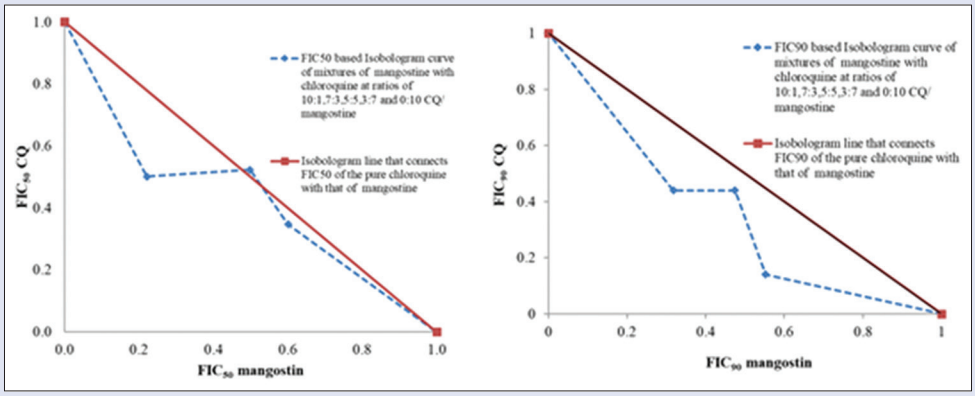

Figure 2: The FIC_{50}- and FIC_{90}-based isobolograms for different combination mixtures of mangostin and chloroquine at (10:0, 7:3, 5:5, 3:7 and 0:10 (chloroquine/mangostin). The red lines in the two graphs represent lines of additively. Synergy is considered for the points located above the line of additivity while antagonism is considered for points located above that line

results of the simulation software, mangostin has been included in lots of *in vitro* study and its action against cellular growth and the molecular machinery of different cells was studied extensively.[12,14,32]

The study revealed a poor effect for mangostin against all the targets except on the nuclear receptors as an enzyme inhibitor. Nuclear receptors are targets of the transcription factors which promote or repress different genes expression. This effect against different cell line was proved previously as its effect on the expression of the inflammatory genes[32] or its effect on genes involved in the differentiation of myeloblasts to myotubules.[33] On the other hand, the software predicted the presence of an inhibitory effect against intracellular enzymes. This action for mangostin was seen against different intracellular enymes, such as; cyclooxygenase enzyme that is involved in inflammation in mammals,[13] sphingomylinase that is involved in apoptosis or on enzymes involved in cellular mitosis like topoisomerase or DNA polymerase.[34] But any way such effects were detected *in vitro* in human and were suggested by the software in the human models. But this suggests their presence in plasmodium as well due to the homology in different intracellular targets with the human model. The selective effect against the parasite can be attributed to having a larger extent of action against the parasite rather than the mammal cells.

The *in vitro* assessment of hemozoin formation inhibition shows that mangostin had a capacity to bind to heme and suppress hemozoin formation in an extent lower than that of CQ. This can be attributed to its quinone group containing structure that qualifies it to establish bindings with heme.[35,38] Ubiquity of the lipophilic alkyl side chains in the mangostin structure might have hindered its binding to heme [Figure 1]. Heme is a toxic byproduct of hemoglobin catabolism. It is detoxified inside the digestive vacuole (DV) through series of biocrystallization and biomineralization steps to produce hemozoin as an innocuous waste product. This step is crucial for the survival of the parasite and may be targeted by many drugs that lead it its accumulation within the plasmodial cytosol. It has a powerful pro-oxidant effect and cellular damage. Interference with heme detoxification is the main mechanism of CQ; the most widely used conventional antimalarial chemotherapy.[36] Hemozoin formation requires establishment of reciprocate iron oxygen bonds between the central iron of the one of the ferroprotoporphyrine moieties and the carboxyl group of the other (π-π bonding).[37] This bonding results in creation of heme dimers that can stack together though establishing hydrogen bonds among the uncoordinated side chains. This process can be inhibited by drugs that can establish π-π bond with the feroprotoporphyrine resulting in halting of heme dimer and the subsequent hemozoin formation. CQ is a good example as it contains hydroxyl moieties that entitle it to undergo this π-π bonding.[37] its ability to reduce Sorret band intensity suggests that it can inhibit heme polymerization. Drugs that interfere with hemozoin formation may reduce its action through establishing the π-π bond, induction of heme aggregation or precipitation or through creation of axial bonds through binding with the feroprotoprphyrine oxygen at an axial position.[38] The similar stoichiometric ratio of mangostin to that of CQ in the heme binding assay, suggests that their binding to the ferroprotoporphyrine occurs through π-π binding.

