International Journal of Medicine and Medical Sciences (ISSN: 2315-9844) Vol. 1(8) pp. 100-106, August, 2014
Available online http://internationalinventjournals.org/journals/IJMMS

Full Length Research Paper

Oncostatic Effect of Streptomyces Crude Extracts on Murine Ehrlich Ascites Carcinoma

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Abstract

To investigate the anti cancer effect of actinomycetes crude extracts on murine Ehrlich Ascites Carcinoma (EAC). Crude extracts of actinomycetes were prepared and investigated their cytotoxic effects and super oxide dismutase (SOD) like activity in vitro. Anti cancer activity was tested in vitro in animal model bearing EAC. The cytotoxicity of the three isolated strain were, 67% *Streptomyces Fradiae,* 64.2% for *Streptomyces clavus* and 75% for *Streptomyces roseoflavus*. The SOD-like activity for crudes extracted from *Streptomyces fradiae*, *Streptomyces clavus* and *Streptomyces roseoflavus* were 84.4 %, 87.3 % and 67.3 % respectively. In vitro studies revealed that, there was a significant increase of gluthation and SOD, and significant decrease of malonaldehyde content of mice bearing tumor and treated with the crude extract isolated from actinomycetes. Crude extracts of *Streptomyces fradiae, Streptomyces clavus* and *Streptomyces roseoflavus* reveals anti cancer activity against EAC.

Keywords: Ehrlich Ascites Carcinoma, streptomyces, Fermentation.

INTRODUCTION

Cancer still represents one of the most serious human health problems despite the great progress in understanding its biology and pharmacology. The usual therapeutic methods for cancer treatment techniques are individually useful in particular situations and when combined, they offer a more efficient treatment for tumors. An analysis of the number of chemotherapeutic drugs and their sources indicates that over 60% of approved drugs are derived from natural compounds and many have been extracted from actinomycetes (*Sudha* and Masilamaniselvam, 2013). Actinomycetes are the well-recognized as the richest source of bioactive compounds including clinically useful antibiotics, anticancer agents and cell function modulators and hence of high pharmacological and commercial interest (Butler, 2008).

The present study was conducted to show the antitumor activity of some actinomycetes, namely streptomycetes crude extracts in the tumor model of Ehrlich ascites carcinoma (EAC) cells implanted in Swiss albino female mice in liquid tumor in which (EACs) inoculated intraperitoneally. Also the antioxidant effect of these extracts in both normal and tumor-bearing mice was investigated.

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MATERIALS AND METHODS

Actinomycetes isolates

The used streptomycetes species during this work were isolated and identified by Dr. Abou-Dobara, Botany department, Faculty of science, Damietta University.

These Streptomycetes species include *Streptomyces fradiae, Streptomyces roseoflavus* and *Streptomyces clavus*

Fermentation

Streptomyces clavus, Streptomyces roseoflavus and *Streptomyces fradiae* were cultivated in starch nitrate agar media to obtain the extracts that were filtered and then dialyzed against poly ethylene glycol. .

Induction of Cancer

Ehrlich ascites carcinoma cells (EAC) was obtained from the National Cancer Institute, Cairo University, Egypt. The tumor cell line was maintained in Swiss albino mice through serial intra-peritoneal transplantation of 1×10^6 viable tumor cells suspended in 0.2 ml of saline. The tumor cells were characterized by moderately rapid growth, which kills mice in 16 to 18 days.

Treatment protocol

Swiss albino mice were randomly divided into four groups (eight mice each), as follow:

1- Normal mice (control, group1)
2- Normal mice *Streptomyces fradiae* extract treated (group 2): Mice were inoculated only with *Streptomyces fradiae* extract.
3- Normal mice *Streptomyces roseoflavus extract* treated (group 2): Mice were inoculated only with *Streptomyces roseoflavus extract.*
4- Normal mice *Streptomyces clavus* extract treated (group 2): Mice were inoculated only with *Streptomyces clavus* extract.
5- Tumor bearing *Streptomyces fradiae* treated mice (group 3): Mice inoculated intraperitoneally with $1xl0^6$ tumor cells/mouse and then treated with *Streptomyces fradiae.*
6- Tumor bearing *Streptomyces roseoflavus* extract treated mice (group 3): Mice inoculated intraperitoneally with $1xl0^6$ tumor cells/mouse and then treated with *Streptomyces roseoflavus* extract.
7- Tumor bearing *Streptomyces clavus* extract treated mice (group 3): Mice inoculated intraperitoneally with $1xl0^6$ tumor cells/mouse and then treated with *Streptomyces clavus* extract.
8- Tumor-bearing mice only (group 4): Mice inoculated intraperitoneally with $1xl0^6$ tumor cells/mouse.

