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Evaluation of Cordyline fruticosa Leaf Extract's *In-Vivo* Anticancer Potential against Swiss Albino Mice Carrying Ehrlich's Ascites Carcinoma (EAC) Cells

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Abstract

Objective:

Cordyline fruticosa plant part has various ethno-pharmacological properties against various body ailments including pain, sore throat, bleeding haemorrhoids, fever, smallpox, inflammation of digestive tract and rheumatoid arthritis.

Methods:

In-vivo anticancer investigation of *Cordyline fruticosa* leaf extract was conducted through various established methods including inhibition of cell growth, average tumor weight and mean survival time, apoptosis by DAPI staining, hematological parameters and brine shrimp lethality bioassay. Anticancer properties of *Cordyline fruticosa* leaf extract were investigated using Ehrlich's ascites carcinoma cell-bearing Swiss albino mice.

Results:

Cordyline fruticosa leaf extract exhibited statistically significant suppression of cell growth and high percentage of apoptotic cells (38.60% and 48.83 ± 4.75 cells, respectively) @ 400 mg/kg dose. Concentration of 400 (mg/kg) showed significant reduction of average tumor weight of 12.67 ± 3.27 g, whereas statistically increased mean survival time. Plant extract significantly restored the hematological parameters to their normal level, however, the effect of crude extract was largely dose-dependent. Median lethal concentration of *Cordyline fruticosa* leaf extract was 329.132 µg/mL

Conclusions:

Phytochemicals of *Cordyline fruticosa* helped to enhance apoptosis of EAC cells and mean survival time to lower cancerous cell growth and average tumor/body weight and restore its biochemical parameters to normal level, owing to its anticancer potential.

Keywords: *Cordyline fruticosa*, Ehrlich's ascites carcinoma, cell growth inhibition, antioxidants, anticancer.

1. Introduction

Due to the large rate of morbidity and mortality, cancer has become a prevailing fatal disease in the world (Al-Mamun et al., 2016). According to a recent study, approximately over 1.7 million new cancer cases had been identified and it was also noted that cancer deaths was anticipated to increase by about 13 million by 2030 (Jose et al., 2020). Worldwide, cancer is the second most common cause of death and causes about 8.7 million deaths every year (Beeby et al., 2020). The peculiar cellular proliferation of cancer distinguishes it from other types of cell diseases (Baharum et al., 2014). Cancer growth and advancement are largely subjected to the cellular gathering of several epigenetic and genetic events (Baharum et al., 2014). The author added that due to the unusual gathering of typical cells developing from additional insufficient apoptosis and proliferation. Normally, cancer growth occurs via modifications to the tumor suppressor gene and

microRNA gene and also for oncogene modification. Cancer carries a severe burden on the public health system, and getting rid of it is significantly challenging, as well as the priority demand of the healthcare professional (Baharum et al., 2014).

Although many cancers initially respond to various chemotherapeutic agents, after a certain time interval, the cells develop resistance to them. Besides, chemotherapeutic agents can also exhibit various adverse drug reactions in patients (Mehdizadeh et al., 2020). In addition, conventional cancer management and treatment, including chemotherapy, radiation, and immunotherapy, have various side and harmful effects for patients (Alsaraf et al., 2019). In this situation, the establishment of an efficient and cheaper way for the treatment of cancer is essential (Mehdizadeh et al., 2020). Current strategies emphasize the establishment of naturally occurring anticancer therapeutics from medicinal plants and phytochemicals that are derived from natural sources and have potent pharmacological effects with minor adverse reactions for the management of versatile life-threatening diseases like cancer and infectious diseases (Al-Mamun et al., 2016). Naturally occurring phytochemicals that contain phenolic compounds have anticancer and anti-metastasis properties (Hassabou and Farag, 2020). Over 60% of today's anticancer medications are derived from herbal products, and over 80% of the world's population mostly receives healthcare from these sources (Cragg and Newman, 2005; Gao and Watanabe, 2011). Studies showed that phytochemicals derived from plant sources possess potential bioactive compounds and show exciting antioxidant and antitumor activity (Fadeyi et al., 2005; Saeed and Shabbir, 2012). Through the modification of the apoptotic signalling pathway in tumour cells, phytochemicals exhibited their anti-proliferative potentials, which are reflected as essential events in antitumor activity (Arul and Subramanian, 2013).

