueh et al., 2004). Hepatic disease is a term that indicates damage to the cells, tissues, structure, or liver function, and this damage can be induced by biological factors (bacteria, virus, and parasites) and autoimmune diseases (immune hepatitis, primary biliary cirrhosis), as well as by the action of different chemicals such as some drugs [high doses of paracetamol and antitubercular drugs], toxic compounds [carbon tetrachloride(CCl4), thioacetamide, dimethylnitrosamine (DMN), D-galactosamine/lipopolysaccharide ride (GaIN//LPS)], and unquestionably excessive consumption of alcohol (Casafont, 2008).

Flavonoids

Flavonoids are a large family of polyphenolic plant compounds that carry out important functions in plants, including attracting pollinating insects; combating environmental stresses, such as microbial infection; and regulating cell growth. They are diverse and the large group of phytonutrients (plant chemicals) found in

almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables (Kumar, 2013). Flavonoids content and hepatoprotective activity of Senna surattensis leaf extract (Caesalpiniaceae) were investigated. Liver damaged was induced thru the intraperitoneal injection of CCl4 and the administration of 100mg/kg of 80% of ethanol extract of Senna surattensis were administered for one month. The significant decrease in the ALT, ALP and AST level in plasma indicates the efficacy of Senna surattensis leaf extract as hepatoprotective (El-Sawi, 2010).

Research Paradigm

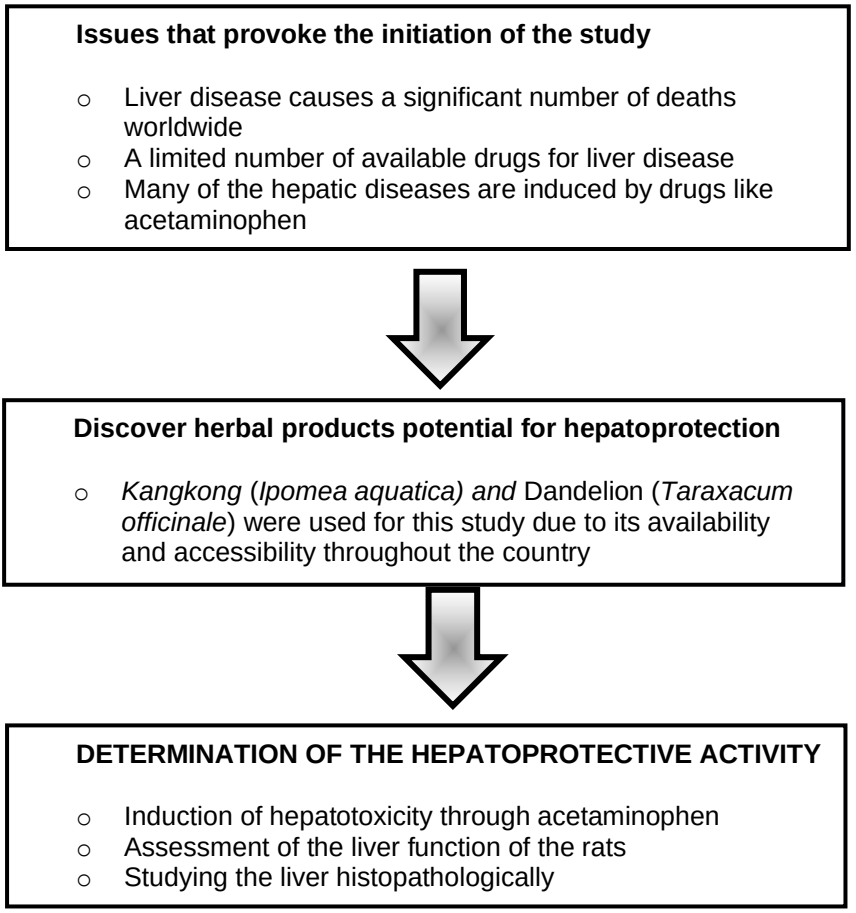

Figure 1. *Research Paradigm*

This paradigm shows the reasons why this study is being conducted and the ways to prove the activity of our extract.

METHODS

Research Design

The research design used in this study is experimental.

Subjects of the Study

Healthy male Wistar rats weighing 120-140 grams were randomly selected. The animals were acquired from Cagayan Valley Herbal Processing Plant Laboratory Carig Sur, Tuguegarao City in a temperature and light controlled room with free access to food and drinking water.