The mice of group 2 and group 3 were i.p. treated with the *Streptomyces clavus* extract or *Streptomyces roseoflavus* extract or *Streptomyces fradiae* extract with a daily dose of 0.52 mg/kg/day (300µLof liquid extract) for *Streptomyces fradiae* extract and *Streptomyces clavus* extract and 0.64mg/kg/day(300µLof liquid extract) for *Streptomyces roseoflavus* extract day after day for 10 days starting from the first day after tumor inoculation. The normal extracts treated mice's group was treated with the same extracts dose as that of group 4.

Viability Test.

Viable EAC cells' counting was carried by trypan blue exclusion by the method of MacLimans et al. (1957).

Antioxidant Status.

Reduced Glutathione in blood was determined by the method of Beutler et al. (1963). Malondialdehyde in RBCs (MDA) was determined by the method of Stocks and Donnandy (1971). In which Malondialdehyde can react with thiobarbituric acid (TBA) to form a Coloured complex, which can be calorimetrically measured. Activity of catalase is determined according to the method of Chance and Mackley (1955), where the rate of decomposition of H_2O_2 by catalase is measured spectrophotometrically at 240 nm. Superoxide dismutase in (SOD) in liver tissue activity was assayed by the procedure of Nishikimi *et al.* (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of NBT dye.

RESULTS

Cytotoxicity effects of actinomycetes extracts

The cytotoxicity effects of the extracts were increased with the increase of the extracts concentration. Also, the results demonstrated that the average cytotoxicity effects of *Streptomyces Fradiae*, *Streptomyces Clavus*, and *Streptomyces roseoflavus* were 67%, 64.2% and 75 respectively. Table 1.

Superoxide dismutase (SOD)-like activity of actinomycetes extract

The SOD-like activities of the extracts were increased with the increase of the extract concentration. Also, the results demonstrated that the SOD-like activity of *Streptomyces fradiae*, *Streptomyces clavus* and *Streptomyces roseoflavus* were 84.4 %, 87.3 % and 67.3 % respectively. Table 2.

The activity of superoxide dismutase in liver homogenate (SOD/Liver)

The statistical results showed that, the mean activity of SOD/Liver of normal mice was significantly increased than that of tumorized-non treated mice group (P=0.01) also the mean activity of SOD/Liver of *Streptomyces fradiae*, treated tumorized mice were significantly increased P1= 0.006.

The activity of catalase in liver tissues:

The statistical results showed that, the mean activities of catalase of normal mice was significantly increased than that of the tumorized non treated-mice (P1= 0.0006).

Table 1. Cytotoxicity of *Streptomyces fradiae*, *Streptomyces clavus* and*Streptomyces roseoflavus* extracts

Volume effects	**Cytotoxicity**		
	Streptomyces fradiae extract	*Streptomyces clavus* extract	*Streptomyces roseoflavus* extract
100 **µL**	60%	59%	70%
20**0µL**	63%	66%	72%
300**µL**	71%	66%	80%
400**µL**	74%	66%	80%
Average effects%	67±6.5	64.25±3.5	75.25±5.5
Effects range%	60-74	594-66	70-80

Table 2. Superoxide dismutase (SOD)-like activity of *actinomycetes extracts*.

Volume Effects	**SOD-like activities**		
	Streptomyces fradiae extract	*Streptomyces clavus* extract	*Streptomyces roseoflavus* extract
50**µL**	74%	79.9%	60%
100**µL**	80.3%	82.3%	64.7%
150**µL**	88%	85.9%	69.6%
200**µL**	89.7%	93.8%	69.6
250**µL**	89.7%	94.6%	72%
Average effects%	84.43±6.9	87.3±6.6	67.3±4.9
Effects range%	74-89.7	79.9-94.6	60-72

Also the mean activity of catalase *Streptomyces fradiae*, *Streptomyces clavus* and *Streptomyces roseoflavus* extracts treated tumorized mice were significantly increased (P1=0.007), (p1=0.002) and (0.008) respectively.