Cordyline fruticosa (L) (Family: Asparagaceae) ordinarily familiar as Palm Lily, Ti plant and Good luck Plant, having various colored leaves including pink, red, borders of whitish leaves and purplish strips leaves (Naher et al., 2019). The plant is mainly found in both subtropical and tropical regions of the world. The plant part has various ethnopharmacological properties like leaves used to get relief from neck pain, sore throat, bleeding haemorrhoids and bark used against diarrhoea, dysentery, cough, bloody cough, bloody urine, high fever, smallpox, inflammation of the digestive tract and pain of rheumatoid arthritis (Naher et al., 2019). *Cordyline fruticosa* has various phytochemicals, including glucofructan, alkaloids, tannins, polyphenols, saponins,

flavonoids, cholestane glycosides, phytosterols and steroidals. Previous literature showed that *Cordyline fruticosa* possesses various pharmacological activities, including antibacterial, antioxidant (Naher et al., 2019) and anti-gastric cancer (Liu et al., 2013). Investigating the in-vivo anticancer potential of Cordyline fruticosa leaf extract against Ehrlich's ascites carcinoma (EAC) cell in Swiss albino mice is the primary goal of this work. Additionally, we made an effort to investigate more affordable, bioactive substances derived from natural plant sources that exhibit anticancer properties despite little drug resistance and toxicity.

2. Materials and Methods

The reagent grade was utilized for all of the chemicals and reagents: trypan blue dye, DAPI; 4' 6—diamidino-2 phenylindole, PBS; phosphate buffered saline, Methanol (Sigma Aldrich, USA), DMSO; dimethylsulfoxide (Merck, Germany); SoloTM 0.9% NaCl solution IV infusion (Square Pharmaceuticals Ltd, Bangladesh); Vacutainer EDTA tube (Thomas Scientific).

2.1 Gathering and identifying the plant

In August 2019, *Cordyline fruticosa* leaves, both young and old, were taken from Jhigorgacha, Jashore, Khulna, Bangladesh. The plant was recognized and verified by taxonomist Naimur Rahman, a senior scientific official at the National Herbarium in Dhaka, Bangladesh. The ethics committee granted the accession number, 46998.

2.2 Preparation of plant extract

For the removal of dirt and microorganisms, the leaves were rinsed properly with both hot and cold water, respectively. To prevent the photooxidation of the bioactive compounds of the plant part, it was shaded dry for 15 days at room temperature. Then, the dried leaves were ground, and fine powder (500 gm) was socked with 3-liter methanol (99.99%) for the extraction of the chemical compound and kept for 14 days with occasional agitation with a shaker. A cold extraction procedure was followed to prevent possible degradation of thermolabile chemical compounds. After 14 days, the filtrate was collected after filtration using cotton and followed the number 1 Whatman filter paper. The filtrate was then dried in the air under the ceiling fan to get the final solid residue and avoided heating dry to prevent the heat-related oxidation of the bioactive. The extraction yield of *Cordyline fruticosa* leaves was 4.64% (w/w).

2.3 Experimental animals and dose selection

Mature adult male Swiss albino mice (weighting 30-32 gm, 8-10 weeks old) were obtained from Jahangirnagar University in Savar, Dhaka, Bangladesh. The mice were kept at a laboratory animal house for one week in a 12-hour light-dark cycle for the adaptation. The mice were fed a conventional lab diet and given access to tap water on a constant basis. There was a total of four groups of mice, including Group I (Negative control group), Group II (positive control group), Group III, and Group IV (experimental group of the plant extract). Each group contains six (n=6) moues to perform the experiment. Group I was given 0.15 mL DMSO and 0.9% normal saline, whereas Group II was given the conventional medication vincristine sulfate at a body weight of 0.3 (mg/kg) and finally, group III received 200 (mg/kg) and group IV received 400 (mg/kg) plant extract respectively through intraperitoneally (i.p.) every day throughout the experiment. The investigations included a mean survival time and average tumor weight that were run for 20 days, as well as a haematological profile, DAPI staining for apoptosis, and cell growth inhibition that was run for 6 days.

2.4 EAC tumor cells inoculation

Ehrlich ascites carcinoma (EAC) cells were injected intraperitoneally (i.p.) (Approximately $1x10^5$ cells/mouse) by Professor Dr. Abu Reza, protein science laboratory, University of Rajshahi, Bangladesh. With a thin syringe, each mouse in each group received an inoculation of around 1x105 cells/mouse at a concentration of 1 ml (10 µl) for the therapeutic evaluation. After the tumor cell injection was given for 24 hours, treatment was initiated.

2.5 Phytochemicals screening

A small amount of plant extract was used to perform the phytochemicals screening test with the conventional method described by (Abioye et al., 2013) with minor modifications to ensure the presence of glycosides, flavonoids, alkaloids, tannins and steroidal saponin.

2.6 Test for alkaloids

Cordyline fruticosa (CF) leaf extract (0.5g) was dissolved in 5 ml (1% HCl) in an Eppendorf tube. After filtering the solution, a few drops of Dragendorffs reagent were added to the 1 mL filtrate.