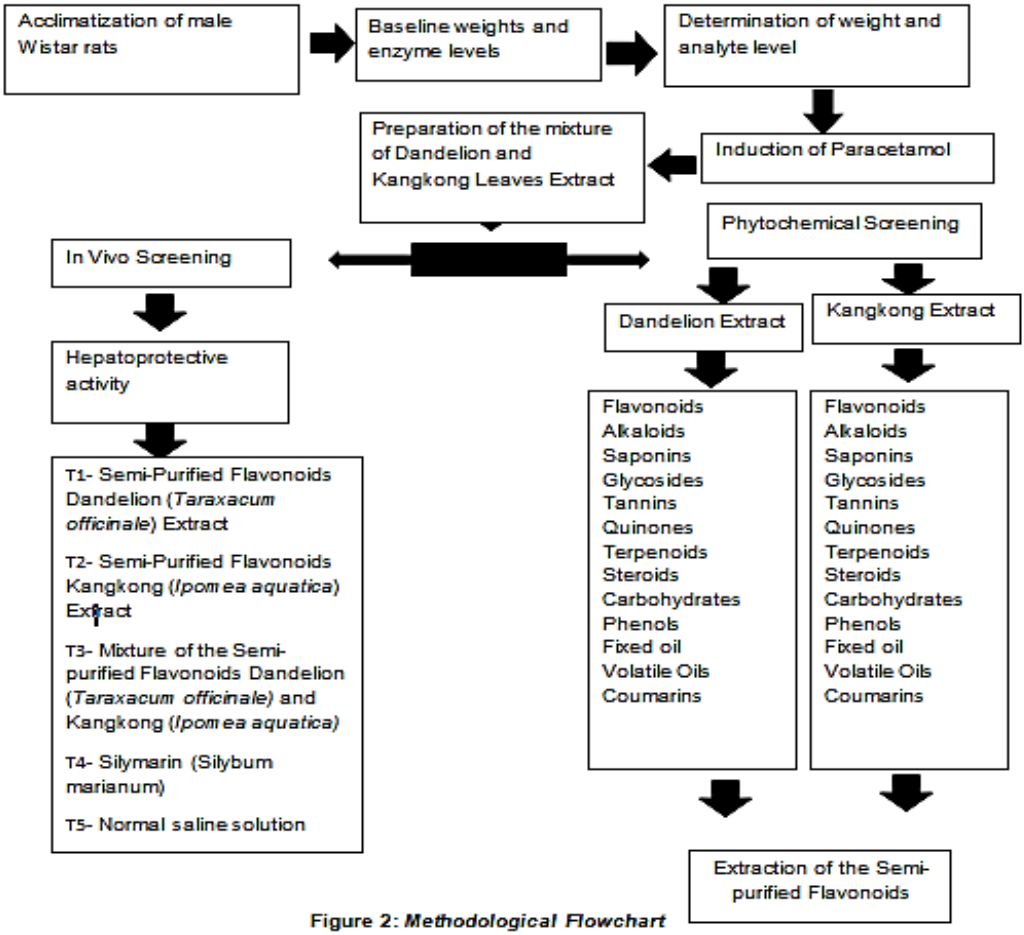

Figure 2: *Methodological Flowchart*

Data Collection, Instruments and Procedures

1. Preparation of the Subjects

The experimental animals were acclimatized for fourteen days before any treatment is administered and by the assistance of Veterinarian. Conditions such as room temperature, pathogen-free, 12-hour light/ 12-hour dark cycle, oxygen, good humidity level and free access to water were observed regarding the housing of the rats to ensure that their condition will not be affected by the lack of these factors.

2. Baseline Enzyme and Weight

The weights of the subjects were evaluated. The Clinical enzymes were determined namely, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST).

3. Collection of Plant Material

The fresh leaves of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) from Baguio City during the month of July were gathered and authenticated at the Department of Agriculture, Carig, Tuguegarao City, Cagayan.

4. Preparation and Extraction of the Semi- purified Flavanoids of Dandelion and Kangkong Ethanolic Leaves Extract

Fresh leaves of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) weighing 500 grams ws used in the experiment. The Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) leaves that were collected were washed in a running tap water to remove the dust. The leaves were dried under the shade, pulverized to produce a powder and then accurately weighed prior to extraction. After weighing, the powdered leaves were macerated with 70% ethanol and evaporated with a sufficient amount of time. The extract was filtered, evaporated to dryness, and weighed. Percentage yield was calculated (Trifunschi, & Ardelean, 2013).