Glutathione reduced form (GSH) in RBCs

The statistical results showed that, the mean level of GSH in RBCs of normal mice was significantly increased than that of tumorized mice non treated group (P=0.0027) also the mean levels of GSH in RBCs of *Streptomyces fradiae* and *Streptomyces clavus* extracts treated tumorized mice were significantly increased than that of tumorized mice non treated group P2= 0.04, P2=0.026, respectively

Malondialdehyde (MDA) in RBCs

The statistical results showed that, the mean level of MDA in RBCs of normal mice was significantly decreased than that of tumorized- non treated mice (P1= 0.0005). Also the mean levels of MDA in RBCs of *Streptomyces fradiae*, *Streptomyces Clavus* and *Streptomyces roseoflavus* extracts treated tumorized mice were significantly decreased than that of tumorized-non treated mice (P2= 0.0023) , (0.004)and (0.0007).

Correlation

SOD in liver was negatively Correlated with GSH in RBCs (r = -0.417, p = 0.007) and catalase in liver (r = -0.459, p = 0.002). Figure 1, 2, 3, 4 and table 3.

DISCUSSION

Cancer is considered to be the public health problem in developed and developing countries. Actinomycetes are the most economical and biotechnologically valuable class of prokaryotes producing bioactive secondary metabolites notablyantibiotics (Blunt et al., 2006) anti tumor agents, immunosuppressive agents (Mann, 2001) and enzymes (Berdy, 2005; Strohl, 2004). Streptomyces is the largest genus known for the production of many secondary metabolites (Maleki and Mashinchian, 2011) which have different biological activities, such as antibacterial, antifungal, antiparasitic, antitumor, anticancer and Immunosuppressive actions (Bizuye et al., 2013).

The present study was conducted to show the antitumor activity of actinomycetes crude extract in the tumor model of Ehrlich ascites carcinoma (EAC) cells implanted in Swiss albino female mice in liquid tumor in which (EACs) inoculated intraperitoneally. The present study also investigated the antioxidant effect of these extracts in both normal and tumor-bearing mice. In the present study, *Streptomyces Fradiae*, *Streptomyces*

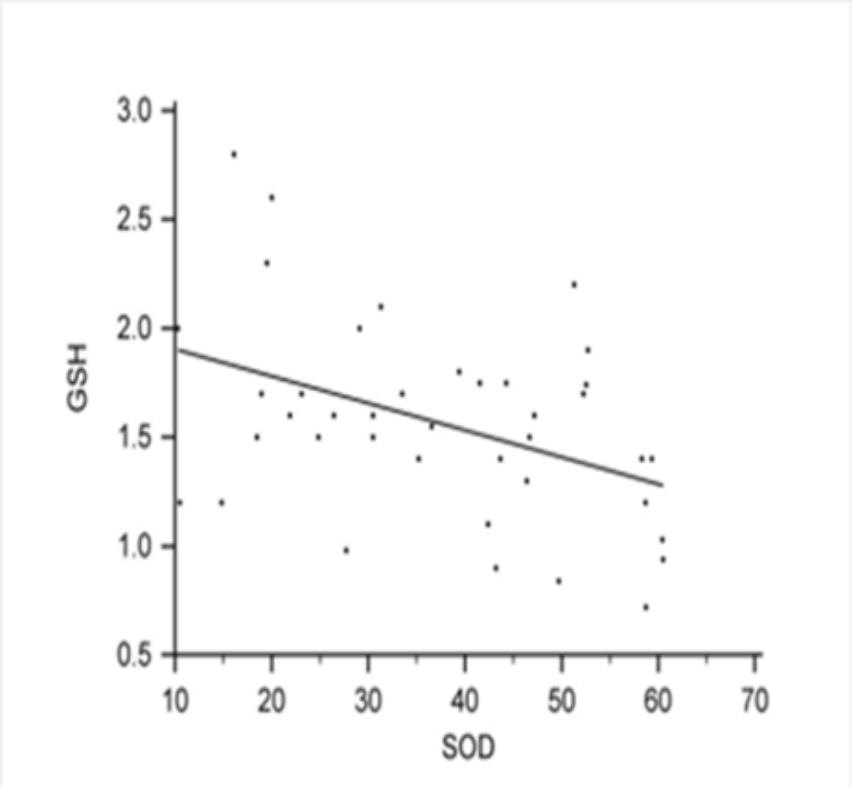

Figure 1. Correlation between SOD in liver homogenate in mice bearing liquid tumor and GSH in blood.

