East African Scholars Journal of Agriculture and Life Sciences
*Abbreviated Key Title: East African Scholars J Agri Life Sci*
ISSN 2617-4472 (Print) | ISSN 2617-7277 (Online) |
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-6 | Jun-2019 |

Research Article

# The pharmaceutical excipient, magnesium stearate, depresses lymphocyte counts in vivo but does not lower humoral immune response in sprague-dawley rats

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**Abstract:** Effects of magnesium stearate (MS) on immunological and haematological responses of rat were investigated. Twenty male rats randomly assigned to four groups (A-D) of five rats were used in the study. Rats in all groups were immunized with heterologous antigen and boosted every 14 days till end of the study. Groups A, B, and C received 2% w/v, 4% w/v, and 8% w/v of MS daily and respectively via gastric gavages from day 0 till day 28, while group D, the negative control, received distilled water. Antibody titres to heterologous antigen challenge were determined on days 0, 14, and 28, while haematological studies were carried out on days 0, 7, 14, and 28. A terminal assay was done on day 42 to observe the effect of withdrawal of treatment with MS. By day 28, groups A and C had significantly ($p < 0.05$) higher antibody titres than the control, while groups B and C had significantly lower total leucocyte and lymphocyte counts than the control. Treatment with MS did not adversely affect the total erythrocyte count, haemoglobin concentration and the packed cell volumes of the groups. Following withdrawal of MS treatment, by day 42, leucocyte counts began to recover but were significantly lower than values in the control group. In conclusion, *in vivo*, certain excipient doses of MS can transiently suppress cellular immunity but not humoral immunity; hence, it may not be the best excipient for agents destined for immunostimulation or treatment of infectious and immunosuppressive conditions.

**Keywords:** Magnesium stearate, excipient, immune response; haematology, toxicity, lymphocytes.

## INTRODUCTION

Magnesium stearate (MS) is a flocculent white powder at room temperature. It remains a critical inert ingredient in the manufacturing process of many pharmaceutical solid dosage forms (Good D & Wu Y, 2017), probiotic preparations, and confectioneries. It serves as a major tablet and capsule lubricant (Johansson ME, 1984; Bolhuis GK & Holzer AW, 1996). MS is widely used as pharmaceutical excipient at concentration between 0.25% to 5.0% w/w (Allen LV & Lunner PE, 2006). Several works have been done on the undesirable effects of MS but the main foci have been on pharmaceutical technology and pharmaceutics, and not on biological systems. Such undesirable effects of MS include the alkalinization and rapid hydrolysis of drugs such as aspirin and ibuprofen (Gordon RE *et al.*, 1984; Miller TA & York P, 1988), magnesium mediated degradation of drug ions (Thakur AB *et al.*, 1993), oxidative damage of drugs (Osawa Z & Ishizuka T, 1973; Good D & Wu Y, 2017), and reduced activity of the antimicrobial cetylpyridinium chloride (Richards RM *et al.*, 1996). Magnesium stearate is generally classifieid as a non-toxic substance whether inhaled or consumed (Allen LV & Lunner PE, 2006), and a non-carcinogen (Boyland E *et al.*, 1964). Earlier workers (Gurr MI, 1983; Erikson KL, 1986) reported that the immunological functions of lymphocytes are modulated by both unsaturated and saturated fatty acids such as stearic acid.

Sondergaard *et al.* (Sondergaard D *et al.*, 1980) from a 90-days feeding study, concluded that the non-toxic or the no-effect concentration of magnesium stearate in rats are levels less than 5 % w/w, corresponding to 2500mg/kg body weight/day; and that liver damage, urolithiasis and emaciation were seen in some rats that received higher levels of MS. Despite widespread use of magnesium stearate in human and

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjals/

Article History
Received: 15.04.2019
Accepted: 28.05.2019
Published: 21.06.2019



DOI: 10.36349/easjals.2019.v02i06.005

veterinary drugs, biopharmaceuticals, foods etc., there remains a paucity of scientific information on the effects of excipient doses of magnesium stearate on the total and differential leucocyte counts, erythrocytic indices, and humoral immune response of *in vivo* biological systems. The foregoing, therefore, forms the basis of this investigation.