5. Phytochemical Screening

Phytochemical screening of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) ethanolic leaf extract were assessed by standard method as described by Guevarra et al. (2005).

5.1. Test for Tannins: One mL of the leaf extract was added to 1 mL of 5% ferric chloride. Formation of dark blue or greenish black indicates the presence of tannins.

5.2. Test for Saponins: One mL of the leaf extract was added to 1 mL of distilled water and shaken in graduated cylinder for 15 min; lengthwise formation of 1 cm layer of foam indicates the presence of saponins.

5.3. Test for Flavonoids: One mL of the leaf extract was to 1 mL 2N sodium hydroxide. Formation of yellow color indicates the presence of flavonoids.

6. Induction of Liver Toxicity

The induction of liver toxicity using acetaminophen was carried out through oral administration using gavage four (4) times a day for seven (7) days.

Acetaminophen LD50= 2402 mg/kg Average weight = 0.130kg
Dose=250mg/kg
(2402mg/kg)/4=600.5 mg/kg
(600.5 mg/kg*0.130kg)/(250mg/5ml)=1.5613 ml daily

7. Administration of Treatments

The male Wistar rats were grouped according to their weight and the level of ALT, AST and TPAG, they were divided for the administration of treatments. Treatment 1 was treated with Semi-Purified Flavonoids from Dandelion (*Taraxacum officinale*) Ethanolic leaf extract (50mg/kg). Treatment 2 was treated with Semi-Purified Flavonoids from Kangkong (*Ipomea aquatica*) Ethanolic leaf extract (50mg/kg). Treatment 3was treated with the mixture of the Semi-Purified Flavonoids.

The Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) leaves that were collected were washed in a running tap water to remove the dust. The leaves were dried under the shade, pulverized to produce a powder and then accurately weighed prior to extraction. After weighing, the powdered leaves were macerated with 70% ethanol and evaporated with a sufficient amount of time. The extract was filtered, evaporated to dryness, and weighed.

Table 1. *Administration of Treatments*

Treatments	Acclimatization for 14 days	Induction of Acetaminophen for 7 days	Baseline weight and enzymes	Day 1 to Day 10	Determination of AST and ALT Levels
1	✓	250mg/5ml	✓	50mg/kg of Dandelion	✓
2	✓	250mg/5ml	✓	50mg/kg of Kangkong	✓
3	✓	250mg/5ml	✓	100mg/kg of Mixed	✓

				Dandelion	
4	✓	250mg/5ml	✓	125mg/kg of Silymarin	✓
5	✓	250mg/5ml	✓	NSS	✓

Table 1 shows the step by step process conducted in the treatments 1 to 5 used in the research study beginning from the acclimatization for 14 days up to the determination of AST and ALT levels. Treatment 4 was treated with 125mg/kg Silymarin (*Silybum marianum*). And treatment 5 was treated with Normal Saline Solution (NSS).

Statistical Tool

The results gathered were tabulated and subjected to statistical treatment utilizing one-way analysis of Variance (ANOVA) using 0.05 level of significance and multi comparisons.

The data presented in tables were analyzed and further interpreted to give a clear and accurate presentation on the hepatoprotective activity of theSemi-Purified Flavanoids from Dandelion and Kangkong ethanolic leaves extract against male wistar rats.

Disposal of Animals

Dead animals, animal tissues and excreta, bedding, unused food were collected in leak-proof metal or plastic containers with leak-proof, disposable liners and tight lids. Liners were essential for animal tissues, carcasses, and radioactive or toxic waste. Infectious wastes were incinerated on the site. All wastes were sterilized or autoclave before removal. Gamma irradiation is a relatively recent method of disinfection of waste products which may come into more prominent use (Garcia, Brooks, Stewart et al. 1987). Wastes which cannot be rapidly disposed of were stored in a cold storage area provided for that purpose. Such areas were vermin-free, easily cleaned and disinfected as well as being physically separated from other storage facilities. The waste storage area was located so that wastes need not be carried through other rooms of the facility. Dead animals should be removed from cages as soon as they are noticed. The laboratory animal Veterinarian, who should have been immediately informed of sick animals, was informed of dead ones. Dead animals were properly identified, placed in disposable plastic bags and were brought to the postmortem area immediately upon discovery. In the postmortem area, they were held under refrigeration for necropsy or for disposal in accordance with the investigator's instructions (HWC/MRC). The disposal of animals was off by PITHAC and with the assistance of the Veterinarian.