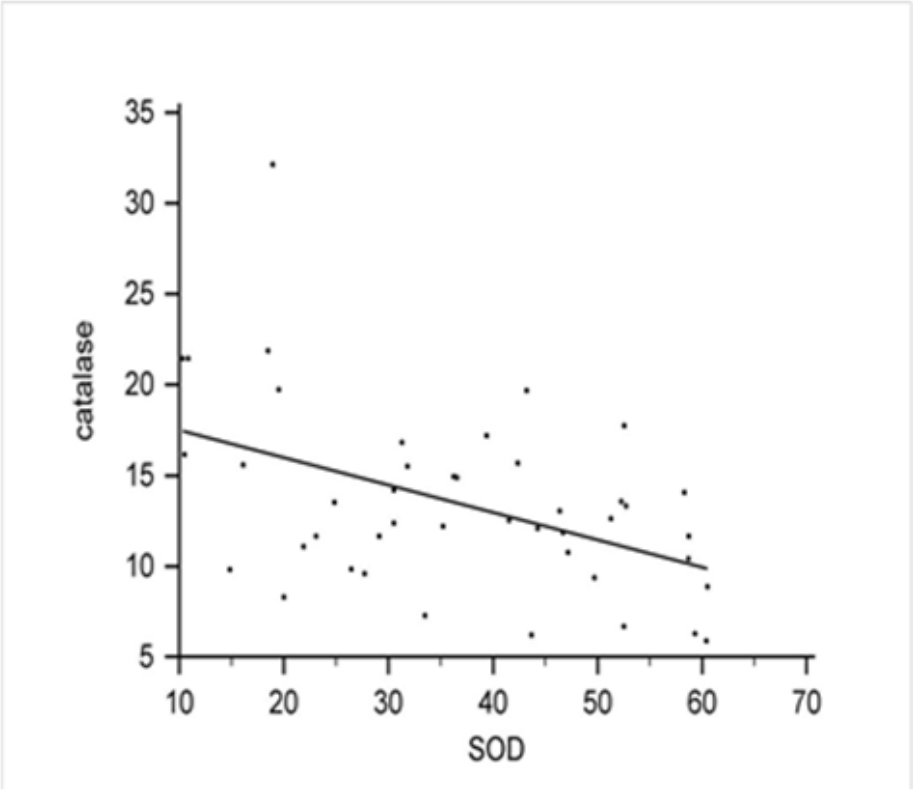

Figure 2. Correlation between SOD in liver homogenate in mice bearing liquid tumor and catalase in liver homogenate.