The inhibitory effect against plasmodial hemozoin formation was not parallel with that against the parasite growth as the former required higher concentration. This discrepancy may be due to factors related to the drugs ability to accumulate inside the DV as each needs to cross the DV membrane and accumulate against the drug efflux mechanisms. Furthermore, this phenomenon suggests that, the antiplasmoial action is not conferred only by their anti hemozoin action but other mechanisms are suggested.

Different antimalarials target different intra-cellular pathways as some act on the DV, viz., the parasite protease enzyme or heme detoxification pathways. Others affect cytoplasmic targets like fatty acid or isoprenoid synthesis pathways, histidin-rich protein, or plasmodial protein kinase.[39] Not only does CQ acts on the hemozoin pathway, recently, it has been found that it may act first as a lysmotropic amine, like CQ. It can bind to the integral proteins of the DV membrane resulting in the permeabilization of its low gram/weight hydrolytic enzymes to the cytosol, viz., cathepsin. The later trigger the sequential cascade of apoptosis induction.[40]

At low doses, CQ induces the apoptotic features at a basal level while at higher doses (micro-molar concentrations), a higher number of apoptotic cells with MOMP and caspase over-activation evolve. Meanwhile, at the physiological nanomolar concentration, CQ accumulates inside the DV and starts interfering with hemozoin formation. It starts appearing in the cytosol when its concentration jumps to micro-molar concentration due to the DV membrane permeabilization.[16,40]

The study excluded any effect for mangostin against the NPP pathway within their effective concentrations against the parasite growth (data not shown). The NPPs evolve due to intraerythrocytic ubiquity of the parasite which induces structural changes in the RBCs membrane characterized by their appearance. NPPs are specific channels that regulate entry of the nutrients and electrolytes and enhance exodus of the waste products within the infected cells only. Its inhibition may compromise the parasite growth.[41,42]

Mangostin showed a prominent antioxidant power. Antioxidants act as double edged sword weapons for the cells. Form one side; they protect the cells through halting the flow of the deleterious free radicals; which are released as by products due to the cellular activities. On the other hand, they may turn into pro-oxidants and release more free radicals at higher concentration. This concentration threshold is different between different cells and it is not sure if there is a discrepancy in this threshold between plasmodia and human cells.[43,44] Previous studies had pointed out to the significance of such discrepancy in eradicating the undeveloped cells.[44] Thus, it is recommended to test their impact on free radicals accumulation at the concentration wherein their antiplasmodium impact had been produced.

Unlike most of the phytochemicals, mangostin had a low toxic effect against two models of mammalian cells; RBCs and Vero cells. But it is still considered as a patent drug as it produced its cytotoxicity at IC_{50s} >30 μg/ml [Table 3]. This rendered it moderately selective drug to the plasmodium. Consequently, caution should be exercised while introducing it to malaria chemotherapy. Its impact on the RBCs can be attributed to its lipophilicity which qualifies it to accumulate in the cell membrane and induces structural changes characterized by disruption of the membrane double layer integrity, membrane speculation, alteration in RBCs morphology and subsequent RBCs hemolysis.[30] Paradoxically, the *in vitro* assessment of its antioxidant potential suggests that mangostin confer some protection to cell membranes through scavenging the deleterious free radicals as it showed a prominent potential to scavenge the lipophilic DPPH. This protection may incur during its early accumulation within the membrane but hemolysis is induced when its accumulation exceeds the thresholds.