Ethical Considerations

The collection of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) leaves followed the protocols provided so as not to harm the it. The plants, instrumental value, relational value, and inherent worth were secured upon the conduct of the study.

The researchers had used rats for the purpose of accomplishing this study and were responsible for responding effectively for the housing and care of these testing animals. The researchers were responsible for the provision of the appropriate measures for the maintenance of the well-being and health of the rats. The researchers had performed test methods (Blood collection, Treatment Induction) that had lessened the pain or distress in animals and had enhanced animal well-being.

RESULTS

Table 2. *Chemical Constituents of the Ethanolic Leaf Extract of Dandelion (Taraxacum offiicinale)*

Chemical Constituent Tested	Result
Flavonoids	+
Tannins	+
Saponins	-

From the information given in the table, Flavanoids and Tannins were positive in the ethanolic leaf extract of Dandelion (*Taraxacum offiicinale*).

Table 3. *Chemical Constituents of the Ethanolic Leaf Extract of Kangkong (Ipomea aquatica)*

Chemical Constituent Tested	Result
Flavonoids	+
Tannins	+
Saponins	-

It is significantly noted that Flavanoids and Tannins were positive in the ethanolic leaf extract of Kangkong (*Ipomea aquatica*).

Table 4.1. *Liver Enzyme Levels of the Different Treatments Groups after Acetaminophen Induction*

Treatment Groups	Trial 1		Trial 2	
	ALT Levels	AST Levels	ALT Levels	AST Levels
1	144.568	167.814	152.218	162.498
2	141.366	155.722	153.396	180.5
3	111.914	163.686	96.984	155.712
4	108.032	127.294	101.72	129.98
5	108.534	130.502	136.94	155.376

The table above presents that levels of AST and ALT obtained from the different groups of subjects which provides a measure of the liver function of test subjects. It can be noted that all test subjects manifested elevated levels of serum ALT and AST after 7 days of Acetaminophen induction which simulates hepatotoxicity.

Table 4.2. *Liver Enzyme Levels of the Different Treatments Groups after Administration of Treatments*

Treatment Groups	Trial 1		Trial 2	
	ALT Levels	AST Levels	ALT Levels	AST Levels
1	113.332	138.402	97.782	153.322
2	87.524	108.674	120.586	160.182
3	72.388	147.338	61.862	100.866
4	74.6	99.018	92.858	116.646
5	104.184	126.6	132.504	151.472

The table above presents the AST and ALT levels of the test subjects according to the different groups after 10 days of administration of the different treatments. It can be observed that the serum ALT and AST levels of the test subjects have decreased compared to the levels in Table 4.1.

Table 5.1. *Test of Significant Difference in the ALT Liver Enzyme Levels before and after Administration of the Different Treatments*

Treatment Groups	t-value	p-value	Decision
1	5.639	.000	Reject Ho
2	6.009	.000	Reject Ho
3	5.117	.001	Reject Ho
4	2.585	.029	Reject Ho
5	.728	.485	Accept Ho

It can be gleaned from the table above that the liver function as manifested by the ALT levels significantly improved for all treatment groups except for the group that was given NSS (Group 5). This therefore indicates that treatments 1-4

significantly improved the liver functions of the test subjects signifying a favorable hepatoprotective activity.

Table 5.2. *Test of Significant Difference in the AST Liver Enzyme Levels Before and after Administration of the Different Treatments*

Treatment Groups	t-value	p-value	Decision
1	4.472	.002	Reject Ho
2	3.437	.007	Reject Ho
3	4.034	.003	Reject Ho
4	6.121	.000	Reject Ho
5	-.967	.359	Accept Ho

It can be gleaned from the table above that the liver function as manifested by the AST levels significantly improved for all treatments groups except for the group that was given NSS (Group 5). This therefore indicates that treatments 1-4 significantly improved the liver functions of the test subjects signifying a favorable hepatoprotective activity.

Table 6. *Test of Significant Difference in the Liver Enzyme Levels of the Different Groups after Administration of the Different Treatments*

Liver Enzyme Level	F-value	p-value	Decision
ALT	9.184	0.000	Reject Ho
AST	1.026	0.393	Accept Ho

The table above indicates that the activity of the different treatments is significantly the same with the positive control in improving the AST levels of the test subjects.