The Dragendorffs reagent includes potassium iodide (0.11 M), bismuth nitrate (0.6 M), and acetic acid (3.5 M). Precipitation creation was thought to be a sign that alkaloids were present.

2.7 Test for tannins

CF leaf extract (1g) was added to 10 mL distilled water and stirred. Then, we used the Whatman number 1 filter paper to filter the solution. Tannins were present because, upon adding two drops of 10% FeCl3 reagent to that filtrate, a blue colouring formed.

2.8 Test for flavonoids

The mixture was repeatedly heated under a spirit lamp after dissolving 0.2g of CF leaf extract in 2 mL of methanol. Few drops of conc. HCl and a chip of magnesium metal were added to mixture. The formation of red or orange coloration was considered an indication of flavonoid presence.

2.9 Test for saponins

A Whatman number 1 filter paper was used to filter the approximately 2 g of pulverized powdered material that had been boiling in 20 mL of distilled water in a water bath. To create a stable, longlasting foam, 10 mL of filtrate was combined with 5 mL of distilled water and shaken vigorously. After adding three drops of olive oil to the froth and giving it a good shake, the creation of foamed emulsions was thought to be a sign that saponins were present.

2.10 Test for glycosides

Concentrated H2SO4 was applied on top of 0.5g of CF leaf extract and 2 mL of glacial acetic acid (one drop of 1% FeCl3). One way to identify the presence of deoxy sugar, a feature of glycosides, near the contact is by the creation of a brown ring.

2.11 The median lethal dose (LD_{50})

LD_{50} was calculated using conventional methods with minor modifications (Litchfield and Wilcoxon, 1949). The extract of *Cordyline fruticosa* (CF) leaf disintegrated in 0.15mL (v/v) DMSO with 5mL (0.9% NaCl) solution at various concentrations, *i.e.*100, 200, 400, 800, 1600 & 3200 mg/kg, through serial dilution and finally injected 500µL intraperitoneally (i.p.) per mouse. The plant extract 3200 mg/kg was used as a stock solution for this experiment. Mice of each group were observed for 24 hours to find out any kind of toxicity, death and for behavioural change.

2.12 Suppression of cell growth

Cell growth inhibition was carried out using the standard procedure (Rana et al., 2020), with a few small adjustments. After 6 days of treatment, mice were sedated with CHCL3 (Chloroform), and then EAC cells were collected using a 5 ml syringe from the intraperitoneal cavity and diluted with normal saline (0.9% NaCl IV infusion). Trypan blue dye staining was performed to observe and calculate the number of viable cells using the following equation:

$$\% \text{ Cell growth inhibition: } (1-Tw/Cw) \times 100$$

Tw denotes the mean EAC cell count in treated mice, whereas the mean EAC cell count in control mice is represented by Cw.

2.13 Apoptosis by DAPI staining

The standard procedure for DAPI staining apoptosis was followed but with a few slight modifications (Al-Mamun et al., 2016). After 6 days of treatment, mice were sedated with CHCL3 (Chloroform), and then EAC cells were collected using a 5 ml syringe from the intraperitoneal cavity. From the stock cells (5 mL) 1 ml of EAC cells were collected and centrifuged for 2 minutes at 1200 rpm (Rotation Per Minute). After centrifugation, the plate was twice cleaned with PBS buffer solution, and the resultant cells were incubated for five minutes in a dark environment with five milliliters of DAPI staining solutions. After adding the PBS solution and the resulting DAPIcontaining pellets, the mixer was centrifuged for two minutes at 1200 rpm. Finally, the pellets were added with 200 µl PBS solution and 10 µl of supernatant was withdrawn to prepare microscopic slides. These microscopic slides were placed under a fluorescence microscope (XDS2FL, Optika, Italy).

2.14 Average tumor weight and average survival time

Average tumor weight and mean survival time were measured using the standard methodology (Islam et al., 2012; Samiron et al., 2018), with a few minor adjustments. After the 20 days of treatment the average tumor weight was measured where weight change was recorded on a daily basis, and host survival data was recorded for the mean survival time and calculated the data through the following equation:

$$\text{Mean survival time: } \sum \frac{\text{Survival times (days) of each mouse group}}{}$$

Total number of mice

% Increase of life span:(——————————−1)x 100

Average tumor weight (%): (——————————————————— ( ))x 100

2.15 Hematological profile

The following traditional approach was used to examine the haematological parameters, with a few minor adjustments (Islam et al., 2014). Following six days of therapy, blood was collected from all the groups of mice by puncturing the tail and keeping it in EDTA tubes. Centrifugation was used to separate the serum from the plasma. (4000 rpm for 10 minutes). Blood was analyzed through the bioanalyzer (micro lab 200) using commercial kits (Atlas Med, UK).