## MATERIALS AND METHODS

### Experimental Design and Experimental Animals

A Randomized controlled experimental designed was used for the study. Twenty adult male sprague-dawley rats weighing between 200-225 grams were used for the experiment. They were acclimatized for two weeks, and then randomly divided into four groups (n = 5). Each group was housed in a fly-proof cage, at room temperature ($20^oC$ - $28^oC$), and supplied with proprietary animal feed, and fresh drinking water *ad libitum*. Ethical considerations for use of the animal models in this study were based on the procedures of the Animal Use and Care Committee of the faculty of veterinary medicine of the university which agrees with the NIH guidelines (National Institute of Health NIH, 2011).

### Immunization with a Heterologous Antigen

On day 7 before start of treatments with magnesium stearate, rats in all the groups were immunized with 10% sheep red blood cell intraperitoneally, and boosted every 14 days till termination of the study.

### Treatment with Magnesium Stearate (MS)

A suspension of reagent grade magnesium stearate (Evans Pharm. Co®, England) was made in distilled water and administered via gastric gavages. Starting from day 0 post treatment (PT), rats in groups A, B, and C received 2% w/v MS, 4% w/v MS, and 8% w/v MS respectively till day 28. Rats in group D, the control, received distilled water throughout the treatment period.

### Blood Collection

About 1ml of blood was collected from the retro-bulbar plexus of the medial canthus of rats. About 0.4 ml of the blood was collected into EDTA- bottles (1.5mg Na-EDTA/5mlbottle) for haematological studies. The remaining blood was collected into Eppendorf tubes, allowed to clot, and centrifuged at 3000 rpm for five minutes to separate the serum for immunological assay.

### Immunological Assay

Immunological responses to heterologous antigen stimulation were detected by direct haemagglutination technique (Ikeme MM & Adelaja AO, 1990) on days 0 (baseline), 14, and 28 post treatment. A terminal sampling was done on day 42 to check the effect of withdrawal of treatment with MS. Briefly, non-specific antibodies in the test sera were inactivated in a water bath at $56^oC$. Thereafter 50µl of serum samples were added to 50µl of phosphate buffered saline in a microtitre plate well and were serially diluted. Then 50µl of 2% sheep erythrocytes was added. The mix was allowed to incubate for one hour at room temperature to allow antigen- antibody reaction. Thereafter the antibody titres were read and recorded.

### Haematology

The differential and total leucocyte counts, total erythrocyte counts, haemoglobin concentration and packed cell volumes were determined on days 0, 7, 14 and 28 post treatments using the Leishman's technique, haemocytometer methods, cyanohaemoglobin methods, and microhaematocrit method respectively (Schalm OW *et al.*, 1975; Coles EH, 1986; Thrall MA & Weisser MG, 2002). A terminal assay was done on day 42 to check the effect of withdrawal of treatment with MS.

### Data Analysis

Data obtained were analyzed with Fisher's one way analysis of variance statistic (Fisher RA, 1952) using SPSS version 18. Means were separated using the least significant difference (LSD) post hoc tests. Significance was accepted at $p < 0.05$. The results were presented as mean ± standard error of mean using tables and chart.

## RESULTS

### Antibody Response

The baseline antibody titre (day 0) did not show any significant variation across all experimental groups (Fig 1). By day 14 the antibody titre varied in a dose response fashion in treated groups, with levels in groups C being significantly ($p < 0.05$) higher than levels in the control group and group A. By day 28 of treatment, groups A and C had significantly higher antibody titres when compared to the control group. Following withdrawal of MS treatment, all the experimental groups had similar antibody titres by day 42.

### Total and Differential Leucocyte Count

Across all the experimental groups, there were no significant variations in total leucocyte counts by days 0, 7, and 14 of treatment with magnesium stearate (Table 1). By days 28 and 42, groups B and C had significantly ($p < 0.05$) lower total leucocyte count when compared to the control (group D). Also, there were no significant variations in mean absolute lymphocyte counts by days 0, and 14 of treatment with magnesium stearate (Table 2). By days 7, 28 and 42, groups B and C had significantly ($p < 0.05$) lower mean absolute lymphocyte counts when compared to the control (group D). Treatment with magnesium stearate did not result in any significant variation in mean absolute neutrophil counts across all the groups by days

0, 7, 14 and 28. By day 42, groups B and C had significantly ($p < 0.05$) lower neutrophil counts when compared to the control (Table 3). No significant ($p < 0.05$) variations in mean absolute eosinophil counts were seen across all the groups by days 0, 7, 14, 28 and 42 (Table 4).