Table 6.1. *Test of Significant Difference in the ALT Liver Enzyme Levels of the Different Groups after Administration of the Different Treatments*

Treatment Group	Mean	1	2	3	4
1					
2		0.861			
3		0.000*	0.000*		
4		0.015*	0.023*	0.059	

**significant at 0.05*

The table above indicates that treatment 3 (mixed Dandelion and Kangkong) exhibited significantly the same activity as the positive control (Silimarin) in improving the serum ALT levels of the test subjects.

DISCUSSION

This research study was intended to determine the Activity of the Semi-Purified Flavonoids from the Ethanolic leaf extract of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) against Acetaminophen-induced Hepatotoxicity in Wistar rats (*Rattus norvegicus*). The researchers conducted phytochemical screening, induction of hepatotoxicity and determination of enzyme levels to attain the objectives of this study.

Based on the results of the gathered data, the phytochemical screening of the ethanolic extract of Kangkong (*Ipomea aquatica*) revealed that flavonoids are present in it. In many research findings, the presence of flavonoids showed evidently the effectiveness of hepatoprotective activity (Dua et al., 2015). Presence of valuable phytoconstituents flavonoids were reported in the ethanolic extract of Dandelion (*Taraxacum officinale*) and showed evidence for activity and that it exhibits strong inhibitory activities against pancreatic lipase in vitroandin vivo (Zhang et al., 2008)

Hepatotoxicity was induced by an oral administration of Acetaminophen with a dose of 250mg/5ml and liver function tests were measured. Silymarin was used as a reference drug for comparing the activity of the ethanolic extract of Dandelion and Kangkong. In the statistical analysis conducted, it showed that there is no significant difference between the positive control (Silymarin) and the mixture of the semi-purified flavonoids from Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) ethanolic leaf extract. The mixture of the semi-purified flavonoids from Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) ethanolic leaf extract has significant difference among the semi-purified flavonoids from Dandelion (*Taraxacum officinale*) ethanolic leaf extract and semi-purified flavonoids from Kangkong (*Ipomea aquatica*) ethanolic leaf extract. As evidence on the results based on the number of days, it was found that it showed significant difference in the lowering of the ALT and AST enzyme levels.

CONCLUSION

According to the result of the study, ethanolic mixed leaf extract of the semi-purified flavonoids of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) has a comparable hepatoprotective agent with Silymarin in dose of 100mg/kg in decreasing liver enzyme parameters. The quantitative similarity of both leaf extracts and Silymarin could be due to their similar mode of action as hepatoprotective agents. In addition, the distinct hepatoprotective activity of the mixed extracts was possibly due to the presence of flavonoids. Thus, Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) ethanolic leaf extracts are good choice to utilize as a medicine in decreasing the liver enzymes of intoxicated liver. Further, studies with individual active compounds occurred in mixed extracts in progress will support the exact mechanism of hepatoprotective action.

RECOMMENDATIONS

Based on the aforementioned findings and conclusions drawn, the following recommendations and suggestions are deemed important:

- Researchers should study more on the effects of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*) components in human clinical trials.
- The community and concerned agency should promote continuous plantation, cultivation and growth of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*).
- Researchers might consider increasing the frequency of administration of the doses prepared.
- Researchers may consider using other varieties of Dandelion (*Taraxacum officinale*) and Kangkong (*Ipomea aquatica*).
- Researchers may use Total protein albumin globulin (TPAG) as baseline enzyme.

REFERENCES

Adeyemi, O. T., Osilesi, O., Adebawo, O. O., Onajobi, F. D., Oyedemi, S. O., & Afolayan, A. J. (2015). Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) Activities in selected tissues of rats fed on processed atlantic horse mackerel (Trachurus trachurus). *Advances in Bioscience and Biotechnology*, *6*(03), 139.

Al-Malki, A. L., Abo-Golayel, M. K., Abo-Elnaga, G., & Al-Beshri, H. (2013). Hepatoprotective effect of dandelion (Taraxacum officinale) against induced chronic liver cirrhosis. *Med Plants Res*, *7*, 1494-505.

Ahsan, M. R., Islam, K. M., Bulbul, I. J., Musaddik, M. A., & Haque, E. (2009). Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Eur J Sci Res*, *37*(2), 302-310.