2.16 Bioassay for lethality of brine shrimp

The traditional approach was used for the brine shrimp lethality test (Asaduzzaman et al., 2015), with a few minor adjustments. Brine shrimp (*Artemia salina*) nauplii was used as the test organism of the present investigation. A stock solution containing 800 µg/mL was created by dissolving 16 mg of plant extract in 200 µl of pure DMSO and adding enough saltwater (up to 20 ml). The plant extract was made at various quantities (6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml) using serial dilutions. Each premarked vial contains 10 living nauplii and were observed for 24 hours, and the number of leaving nauplii was counted using light and magnifying glass.

2.17 Euthanasia and Anesthesia

After the study, all of the live mice utilized in the lab experiments were euthanised and given anaesthesia using a standard procedure with a few minor adjustments (Makowska et al., 2009).

2.18 Statistical analysis

Every single outcome was displayed as mean ± standard deviation (SD). A one-way ANOVA and the Bonferroni test were used for all statistical analyses, with *P<0.05, **P<0.01, and ***P<0.001

being considered statistically significant when compared to the control group. Additionally, SPSS software (version 16; IBM Corporation, New York, USA) was used to analyze all the data. The program Graph Pad Prism 8.0.1 version was used to create the graph. LC_{50} values were calculated by linear regression equations using Microsoft Excel 2013 (Microsoft, USA). A graphical abstract was prepared by using chem bio draw software (Version:14, PerkinElmer). References were arranged through the Endnote software (Version X9).

3. Results

3.1 Phytochemicals screening

Table 1 shows the he phytochemical screening of methanolic CF leaf extract exhibited the presence of flavonoids, glycosides, saponin, alkaloids and tannins as observed during the study.

3.2 The median lethal dose (LD_{50})

After 24 hours of observation, we did not find any kind of abnormal behaviors or mortality after the treatment with CF leaf extract at concentrations of 100, 200, 400, 800, or 1600 mg/kg body weight. This may indicate that during the experiment, the mice were not experiencing any kind of acute toxicity.

3.3 Inhibition of Cell Growth

The effects of *Cordyline fruticose* (CF) leaf extract and standard drug vincristine sulfate on cancer treatment in inhibition of cell growth are shown in Figure 1. Treatment with CF at the concentration 400 (mg/kg) body weight showed maximum cell growth inhibition 38.60% and standard drug vincristine sulphate 0.3 (mg/kg) Body weight indicated 58.51%, which was statistically significant (***P< 0.001).

3.4 Apoptosis by DAPI staining

The effects of CF leaf extract and vincristine sulfate on cancer treatment in the process of apoptosis by DAPI staining shown in Figure 2a and 2b. Treatment with CF at the concentration 400 (mg/kg) body weight showed the maximum number of apoptotic cells 48.83 ± 4.75 and

standard drug vincristine sulfate 0.3 (mg/kg) body weight showed 62.67 ± 2.58 respectively and there was statistical significance in the data (***P< 0.001).

3.5 Average tumor weight and average survival time

The effects of CF leaf extract and vincristine sulfate on cancer treatment in mean survival time and average tumor weight of experimental mice shown in Figure 3a, 3b and 4 respectively.

The mean survival time was raised to 16 ± 1.84 days with treatment with CF at 400 mg/kg body weight, while the mean survival time was enhanced to 18.67 ± 1.97 days with standard drug vincristine sulfate at 0.3 mg/kg body weight. Treatment with CF leaf extract at the concentration 400 (mg/kg) body weight reduced average tumor weight 12.67 ± 3.27 g and vincristine sulfate 0.3 (mg/kg) body weight 9.5 ± 2.07 g respectively. At the end of 20 days treatment, no solid tumor was found from the experimental mice but abnormal weight gain and enlargement in size may be the indication of fluid accumulation within cancer-affected mice that are shown in Figure 5.

3.6 Hematological profile

Table 2 shows the effects of CF leaf extract and standard drug vincristine sulfate on cancer treatment to restore biochemicals profile to the normal level. Among the two concentrations of CF leaf extract 400 (mg/kg) significantly restored the biochemicals parameter to its normal level as compared to the 200 (mg/kg) dose of extract. The CF leaf extracts 400 mg/kg body weight restored haemoglobin content 8.2 ± 1.92 (*P< 0.05), RBC content 6.4 ± 1.14 (**P< 0.1) and WBC content 17.8 ± 1.92 (***P< 0.001) respectively and the values were statistically significant.