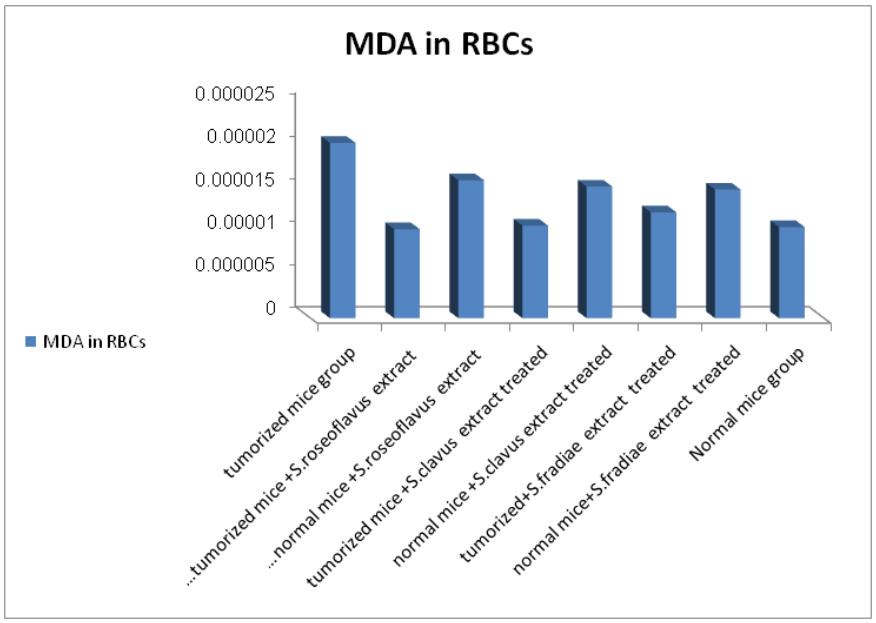

Figure 3. Mean level of MDA in RBCs in different experimental group.

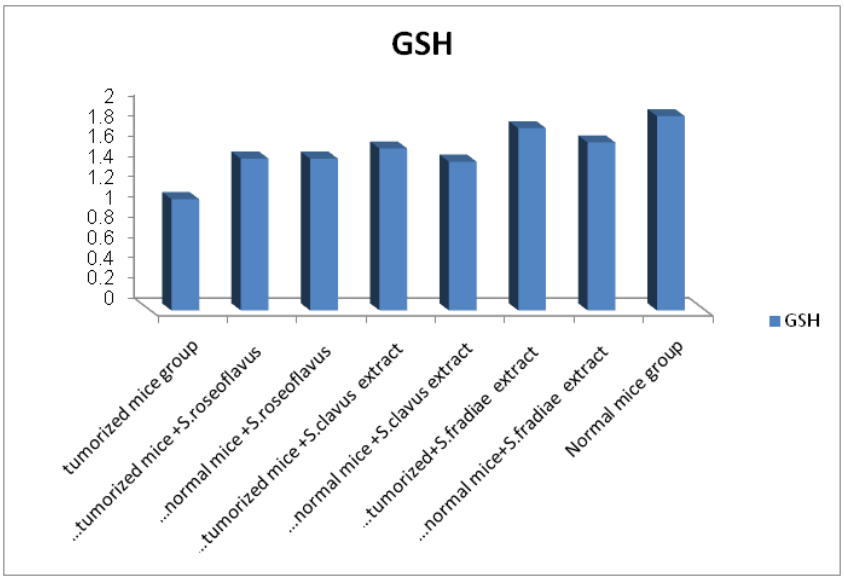

Figure 4. Mean level of GSH in different experimental groups.

Table 3. Mean activities of superoxide dismutase in liver tissues (SOD/Liver), catalase in liver tissues and the mean levels of glutathione reduced form (GSH) and malondialdehyde in RBCs and in liver tissues of mice treated with *Streptomyces fradiae*,*Streptomyces clavus* and *Streptomyces roseoflavus* extracts.

Parameters ***Group***	**SOD/Liver** **(% of inhibition/0.01 gm tissue)**	**Catalase** **(U/0.01 gm tissue)**	**GSH /RBCS** **(Mol/ l cells)**	**MDA/RBCs** **(Mol/ml packed cells)X10^{-5}**
Normal	54.34±6.9 (43.6-60.5)	22.2±5.1 (16.8-31.1)	1.92±0.3 (1.7-2)	1.06±0.25 (0.7-1.4)
Normal + *Streptomycesfradiae* extract treatment	24.53±9.6 [ii] (10.5-35.2) P1=0.00062	12.1±3.17 [ii] (8.3-16.15) P1=0.0038	1.66±0.54 (1.2-2.6) P1=0.39	1.5±0.90 (0.7-2.5) P1=0.40
Normal + *Streptomyces clavus* extract treatment	47.3±10.6 (31.8-58.7) P1=0.25	14.2±2.4 (11.65-17.7) P1=0.011	1.47±0.50 (0.72-1.75) P1=0.19	1.5±0.734 (0.7-2.3) P1=0.224