**Erythrocytic Indices**

Across all the experimental groups, there were no significant variations in mean haemoglobin concentrations by days 0, and 7 of treatment with magnesium stearate, and also by day 42 following withdrawal of treatment (Table 5). Groups A, C, and D had similar haemoglobin concentrations on days 14, while groups A, B, D, had similar haemoglobin concentrations by day 28 of treatment. No significant ($p < 0.05$) variations in total erythrocyte counts and packed cell volumes were seen across all the groups by days 0, 7, 14, 28 and 42 (Tables 6 and 7).

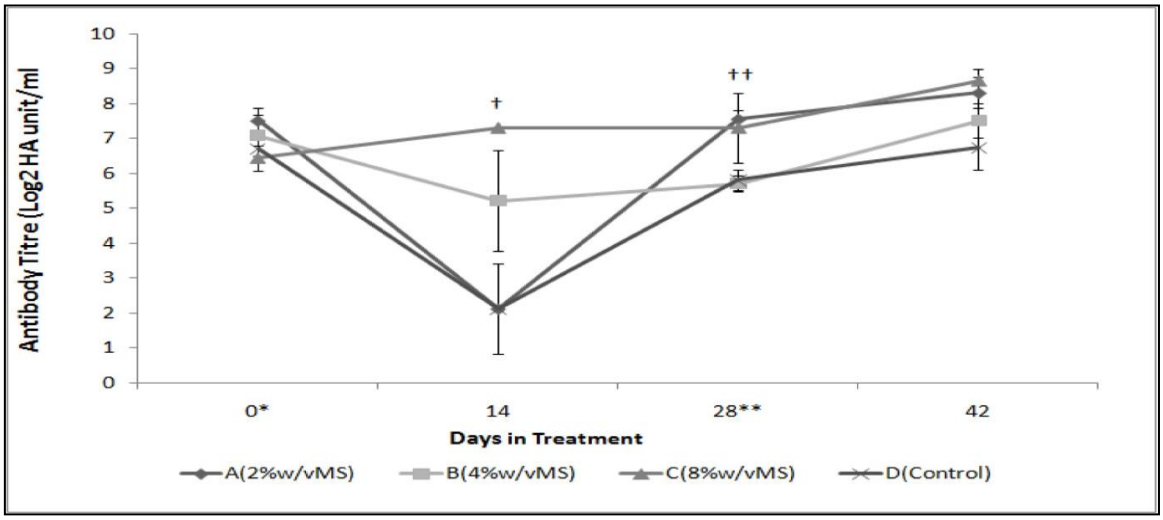


**Figure.1 Humoral immune response to heterologous antigen challenge in rats treated with magnesium stearate. Each data point represents the mean ± standard error of mean of antibody titres of five rats in each group. † (single dagger) represent significantly ($p < 0.05$) higher antibody titre in group C compared to the control and group A. †† (double dagger) represents significantly ($p < 0.05$) higher antibody in groups A and C compared to the control.**

**Table.1Mean total leucocyte count (x$10^3$ cells / µL of blood) of rats treated with magnesium stearate**

| DAYS | A (2% W/V MS) | B(4% W/V MS) | C (8% W/V MS) | D (CONTROL) |
|---|---|---|---|---|
| 0* | 12.28 ± 0.43 | 12.12 ± 1.97 | 14.48 ± 2.33 | 11.94 ± 1.32 |
| 7 | 7.20 ± 1.71 | 5.58 ± 1.60 | 4.94 ± 1.39 | 8.96 ± 1.83 |
| 14 | 12.40 ± 1.20 | 12.22 ± 1.91 | 9.58 ± 2.40 | 11.82 ± 1.45 |
| 28** | 3.50 ± 0.64$^{ab}$ | 1.50 ± 0.23 $^{a}$ | 1.93 ± 0.46 $^{a}$ | 5.33 ± 1.50 $^{b}$ |
| 42 | 9.93 ± 2.05$^{a}$ | 4.95 ± 1.21$^{b}$ | 2.23 ± 0.42$^{b}$ | 10.43 ± 1.12$^{a}$ |

*W/V = weight/volume; MS = magnesium stearate; *represents start of treatment; **represents end of treatment; different superscripts (a,b) flag statistically significant ($p < 0.05$) variations across the groups.*