3.7 Lethality bioassay of brine shrimp

The median lethal concentration (LC_{50}) of CF leaf extract was found to be 329.132 (µg/mL) in this experiment, that may have significant anticancer activity shown in Figure 6. The inhibition of tumor cell growth, increased number of apoptotic cells, decreased average tumor weight and increased life span and also maintaining the standard level of all biochemical parameters, indicating the potent anticancer properties of *Cordyline fruticosa* (CF) leaf extract (Islam et al., 2014). The anticancer potentiality of CF leaf extract was compared with the controlled group, and the result was statistically significant (**P< 0.01 and ***P<0.001).

4. Discussion

Our results findings depicts a higher number of cell growth inhibition, and apoptotic cells seen in the CF leaf extract at 400 (mg/kg) and conventional medicine vincristine sulfate at 0.3 (mg/kg) body weight, respectively, both of which were statistically significant (***P<0.001). The CF leaf extract contains various phytochemicals, including alkaloids, tannins, flavonoids, saponin and glycosides that may act as anticancer agents. The CF leaf extract contains steroidal saponin, which may be a potent source of the anticancer agent (Aumsuwan et al., 2016). By taking a sufficient number of antioxidants through a dietary supplement, oxidation and cancer may be prevented (Mut-Salud et al., 2016). Antioxidants prevent cellular oxidation and also inhibit abnormal cell proliferation ((Mut-Salud et al., 2016). There are many polar solvents, including methanol, which can extract a large number of polyphenols and antioxidants containing various bioactive compounds having exciting antioxidants and anticancer properties . Cordyline fruticosa leaf powder was extracted with polar solvent methanol, which may contribute to the extract of various polyphenols, and antioxidants that may act as anticancer agents in this experiment (Thouri et al., 2017). Cell death occurs in two different ways: necrosis and apoptosis. The planned death of cells is called apoptosis. Through the apoptosis process, the body can remove malignant or cancerous cells without damaging any kind of normal cells in the human body (Ashkenazi. 2008). In contrast to normal cells, which are round-shaped and less brightly stained under blue fluorescence light, apoptotic cells can be identified by membrane blebbing, cell shrinkage, aggregation of chromatin condensation, apoptotic bodies, and brightly stained nuclei under blue fluorescence light (AlMamun et al., 2016). From the experimental data of this experiment, “apoptosis by DAPI staining”, we may suggest that the anticancer activity of *Cordyline fruticosa* leaf extract was performed through apoptosis. The concentration of the leaf extract was 400 mg/kg, while the standard medicine vincristine sulfate was 0.3 mg/kg, body weight lowered the EAC cancerous cell growth and increased the number of the apoptotic cells 38.60% and 48.83 ± 4.75 cells and 58.51% and 62.67 ± 2.58 cells respectively and the data were statistically significant (***P< 0.001), (Figure 1 & 2). The 400 mg/kg concentration of plant extract and vincristine sulfate at the concentration of 0.3 (mg/kg) lowered the average tumour weight (mg) and increased mean survival time, and there was statistical significance in the data. (***P< 0.001) Fig. 3 and 4. This

enhancement of life span and reduction of tumour weight is a crucial indication and judgment for selecting a medicinal plant for the treatment of cancer as an anticancer agent (Price and Greenfield, 1958). The concentration of chemotherapeutic agents at the target site for cancer treatment may be lowered due to the resistance of chemotherapy and chemotherapeutic agents that may be improved through the combination of natural antioxidants (Chinery et al., 1997). During the treatment of cancer patients with chemotherapeutic agents' anemia is a common phenomenon. It occurs due to the breakdown of hemoglobin within the RBC, and at the same time, the number of WBCs increases to provide defensive actions against the abnormal signal of the body (Hoagland. 1982). When CF extract (400 mg/kg) and vincristine sulfate (0.3 mg/kg) were administered to the body weight, all biochemical parameters returned to normal in comparison to the control group. This experiment was performed to explore the anticancer properties of *Cordyline fruticosa* leaf. From the above evidence and discussion, we may suggest that *Cordyline fruticosa* leaf may be a potent natural source of an anticancer agent.

5. Conclusion

Conclusively, from the above-represented data, we may suggest that Cordyline fruticosa may be a potent natural source for cancer treatment. It altered all the promising factors, including cancerous cell growth, apoptosis of EAC cells, average tumour/body weight and mean survival time and also biochemical parameters that are crucial for cancer. It is the first experiment performed on in vivo anticancer activity on EAC cancer in *Swiss albino* mice model. Further research needs to be conducted for the isolation, purification, and identification of the chemical molecules that are bioactive and to elucidate the in vivo mechanism of anticancer activity of bioactive compounds of *Cordyline fruticosa.*

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Table. 1. Phytochemical screening of methanolic leaf extract of *Cordyline fruticosa*.