Alkiyumi, S. S., Abdullah, M. A., Alrashdi, A. S., Salama, S. M., Abdelwahab, S. I., & Hadi, A. H. A. (2012). Ipomoea aquatica extract shows protective action against thioacetamide-induced hepatotoxicity. *Molecules*, *17*(5), 6146-6155.

Baghbahadorani, F. K., & Miraj, S. (2017). The impact of silymarin on improvement of hepatic abnormalities in patients with severe preeclampsia: A randomized clinical trial. *Electronic physician*, *9*(8), 5098.

Berezi, E. P., Monago, C., & Adelagun, R. O. A. (2013). Haematological profile of rats treated with aqueous extracts of common dandelion leaf (Taraxacum officinale Weber) against carbon tetrachloride (CCl4) toxicity. *Inter J Biochem Biotech*, *2*(1), 263-267.

Celinski, K., Konturek, P. C., Slomka, M., Cichoz-Lach, H., Brzozowski, T., Konturek, S. J., & Korolczuk, A. (2014). Effects of treatment with melatonin and tryptophan on liver enzymes, parameters of fat metabolism and plasma levels of cytokines in patients with non-alcoholic fatty liver disease–14 months follow up. *J Physiol Pharmacol*, *65*(1), 75-82.

Choi, U. K., Lee, O. H., Yim, J. H., Cho, C. W., Rhee, Y. K., Lim, S. I., & Kim, Y. C. (2010). Hypolipidemic and antioxidant effects of dandelion (taraxacum officinale) root and leaf on cholesterol-fed rabbits. *International journal of molecular sciences*, *11*(1), 67-78.

Dua, T. K., Dewanjee, S., Gangopadhyay, M., Khanra, R., Zia-Ul-Haq, M., & De Feo, V. (2015). Ameliorative effect of water spinach, Ipomea aquatica (Convolvulaceae), against experimentally induced arsenic toxicity. *Journal of translational medicine*, *13*(1), 81.

Devaraj, E. (2016). Hepatoprotective properties of Dandelion: recent update. *Journal of Applied Pharmaceutical Science*, *6*(04), 202-205.

Dong, N. T. K., Van Thu, N., Ogle, B., & Preston, T. R. (2006). Effect of supplementation level of water spinach (Ipomoea aquatica) leaves in diets based on para grass (Brachiaria mutica) on intake, nutrient utilization, growth rate and economic returns of crossbred rabbits in the Mekong Delta of Vietnam. *Livestock Research for Rural Development*, *20*(9).

El-gengaihi, E, Hassan, M., Farouk, A., Refaee, V.et al. (2016). Hepatoprotective of Dandelion (Taraxacum officinale) against liver damage induced by carbon tetrachloride in male rats ISSN: 0975-7384 CODEN (USA): JCPRC5

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., & Guo, Z. (1985). Medicinal plants in therapy. *Bulletin of the world health organization*, *63*(6), 965.

Feher, J., & Lengyel, G. (2012). Silymarin in the prevention and treatment of liver diseases and primary liver cancer. *Current pharmaceutical biotechnology*, *13*(1), 210-217.

Hu, C., & Kitts, D. D. (2003). Antioxidant, prooxidant, and cytotoxic activities of solvent-fractionated dandelion (Taraxacum officinale) flower extracts in vitro. *Journal of agricultural and food chemistry*, *51*(1), 301-310.

Hu, G., Wang, J., Hong, D., Zhang, T., Duan, H., Mu, X., & Yang, Z. (2017). Effects of aqueous extracts of Taraxacum Officinale on expression of tumor necrosis factor-alpha and intracellular adhesion molecule 1 in LPS-stimulated RMMVECs. *BMC complementary and alternative medicine*, *17*(1), 38.

Gulfraz, M., Ahamd, D., Ahmad, M. S., Qureshi, R., Mahmood, R. T., Jabeen, N., & Abbasi, K. S. (2014). Effect of leaf extracts of Taraxacum officinale on CCl 4 induced Hepatotoxicity in rats, in vivo study. *Pakistan journal of pharmaceutical sciences*, *27*(4).

Horejsová, M., & Urban, J. (1994). The effect of polyene phosphatidylcholine (Essentiale forte) in the treatment of liver steatosis and ultrasound findings--preliminary study. *Casopis lekaru ceskych*, *133*(12), 366-369.

Kanchana, N., & Sadiq, A. M. (2011). Hepatoprotective effect of Plumbago zeylanica on paracetamol induced liver toxicity in rats. *Int J Pharm Pharm Sci*, *3*(1), 151-154.