Table 3. Continue

Normal + *Streptomyces roseoflavus* extract treatment	42.6±10.6 (23.09-58.6) P1=0.214	13.03±2.1 ii (10.4-15.6) P1=0.005	1.5±0.45 (1.1-2.2) P1=0.132	1.6±0.25 (1.3-1.8) P1=0.036
Tumor only	18.2±7.6 ii (10.3-31.3) P1<0.0001	7.2±1.5ii (5.9-9.35) P1=0.0006	1.1±0.26 ii (0.84-1.4) P1=0.00277	2.05±0.38 ii (1.35-2.39) P1=0.0005
Tumor + *Streptomyces fradiae* extract treatment	32.1±12.8ii (16.1-51.2) P1=0.0061 P2=0.050	13.5±2.8ii (9.5-17.2) P1=0.0071 P2=0.00179	1.82±0.62* (0.98-2.8) P1=0.744 P2=0.04	1.2±0.25 (1-1.6) P1=0.29 P2=0.0023
Tumor + *Streptomyces clavus* extract treatment	28.1±7.5* (14.8-36.2) P1=0.250 P2=0.047	11.6±2.7**ii (9.8-14.9) P1=0.0024 P2=0.0092	1.6±0.29* (1.2-2) P1=0.136 P2=0.026	1.1±0.42** (0.6-1.7) P1=0.93 P2=0.0038
Tumor + *Streptomycesroseoflavus* extract treatment	48.7±5.7* (23-58.6) P1=0.214 P2=0.05	13.6±3.18** ii 10.7-19.6)(P1=0.0080 P2=0.0028	1.5±0.38 (0.9-1.9) P1=0.07 P2=0.06	1.0±0.28** (0.72-1.4) P1=0.88 P2=0.00072

¡= Significant and ¡¡= highly significant compared with those of the control and *= Significant and **= highly Significant compared with those of tumor only. (P1 compared with control, P2 compared with tumor only)

Clavus, *Streptomyces Roseoflavus* extracts showed SOD- like activities of about 84.4%, 87.3 and 67.3.%. Such results were due to presence of Ni –SOD in such species. One of the characteristics of tumor growth and invasion is the increased flux of oxy-radicals and loss of cellular redox homeostasis. Cancer cells can generate large amounts of hydrogen peroxide which may contribute to their ability to mutate, damage normal tissues and invade other tissues. Thus, changes in the rate of cancer cell proliferation could be reflected by changes in the antioxidant machinery and some anticancer agents can act as antioxidants (Gupta et al., 2004).Usually, the tumor cells which have smaller amount of both CuZn- SOD and Mn-SOD proliferate than their normal cell counterparts (Ambrosone et al., 1999). Superoxide dismutases (SODs) and reduced glutathione activities in the liver were reported to be lowered in the tumorized cells of the tumorized mice. In addition the treatment of the tumorized mice with actinomycetes extracts result in significant increases in the mean activities of SOD. In our opinion, the decrease in the SOD of the tumorized mice can help in the reduction of superoxide anion H_2O forming H_2O_2. The later in the absence of catalase in interapertoneal cavity of the tumorized animals may contribute in the ability of EAC carcinoma to mutate, damage the adjacent normal cells and stimulate the activities of EAC to invade other tissues ,which it is actually the case in this study (Gupta et al., 2004). In our study the treatment of mice with actinomycetes extracts relevant to SOD activity causing the previous steps to be reversed. The conclusion were results in line with those of Ridnour et al. (2004) and Zhang et al. (2008) who recognized that cancer cell always has allow Mn-SOD and glutathione activities and usually had high oxidative stress and presence of large quantities of free radicals. Significant results of the antioxidants and lipid peroxidation were obtained after treatment with actinomycetes extracts accompanied with reduction in tumor weight, revealing their protective mechanism in tumor prevention. The status of antioxidants and lipid peroxidation were correlated with the pathophysiology of the cancer (Bandebuche and Melinkeri, 2011). This means that, in tumor control, the increased serum MDA levels indicate oxidative stress which may cause DNA damage which is one of the causative factors for cancer while low levels of SOD and CAT could be due to the increased utilization of these antioxidants in scavenging the lipid peroxides production which overrides the antioxidant defense leading to increased MDA in serum (El-Sabbagh et al., 2013)

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How to cite this article: El-Taweel F, Abou-Doubara MI, Fouda G, 3, El-mezayen HA (2014). Oncostatic Effect of Streptomyces Crude Extracts on Murine Ehrlich Ascites Carcinoma. Int. J. Med. Med. Sci. Vol. 1(8): 100-106