Chemical Test	Result
Alkaloids	++
Tannins	-
Flavonoids	+
Saponins	++
Glycosides	++

Key: + = Present ++ = Abundant - = Absence

Table: 2. The effect of *Cordyline fruticosa* leaf extract on EAC cell bearing *Swiss albino* mice to restore the biochemical parameters after 6 days of the treatment. Standard drug vincristine sulfate 0.3 (mg/kg) and 400 (mg/kg) leaf extract restored the biochemical parameters to its normal level and the data were statistically significant (*P<0.001) and all the data were compared to control group.

Treatment Groups	**% of Hb (gm/dL)**	**RBC (×10^{9}cells/mL)**	**WBC (×10^{6}cells/mL)**
Control mice (EAC bearing)	5 ± 1.02	3±1.22	25.4±1.14
Standard (Vincristine sulphate)	13.4±1.52***	9.8±1.92***	9.6±1.14***
400 mg/kg (Plant Extract)	8.2±1.92*	6.4±1.14**	17.8±1.92***
200 mg/kg (Plant Extract)	6.6±1.52	4.4±1.34	24±1.22

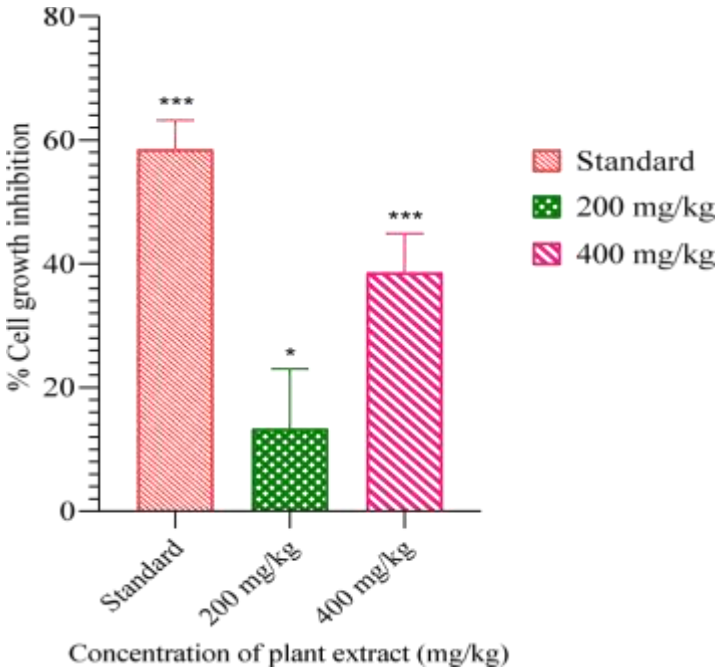

Figure: 1. Percentage of EAC cell growth inhibition in *Swiss albino* mice after the treatment with *Cordyline fruticosa* leaf extract at concentration 200 (mg/kg) and 400 (mg/kg) body weight. The leaf extract at concentration 200 (mg/kg) and 400 (mg/kg) body weight inhibited EAC cells growth in *Swiss albino* mice that showed statistically significant (*P< 0.05) and (*P<0.001) respectively. Vincristine sulfate (0.3 mg/kg) was taken as standard drug and data was statistically significant (*P<0.001), all data were compared to the control group.

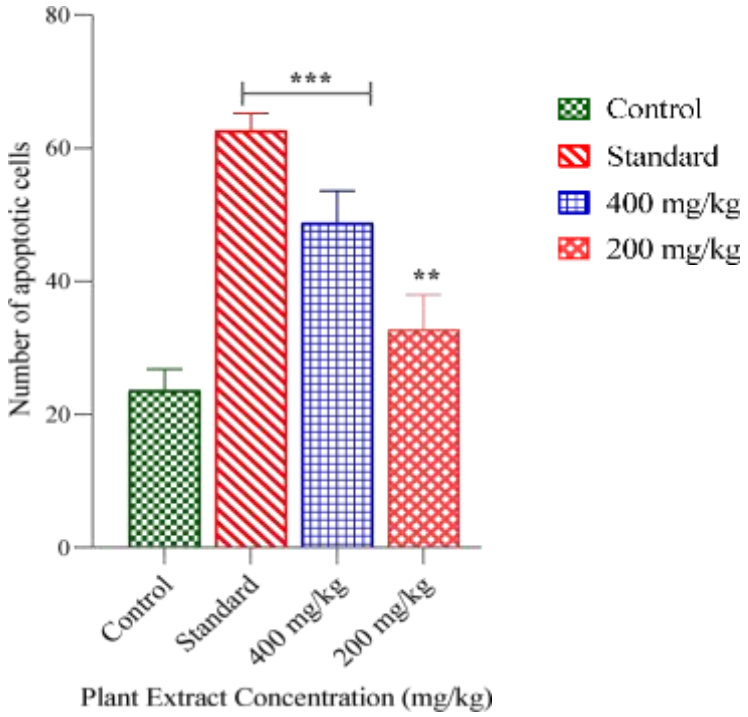

Figure: 2a. The Number of apoptotic cells per slide was measured by counting apoptotic cells after 6 days of treatment with *Cordyline fruticosa* leaf extract at different doses including 200 (mg/kg) and 400 (mg/kg) and standard drug 0.3 (mg/kg) vincristine sulfate. Standard drug vincristine sulfate 0.3 (mg/kg) and plant extract 400 (mg/kg) were statistically significant (***P< 0.001) and all the data were compared to control group.