**Table.2 Mean absolute lymphocyte counts (x$10^3$ cells / µL of blood) of rats treated with magnesium stearate**

| DAYS | A (2% W/V MS) | B(4% W/V MS) | C (8% W/V MS) | D (CONTROL) |
|---|---|---|---|---|
| 0* | 6.36 ± 0.26 | 7.52 ± 2.50 | 9.88 ± 1.74 | 7.13 ± 1.31 |
| 7 | 4.16 ± 0.90$^{ab}$ | 2.14 ± 0.58$^{a}$ | 3.04 ± 0.95$^{a}$ | 6.71 ± 1.40$^{b}$ |
| 14 | 8.04 ± 1.08 | 8.17 ± 1.53 | 6.08 ± 2.52 | 8.19 ± 1.48 |
| 28** | 1.98 ± 0.20$^{ab}$ | 0.72 ± 0.07$^{a}$ | 1.15 ± 0.30$^{a}$ | 3.54 ± 1.08$^{b}$ |
| Day 42 | 6.32 ± 1.43$^{ac}$ | 3.67 ± 1.04$^{bc}$ | 1.64 ± 0.47$^{b}$ | 7.28 ± 1.08$^{a}$ |

*W/V = weight/volume; MS = magnesium stearate; * start of treatment with MS; ** end of treatment with MS; different superscripts (a,b,c) flag statistically significant ($p < 0.05$) variations across the groups.*

**Table.3 Mean absolute neutrophil counts (x$10^3$ cells / µL of blood) of rats treated with magnesium stearate**

| DAYS | A (2% W/V MS) | B (4% W/V MS) | C (8% W/V MS) | D (CONTROL) |
|---|---|---|---|---|
| 0* | 4.75 ± 0.35 | 3.81± 0.66 | 5.66 ± 0.83 | 4.34 ± 0.47 |
| 7 | 3.09 ± 0.87 | 2.21 ± 0.80 | 1.85 ± 0.44 | 2.42 ± 0.88 |
| 14 | 3.78 ± 0.68 | 3.83 ± 0.64 | 5.06 ± 0.10 | 3.53 ± 0. 43 |
| 28** | 1.48 ± 0.71 | 0.69 ± 0.18 | 0.78 ± 0.17 | 1.71 ± 0.48 |
| 42 | 3.40 ± 0.64$^{a}$ | 1.19 ± 0.26$^{b}$ | 0.61± 0.05$^{b}$ | 2.95 ± 0.56$^{a}$ |

*W/V = weight/volume; MS = magnesium stearate; * start of treatment with MS; ** end of treatment with MS; different superscripts (a,b) flag statistically significant ($p < 0.05$) variations across the groups.*

**Table.4 Mean absolute eosinophil count (x$10^3$ cells / µL of blood) of rats treated with magnesium stearate**

| DAYS | A (2% W/V MS) | B(4% W/V MS) | C (8% W/V MS) | D (CONTROL) |
|---|---|---|---|---|
| 0* | 2.24 ± 1.74 | 0.08 ± 0.05 | 0.35 ± 0.12 | 0.32 ± 0.06 |
| 7 | 0.01 ± 0.05 | 0.12 ± 0.05 | 0.03 ± 0.03 | 0.09 ± 0.07 |
| 14 | 0.70 ± 5.02 | 0.17 ± 0.06 | 0.21 ± 0.68 | 0.22 ± 0.04 |
| 28** | 0.04 ± 1.22 | 0.09 ± 0.04 | 0.01 ± 0.00 | 0.04 ± 0.02 |
| 42 | 0.26 ± 0.09 | 0.01 ± 0.06 | 0.01 ± 0.01 | 0.19 ± 0.09 |

*W/V = weight/volume; MS = magnesium stearate; * start of treatment with MS; ** end of treatment with MS; different superscripts (a,b,c) flag statistically significant (p < 0.05) variations across the groups.*

**Table.5 Mean haemoglobin concentration (g/dl) of rats treated with magnesium stearate**

| DAYS | A (2% W/V MS) | B(4% W/V MS) | C (8% W/V MS) | D (CONTROL) |
|---|---|---|---|---|
| 0* | 9.72 ± 1.37 | 11.28 ± 1.45 | 12.02 ± 1.42 | 12.54 ± 0.56 |
| 7 | 14.68 ± 1.00 | 11.60 ± 1.28 | 13.82 ± 0.54 | 14.28 ± 0.49 |
| 14 | 11.96 ± 0.79$^a$ | 17.50 ± 2.25$^b$ | 11.76 ± 0.50$^a$ | 13.02 ± 0.18$^a$ |
| 28** | 14.53 ± 0.73$^b$ | 15.74 ± 0.85$^b$ | 9.57 ± 1.63$^a$ | 14.32 ± 0.26$^b$ |
| 42 | 16.10 ± 2.13 | 16.32 ± 1.85 | 17.93 ± 0.68 | 15.82 ± 2.16 |