On the other hand, its effect against Vero cells can be ascribed to its aptitude to induce the apoptotic pathway and cell cycle arrest.[45] Its lipophilicity qualified it to disrupt the functional characters of the membranous organelles like the mitochondria and the lysosomes resulting in the induction of the cascade pathway of the apoptosis. Furthermore, it may disrupt the integrity of the cell membrane resulting in changing the physiological function of the cells. The results revealed a comparable cytotoxic effect against the teste mammalian cells but this does not exclude a discrepancy in the mechanism through which the cytotoxicity was produced in the RBCs and the vero cells.

Different drugs were tested for the CQ -resistance reversal using different CQ resistant strains of *P. falciparum*. Some showed good effect like; calcium channel blockers, antipsychotics, tricyclic antidepressants, antihistamines, and nonsteroidal anti-inflammatory drugs.[46] It is suggested that their action is through inhibition of the functional activity of *pf*crt; the channel protein involved in the accumulation of CQ within the DV.[47] Previously, it was reported that CQ resistance is closely associated with mutational changes in the *pf*crt structure[48] and its function is affected by the biochemical changes within the surrounding environment. For instance, the -reversing-effect of verapamil was ascribed to its binding to certain allosteric sites within the *Pf*crt. But till now, a clear molecular elucidation for its claimed action has not been achieved yet.[49]

All in all, any drug; that may enhance the mentioned mechanisms, may synergize CQ and reverse its resistance. Since phytochemicals are Janus molecules and can act on multiple intracellular targets. They may chemo sensitize CQ and reverse its resistance.

Furthermore, CQ synergism may be conferred by drugs that enhance its binding to the heme moiety or its intra-vacuolar accumulation or those that compromise the DV membrane (DVM) stability and increase its permeabilization. This is followed by seeping of the hydrolytic enzymes into the parasite cytoplasm and induction of the apoptosis. It was suggested that drugs which augment CQ induced apoptotic pathway may confer synergy.[40,50]

Results of the isobologram analysis revealed absence of any antagonism between CQ and mangostin as none of the combinations produced a sum for FIC_{50} tot or FIC_{90} >2. Antagonism with CQ may occur in the presence of any agent that interfere with access of CQ to the DV or inhibits the CQ induced oxidative stress through moping out the free radicals. Although, both phytochemicals have had good antioxidant potential. But this could not have entitled them to antagonize CQ effect.

Synergy was obtained only when mangostin was combined with CQ especially when both were combined at a ratio of 7:3 (CQ/mangostin). At this ratio, mangostin concentration was as little as 3 μM suggesting higher selectivity for its synergy with CQ. Previous studies have attributed the potential of CQ resistance reversing agents to their ability to reduce CQ exodus outside the DV by inhibiting the DV membrane transporters. Others suggested that CQ induced apoptotic pathway can be set as a target for some drugs to sensitize CQ.[40,41] It is noteworthy that its synergy was somehow more obvious in the FIC_{90}-based isobologram rather than the FIC_{50} based one suggesting that its potential to inhibit CQ tolerance is higher than its effect on the resistance.

Mangostin effect against hemozoin formation can be set as another reason for the observed synergy at the mentioned ratio or the additive effect that was obtained at the combinations of the higher mangostin ratio. Previous studies have pointed out to the role mangostin in induction of the apoptotic pathway and induction of cell cycle arrest in human cancer cells. Such action may be conferred by drugs that can bind to nuclear receptors; an action which is suggested for mangostin as per results of Molinspiration simulation software. This suggests that this pathway might have imparted in the induction of the CQ synergy with mangostin, but further studies are required to confirm this notion.

CONCLUSION

Overall, although, it is unsuitable to use mangostin as a substituent for CQ, it can be considered as an important pharmacophore to develop new antimalarials in the future. The inappropriateness stems from its strongly lipophilic properties that interferes. Mangostin has a promising effect against hemozoin formation both *in vivo* and *in vitro* and this paves the way to develop new derivatives that retain this activity. Its synergy with CQ suggests its use as a sensitizer but further structural or pharmaceutical modifications are required to improve this action.

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Conflicts of interest

There are no conflicts of interest.

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