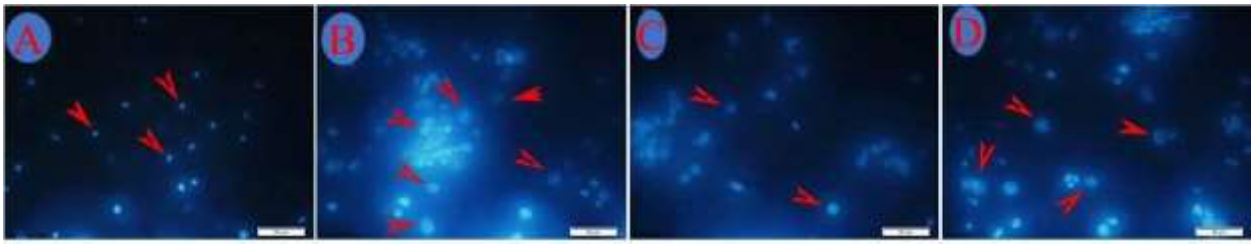

Figure: 2b. Microscopic images represent apoptotic cells detected by using DAPI staining under fluorescence microscope at the end of 6 days treatment with *Cordyline fruticosa* leaf extract at different doses and standard drug vincristine sulphate. Apoptotic cells having characteristics including cell shrinkage, membrane blebbing, chromatin condensation, aggregation of apoptotic bodies and brightly stained nucleus under blue fluorescence were observed in the treated groups as compared to the control group having round shape and less brightly controlled cells. Figure A represents control group, B; standard group (0.3 mg/kg vincristine sulfate), C; 200 (mg/kg) and D; 400 (mg/kg) *Cordyline fruticosa* leaf extract.

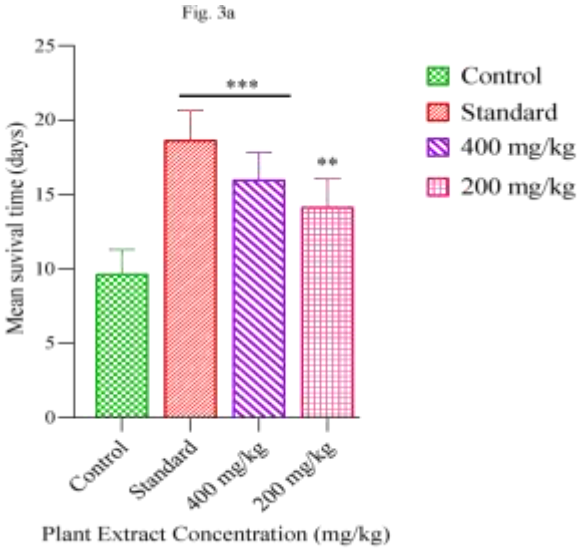

Figure: 3a. Mean survival time (days) of EAC cell bearing *Swiss albino* mice after 20 days of the treatment with *Cordyline fruticosa* leaf extract. Standard drug vincristine sulphate 0.3 (mg/kg) and CF leaf extract at concentration 400 (mg/kg) were statistically significant (***P< 0.001) and at concentration 200 mg/kg (**P< 0.01) and all the data were compared to control group.

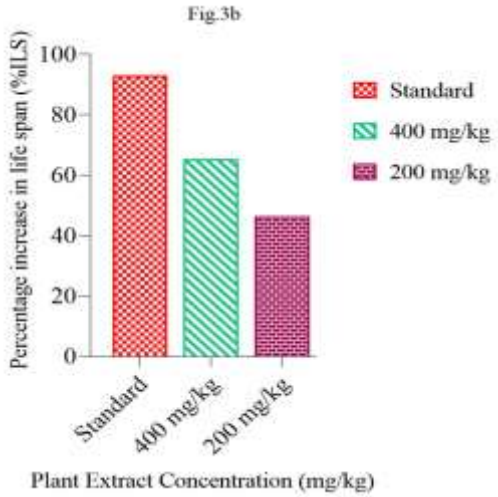

Figure: 3b. Percentage increase in life span (%ILS) of EAC cell bearing *Swiss albino* mice after 20 days of treatment with *Cordyline fruticosa* leaf extract. The mice were treated with CF leaf extract at concentration 200 (mg/kg) and 400 (mg/kg) and standard drug vincristine sulphate 0.3 (mg/kg) body weight and all the data were compared to control group.