*W/V = weight/volume; MS = magnesium stearate; * start of treatment with MS; ** end of treatment with MS; different superscripts (a,b) flag statistically significant (p < 0.05) variations across the groups.*

**Table.6 Mean total erythrocyte count (x$10^6$ cells / µL of blood) of rats treated with magnesium stearate**

| DAYS | A (2% W/V MS) | B(4% W/V MS) | C (8% W/V MS) | D (CONTROL) |
|---|---|---|---|---|
| 0* | 5.67 ± 0.37 | 5.45 ± 0.74 | 6.92 ± 0.63 | 7.33 ± 0.85 |
| 7 | 5.84 ± 0.52 | 6.01 ± 0.46 | 5.46 ± 0.40 | 5.56 ± 0.60 |
| 14 | 4.19 ± 0.43 | 3.89 ± 0.51 | 5.61 ± 0.68 | 5.15 ± 0.87 |
| 28** | 3.15 ± 0.36 | 3.49 ± 0.59 | 2.83 ± 0.38 | 4.20 ± 0.54 |
| 42 | 4.31 ± 0.60 | 4.90 ± 1.18 | 5.42 ± 1.47 | 4.60 ± 0.55 |

*W/V = weight/volume; MS = magnesium stearate; * start of treatment with MS; ** end of treatment with MS; different superscripts (a,b,c) flag statistically significant (p < 0.05) variations across the groups.*

**Table.7 Mean packed cell volumes (%) of rats treated with magnesium stearate**

| DAYS | A (2% W/V MS) | B(4% W/V MS) | C (8% W/V MS) | D (CONTROL) |
|---|---|---|---|---|
| 0* | 42.20 ± 2.31 | 37.80 ± 2.08 | 41.2± 4.97 | 45.40 ± 1.33 |
| 7 | 41.40 ± 2.09 | 41.60 ± 1.50 | 42.80 ± 4.97 | 41.00 ± 2.02 |
| 14 | 45.20 ± 1.10 | 44.00 ± 1.26 | 42.80 ± 1.83 | 45.00 ± 1.58 |
| 28** | 46.50 ± 0.65 | 48.80 ± 1.93 | 38.00 ± 8.14 | 48.25 ± 0.63 |
| 42 | 48.50 ± 2.36 | 49.40 ± 1.72 | 45.33 ± 2.03 | 47.50 ± 1.80 |

*W/V = weight/volume; MS = magnesium stearate; * start of treatment with MS; ** end of treatment with MS; different superscripts (a,b,c) flag statistically significant (p < 0.05) variations across the groups.*

## DISCUSSION

The graded concentrations of MS that were used for this study (2%, 4%, and 8% w/v) were informed by the classic concentration range permissible in the industry, which lies between 0.25% to 5.0% w/w (Allen LV & Luner PE, 2006).

Prior to treatments with MS, animals in all the experimental groups had similar antibody titre in response to stimulation with a heterologous antigen (Fig.1). On day 14, treatment with magnesium stearate resulted in a significantly higher antibody titres in groups C (8% w/v M/S) compared to the control. On the same day, the pattern of variations in antibody titres recorded across the treated groups reflected a dose-response relationship between MS treatment levels and antibody titres. Further stimulation with the same antigen, by day 28 of treatment caused an elevated antibody response across all the groups thus dismantling the dose-response pattern. This elevation in antibody titres even in the control group is probably an anamnestic response to secondary stimulation with the same antigen. Anamnestic immune response is a well-established concept in immunology (Lightowler MW *et al*., 2016; Jonker EF & Visser LG, 2017). These findings suggest that excipient doses of MS do not adversely affect humoral immune response in rat *in vivo*; in fact, it appears that during the early stages (about 14 days) following challenge with heterologous antigen, treatment with magnesium stearate enhanced humoral immune response (Fig. 1). However, this is not to be the case with cell-mediated immunity.

By day 28, treatment with magnesium stearate resulted in significantly lower total leucocyte counts in groups B (4%w/v MS) and C (8% w/v MS) compared to the control (Table 1). The absolute neutrophil counts were marginally depressed in all the treated groups

compared to the control by day 28 (Table 3). A sharper variation was noted in the absolute lymphocyte counts across the groups: by days 7 and 28, treatment with magnesium stearate resulted in a significantly lower absolute lymphocyte counts in groups B (4% w/v MS) and C (8% w/v MS) compared to the control group (Table 2). These findings indicate that excipient doses of MS induced depression in leucocyte counts in rats *in vivo*. The results also indicate that the lymphocytic sub-population is more severely affected than the granulocytic sub-population, tending towards lymphocytic leucopenia.