Karakuş, A., Değer, Y., & Yıldırım, S. (2017). Protective effect of Silybum marianum and Taraxacum officinale extracts against oxidative kidney injuries induced by carbon tetrachloride in rats. *Renal failure*, *39*(1), 1-6.

Kenny, O., Smyth, T. J., Hewage, C. M., & Brunton, N. P. (2014). Antioxidant properties and quantitative UPLC-MS/MS analysis of phenolic compounds in dandelion (Taraxacum officinale) root extracts. *Free Radicals & Antioxidants*, *4*(1).

Khan, A., & Ghosh, K. (2013). Evaluation of phytase production by fish gut bacterium, Bacillus subtilis, for processing of Ipomea aquatica leaves as probable aquafeed ingredient. *Journal of Aquatic Food Product Technology*, *22*(5), 508-519.

Madrigal-Santillán, E., Madrigal-Bujaidar, E., Álvarez-González, I., Sumaya-Martínez, M. T., Gutiérrez-Salinas, J., Bautista, M., ... & Morales-González, J. A. (2014). Review of natural products with hepatoprotective effects. *World Journal of Gastroenterology: WJG*, *20*(40), 14787.

Mahesh, A., Jeyachandran, R., Cindrella, L., Thangadurai, D., Veerapur, V., & Muralidhara Rao, D. (2010). Hepatocurative potential of sesquiterpene lactones of Taraxacum officinale on carbon tetrachloride induced liver toxicity in mice. *Acta Biologica Hungarica*, *61*(2), 175-190.

Mandal, A. K., Das, S., Basu, M. K., Chakrabarti, R. N., & Das, N. (2007). Hepatoprotective activity of liposomal flavonoid against arsenite-induced liver fibrosis. *Journal of Pharmacology and Experimental Therapeutics*, *320*(3), 994-1001.

Petkova, N., Ivanov, I., Topchieva, S., Denev, P., & Pavlov, A. (2015). Biologically active substances and in vitro antioxidant activity of different extracts from dandelion (Taraxacum officinale) roots. *Sci. Bull. Ser. F. Biotechnol*, *19*, 190-197.

Ruepp, S. U., Tonge, R. P., Shaw, J., Wallis, N., & Pognan, F. (2002). Genomics and proteomics analysis of acetaminophen toxicity in mouse liver. *Toxicological sciences*, *65*(1), 135-150.

Samkol, P., Preston, T. R., & Ly, J. (2006). Effect of increasing offer level of water spinach (Ipomoea aquatica) on intake, growth and digestibility coefficients of rabbits. *Livestock Research for Rural Development*, *18*(2).

Schütz, K., Carle, R., & Schieber, A. (2006). Taraxacum—a review on its phytochemical and pharmacological profile. *Journal of ethnopharmacology*, *107*(3), 313-323.

Sivaraman, D., Panneerselvam, P., Muralidharan, P., Prabhu, T. P., & Kumar, R. V. (2013). Identification of potential plant sterols from ipomoea aquatica forsk for Alzheimer's disease by GC-MS analysis.

Timbrell, J.A. (1983). Drug Hepatotoxicity; Br.J.Clinical Pharmacy

Umar, K. J., Muhammad, M. J., Sani, N. A., Muhammad, S., & Umar, M. T. (2015). Comparative Study of Antioxidant Activities of the Leaves and Stem of

Ipomoea aquatica Forsk (Water Spinach). *Nigerian Journal of Basic and Applied Sciences*, *23*(1), 81-84.

Wellington, K., & Jarvis, B. (2001). Silymarin: a review of its clinical properties in the management of hepatic disorders. *BioDrugs*, *15*(7), 465-489.

Vargas-Mendoza, N., Madrigal-Santillán, E., Morales-González, Á., Esquivel-Soto, J., Esquivel-Chirino, C., y González-Rubio, M. G. L., ... & Morales-González, J. A. (2014). Hepatoprotective effect of silymarin. *World journal of hepatology*, *6*(3), 144.

Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*.

Zhang, J., Kang, M. J., Kim, M. J., Kim, M. E., Song, J. H., Lee, Y. M., & Kim, J. I. (2008). Pancreatic lipase inhibitory activity of taraxacum officinale in vitro and in vivo. *Nutrition Research and Practice*, *2*(4), 200-203.