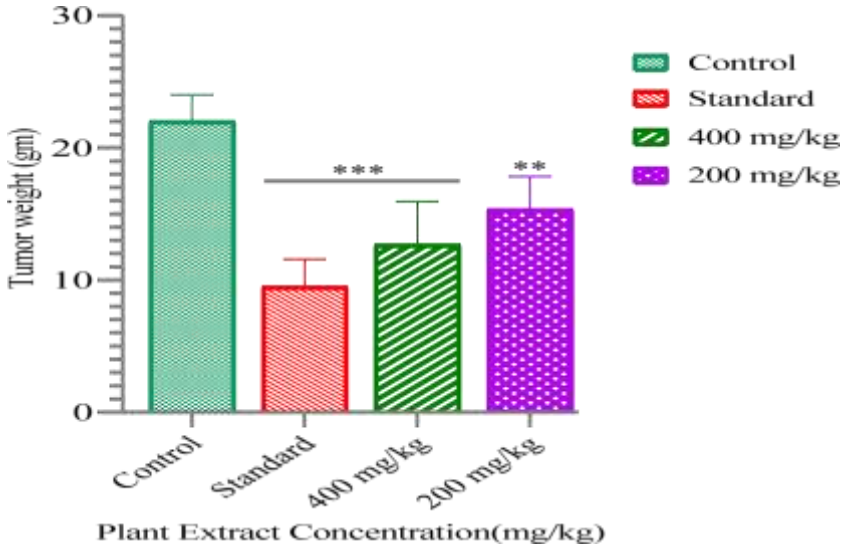

Figure: 4. Tumor weight (g) of EAC cell bearing *Swiss albino* mice after 20 days of the treatment with *Cordyline fruticosa* leaf extract at different doses. Standard drug vincristine sulfate 0.3 mg/kg and plant extract 400 mg/kg were statistically significant (***P< 0.001) and 200 mg/kg (**P< 0.01) and all the data were compared to control group.

Figure: 5. A represents control group, B represents 0.3 (mg/kg) vincristine sulfate (standard drug), C represents 400 (mg/kg), D represents 200 (mg/kg) dose of *Cordyline fruticosa* leaf extract. Pictorial presentation of EAC cell bearing *Swiss albino* mice after treating with *Cordyline fruticosa* leaf extract at concentration 200 (mg/kg), 400 (mg/kg) and standard drug 0.3 (mg/kg) vincristine sulfate (VS) and with the increasing of the dose the tumor bearing mice size and weight was reduced that indicated the dose dependence manner. The enlargement of body size and increased weight may occur due to the fluid accumulation within the body but solid tumor was not found.

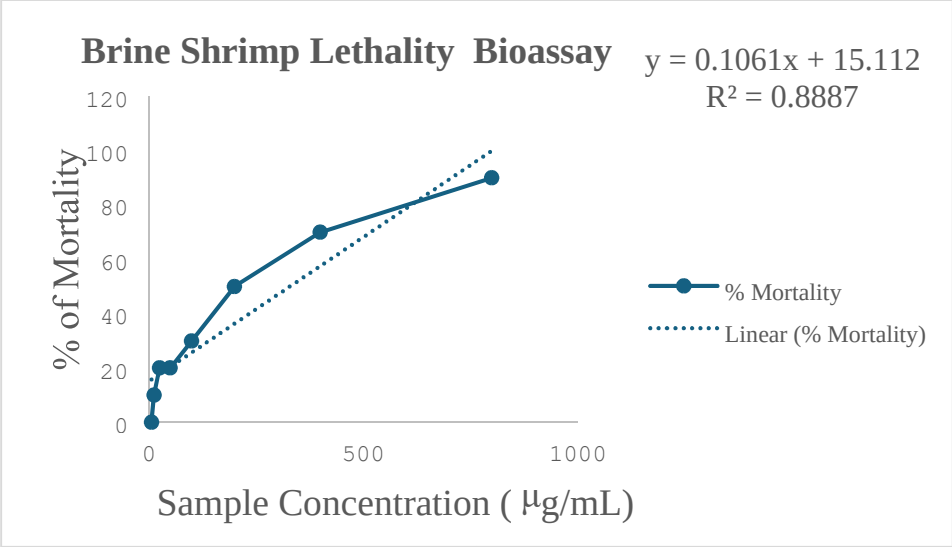

Figure: 6. Brine shrimp lethality bioassay of *Cordyline fruticosa* leaf extract at different concentration.