A paradox is clearly seen from the results of humoral immune response and lymphocyte counts presented above. In this work, it was earlier shown that excipient doses of magnesium stearate do not adversely affect humoral immune response—a function carried out by the B- lymphocyte sub-population; later on, it was also shown that MS depressed lymphocyte counts within the same period; this constitutes a paradox. A conceivable explanation is that while MS depletes the T-cell sub-population (thereby precipitating low lymphocyte counts), it neither depletes the B-cell sub-population nor disrupt their function (humoral immune response) *in vivo*. In the body, magnesium stearate is broken down into magnesium ion and stearic acid. It has been shown that stearic acid is selectively cytotoxic to T -cells (Buttke TM & Cuchens MA, 1984). When mitogen-activated T and B cells were incubated with stearic acid, T -cells replaced their normal membrane phoshatidylcholine with large amounts of desaturated stearic acid-containing phosphatidylcholine (PC) which resulted in the collapse of T- cell membrane integrity within 8 hours; B-cells did not accumulate large amounts of stearic acid within their membrane because of their desaturating ability, and were able to maintain their membrane integrity and function (Tebbey PW & Buttke TM, 1990). It has long been suggested that the alteration of membrane lipid composition could lead to defect in cell membrane integrity and function (Kuyper *et al.*, 1984). It was proposed that T cells lack the enzyme stearoyl-CoA desaturase found in B cells which is responsible for desaturation of stearic acid (Tebbey PW & Buttke TM, 1990).

Another remarkable finding is that significant lymphocyte-count lowering effect of magnesium stearate was seen by day 7 of exposure. The clinical importance of this is that even short term continued use (lasting for about 7 days) of agents containing excipient doses of magnesium stearate could be potentially unsafe with respect to cellular immune response.

Treatment with excipient doses of magnesium stearate did not adversely affect the erythrocytic profile of rats across the groups. In fact, while total erythrocyte counts and the packed cell volumes of MS-treated groups compared with those of the control group, by day 14 of treatment, group B (4% w/v MS) had significantly higher haemoglobin concentration in comparison to other groups. This agrees with the conclusions of Sondergarrd *et al.* (Sondergaard D *et al.*, 1980) that magnesium stearate is non-toxic *in vivo* up to a concentration of 5%w/v.

Following the withdrawal of treatment with MS on day 28, all the treated groups, except group C, (8% w/v MS) began to recover from depressed total and differential leucocyte counts as shown by the their leucocytic indices on day 42. In addition, similar antibody titres to antigenic stimulation were recorded across all the groups by day 42 following withdrawal of treatment with MS. These suggest that the effects of excipient doses of magnesium stearate on cellular and humoral immunity are transient.

The veterinary and public health importance of these findings may continue to face intellectual controversies, where one group considers MS to be at least suspicious whilst the other group considers MS to be completely safe. For the 'completely safe' group, the major criticism against the report of Tebbey and Buttke (Tebbey PW & Buttke TM, 1990) could be that the work demonstrated the immunosuppressive effect of stearic acid on T-lymphocytes *in vitro* and not *in vivo*, and that the study was done using mice T-cells; hence, the results may not be accurately extrapolated for humans and other animal species. In addition, some workers were able to demonstrate that activated human T-cells show reasonable desaturase enzymes activity, and are able to avoid accumulation of stearic acid within their cell membrane (Anel A. *et al.*, 1990); hence it is unlikely that immunosuppression would develop in humans exposed to MS. While this experimental trial may not be sufficiently conclusive for making generalized statements on the toxicity of MS for various human races and animal species; it suffices to say that this study was carried out using *in vivo* experimental models (rats), and there are reasons, from our findings, to think that certain excipient doses of magnesium stearate are toxic to T-lymphocytes *in vivo.*

## CONCLUSION

From this study, evidence suggesting T-lymphocyte toxicity was seen following treatment with certain excipient doses of MS over a period of time. Based on the foregoing, it is recommended that to be on the side of safety, magnesium stearate may not be the best excipient for drugs or biopharmaceuticals destined for immunostimulation, treatment of immunocompromised patients, or for patients suffering infectious diseases that may require the induction cell mediated immunity for cure, especially when such products could be used continuously over a long period. When magnesium stearate must be used for such drugs or biopharmaceuticals, perhaps concentrations below 1% w/v or w/w should be used.

**Conflict of Interests:** none

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